

**INTERNATIONAL JOURNAL OF SCIENTIFIC  
RESEARCH IN  
SCIENCE & TECHNOLOGY**

**VOLUME 1 ISSUE 3 JULY-AUGUST-2015**



**PRINT ISSN : 2395-6011  
ONLINE ISSN : 2395-602X**

**Web Site : [www.ijsrst.com](http://www.ijsrst.com)**

**Email : [editor@ijsrst.com](mailto:editor@ijsrst.com)**



# **International Journal of Scientific Research in Science and Technology**

**Print ISSN: 2395-6011 Online ISSN : 2395-602X**

**Volume 1 Issue 3 July-August-2015**

**International Peer Reviewed, Open Access Journal  
Bimonthly Publication**

**Published By**

**Technoscience Academy**

**(The International Open Access Publisher)**

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# Evaluation the Effect of Pomegranate Pulp of Mixed Alag type with Cow Manure on Eisenia Fetida Worms

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## ABSTRACT

In order to use produced pomegranate pulp in food industry, it is better to consume these remnants to produce organic fertilizers such as Vermicomposting. In this experiment after drying and grinding, pomegranate with ratios of 50%, 40%, 30%, 20%, 10% and control (without pomegranate pulp) mixed with cow manure. Then 100 *Eisenia fetida* mature earthworms were added to each six experimental unit. These experimental units were kept at 25 °C, 75% moisture –holding capacity of water during 80 days. The results showed that higher percentages of pomegranates pulp prevent *E. fetida*'s proliferation and development and it disrupts vermicomposting. Maximum pomegranate pulp which does not reduce *E. fetida* worms' population is its 30% density. To accelerate the vermicomposting process, it is better that pomegranate pulp density is between 10% to 20%, because it is less effective on worms' proliferation and development.

**Keywords:** Pomegranate Pulp, Proliferation, Cow Manure, Vermicomposting, *Eisenia Fetida*

## I. INTRODUCTION

Pomegranate with scientific name as *Punica grantum* belongs to punicea species and it is one of the oldest known fruits which is widely cultivated in Mediterranean countries such as Turkey, Egypt, Tunisia, Spain, Morocco and countries like Iran, Afghanistan, India and partially in China, Japan and Russia (Onur, 1995). According to statistics of Ministry of agriculture in 2003, the most important producing provinces on the basis of cultivation and in descending order are Fars, Khorasan, Isfahan, Markazi, Yazd, Kerman, Semnan and Tehran. Pomegranate is one of the fruits which can be consumed as fresh fruit like citrus and apple. This fruits has vitamins such as B1, B2, B6 and C and also elements like Calcium, phosphorus and Manganese which balance body fluids, especially blood. Various experiment indicate that experimentally 80-85% of the weight of the pomegranate pellets includes its juice or extract form including glycoside, lipids, organic acid, tannins, and various vitamins and soluble minerals

(Mirjalili, 2003). Due to the beneficial properties of pomegranate tend to use this fruit, in particular, pomegranate juice and other processed products such as fruit concentrate, fruit paste, etc has increased day by day which consequently, related industries dependent on production of this fruit has also increased. This factor has caused many pomegranate juice and concentrate factories produce significant amounts of waste. In addition to environmental problems, these remains cause sad and unpleasant sights in nature (Garcia, 2010). The waste produced by these plants, pomegranate peel contains polyphenols substances like Alajyk acid, Tannins and Gallic acid and Anthocyanin (Farhush, 2003; Antoun et al., 1997; Gu et al., 2001; Kaya et al., 1993; Mohamed et al., 1998; Mohd Zin et al., 2002). But wastes produced in these factories are much more than their need in the mentioned industries. Therefore, producing pomegranate factories proceed to bury burn or abandon their waste in the nature which this leads to damage environment. To avoid this phenomenon, it is better that these remains are used to produce organic fertilizers such as vermicomposting. In addition, the

production of these fertilizers prevents environment pollution, create jobs, generate added value and avoid waste of energy and country's capital. Some changes in the remains of organic minerals can proportionate them for use on land and optimized usage in environment. The compost of natural material is a mixture with an organic material which is done by micro-organisms continuous process in a hot and humid environment with air flow and organic materials change into sustainable material which is called humus or compost; in the other hand, compost production is a process of biodegradation of waste under controlled conditions (Haimi & Hutha, 1987). The organic materials in one place for proliferation and subsequent use in agriculturally is done by farmers in different cultures in centuries. In Europe, the use of waste compost to enhance soil fertility goes back to Roman times. It is reported that compost making is the most effective approach to control and manage the remains of organic materials (Bhattacharjeetal, 2002; Dickerson, 2001; Aira et al., 2006). Vermicomposting, as the term indicates, is a kind of produced vermicomposting with the help of earthworms which creates as a result of change in survivors partial digestion through cross in digestive organ of these animals. Because earth worms can grow and multiply fast and have significant potential for the use of waste organic material, such materials are often annoying and polluting the environment, convert to a high quality organic fertilizer. Products which are labeled vermicomposting are organic materials with adjusted PH and full of humus substances and nutrients in the absorbable form for a plant and a variety of different vitamins and growth promoting hormones of plant and different enzymes (Kale et al., 1992). The most important benefits of vermicomposting are buffering property that prevents PH fluctuations during ingredients uptake by plant (Bowman & Reinecke, 1991). Vermicomposting has a potential property to hold water and it can, because of organic acid, solve the nutrients in the soil, especially micro-nutrient elements such as iron through the complex process and provides it to the plant (Reinecke & Vilijoen, 1990). Also, vermicomposting is able to stabilize heavy elements in the soil and prevent excessive absorption by the plant and improve the biological, physical and chemical properties of soil that its results are a positive impact on the quality and quantity performance of product. Vermicomposting has high and specific surface area and provides strong absorption and maintenance of nutrients (Cardetral,

2004). Humus-vermicomposting has organic materials stability, absorption of water and nutrients to feed plants and release water during drought. Vermicomposting contains nutrients in the absorbable form for plants such as Nitrogen, exchangeable Phosphorus, Potassium, and Calcium and solved Magnesium (Edwards et al., 1972). In addition to producing organic fertilizer, vermicomposting production process also is able to produce a second product called biomass earthworm which can be used as a source of protein to feed the livestock and poultry (Alikhani, 2006). Vermicomposting does not have environmental pollution and adverse effects of chemical fertilizers. This fertilizer is extremely useful for growing crops and increases the performance of cultivated crops (Alikhani, 2006). Vermicomposting is a harmless fertilizer and does not have any negative effect on the ecosystem. Repellents by worms often contain Nitrogen, Phosphorus and Potassium at a rate of 5 to 11 times is higher than soils without worm. Therefore, worms play a beneficial and constructive role in the soil. Vermicomposting can control plant diseases. Another benefit of vermicomposting is that during its production the population of pathogens greatly reduces (Atiyeh et al., 2000). According to research, one of the most famous compost maker worms is *Eisenia fetida* that its body composition includes 63% protein, 11% fat, 6%ash, and nitrogen-free extract 19% (Ebadi et al., 2005). Vermicomposting generally lasts 6 to 12 weeks. The rate of decomposition of organic materials depends on many factors such as the nature and amount of organic materials, temperature, the used system and the ration of the amount of worm to the amount of organic materials (Ebadi et al, 2005). In recent years, various studies are done on vermicomposting from organic waste materials. In a study by Kavshyk and Grag, the potential of *Eisenia fetida* earthworm was investigated to decompose textile sludge mixed with cow manure and agricultural waste materials. In this study, the highest rates of growth and reproduction of earthworms was observed in area which only contained cow manure. It was also found that the vermicomposting process has significant impact in reducing the C/N ration and increasing the percentage of K, P, N and Ca bedding materials. Despite studies in the field of compost and vermicomposting production of different organic residues, fertilizers production from pomegranate pulp is not considered. Moreover, related industries to those products generate significant quantities of pomegranate pulp in the country each year.

Vermicomposting can be produced from these remnants, in addition to preventing pollution, provides employment, generates added value and avoids waste of energy and country's capital. To do this, investigation of chemical properties of pomegranate pulp for the production of compost and vermicomposting is significant.

## II. METHODS AND MATERIAL

First, pomegranate type of algal samples collected from Saveh gardens and resulted pulp dried away from sunlight for a week. Then, pulps by using grinding have been completely powered until digestion will be easy for worms. In order to leave harmful compounds and come down EC, cow manure was completely rotten and four times washes with tap water with intervals of 12 hours. In this experiment, *Eisenia fetida* earthworm was used for vermicomposting. This experiment with different ration of pomegranate pulp and cow manure was designed and implemented in six pilot units weighing three kilograms. The first pilot units, 50% fruit pulp contains 1500 grams of fruit pulp; second pilot unit 40% fruit pulp contains 1200 grams of fruit pulp, the third pilot, 30% of the pulp contains 900 grams of fruit pulp, the fourth pilot unit, 20% of the fruit pulp contains 600 grams of fruit pulp, the fifth pilot test, 10% of fruit pulp contains 300 mg and sixth only contains 3 kilograms of cow manure (control). After producing the desired ration of manure and pulp, provided content was thoroughly mixed to wet saturated surface. In each experiment, 100 manure worms were added after 24hours. All experimental units were kept in a room at 25°C with 75% moisture-holding capacity of water for 80 days and were gently mixed for aeration of organic materials in the pilot units with one week intervals. After the end of vermicomposting, mature worms population, the number of cocoons (worm eggs) and infant and immature worms in each of the experimental units were counted.

## III. RESULT AND DISCUSSION

Considering that the aim of this study was to evaluate the effect of pomegranate pulp on biomass earthworms, the investigation of earthworms' reproductive changes was important in the first place. The number of eggs and infant worms' population determine breeding rate of earthworms. The number eggs represents earthworms

tend to reproduce and the number of population of infant worms indicates that context conditions are suitable to change these eggs to earthworms. Regarding to different percentages of pomegranate pulp, worms showed different responses so that in the first test unit (the highest rate of fruit pulp) were completely destroyed. Also by reducing the amount of pomegranate pulp in bed, proliferation and growth of earthworms increased as well. Table [1] shows the population and the number of worms and infants in this experiment.

**Table 1** Population and the number of worms cocoons *E. fetida* different than cow manure and pomegranate

Experiment Unit	1 pomegranate pulp %50	2 pomegranate pulp %40	3 pomegranate pulp %30	4 pomegranate pulp %20	5 pomegranate pulp %10	6 control (cow manure)
No. mature earthworms	--	24	44	105	124	134
No. cocoons	--	101	189	292	354	382
NO. Newborn earthworms	--	189	346	810	1101	1149

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# An Analysis of Mean Monthly Soil Temperature Fluctuation at Different depths at Compton Experimental Site, West Midlands, (UK), Between 1975 - 2008

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## ABSTRACT

The study analyzed mean monthly soil temperature fluctuation at different depths at Compton research site over the period (1975-2008), West Midlands; (UK) based on the statistical analysis of the data base of (12,045) days of individual soil temperature measurements in sandy-loam of the salwick series soils. It was found that the mean monthly soil temperature at all depths significantly increased from 4.80C, although a dramatic fluctuation at 5 cm depth was evident. The soil temperature decreased to around mid-April and then increased steadily, where it attained the maximum in August. However, it has also been observed that soil temperatures fluctuated at 5 and 10 cm depths. The temperature range at 5 cm depth was 16.50C and 10.50C at 10 cm depth. The study concludes that variation occurs at different soil depths and the pattern of fluctuation was in response to periodic shifts in meteorological variables acting on the soil system.

**Keywords:** soil temperature, temperature fluctuation, diurnal temperature variation, seasonal trend

## I. INTRODUCTION

Bai *et al.* (2010), Soil temperature is a very significant environmental factor that control the exchange of heat energy between the land surface and atmosphere. The speed of biological, physical and chemical response in soils have powerful controls on plant growth and soil formation.

Wu and Subbaru (2008) argued that sub soil temperatures are influenced by meteorological conditions and agricultural practices. Diurnal variations in soil temperature occur especially in the sub soil, and frequently soil moisture fluctuates. Similarly, Tang *et al.* (2011) observed that the pattern of soil temperature fluctuations with depths and time are the result of heat transfer processes functioning in the soil system.

In soil classification, soil temperature is an important factor for consideration (Soil Survey Staff, 1999; cited

in Bai *et al.* 2010). It is integrated into soil taxonomy through the soil temperature system, such as the variation between mean summer temperature and mean winter soil temperature, as well the mean annual soil temperature.

Over the years, scientists for example, Lambert *et al.* (2005), Martinez *et al.* (2008), Ghahreman *et al.* (2010) and Tang *et al.* (2010) described the patterns of soil temperature variations at different depths, and seasonal trends, with the emerging view that soil temperatures fluctuates dramatically.

Hu and Feng (2003) argued that fluctuation in soil temperature and moisture change the pattern of heat transfer from the surface and influence atmospheric boundary layer processes and regional circulation (Pan and Mahrt 1987; Peters-Lidard *et al.* 1998; cited in Hu and Feng 2003). Soil temperature fluctuation results mostly from shallow radiation and

latent heat exchange and vertical heat transfer in soils with diverse thermal properties. Thus, soil temperatures at different depths are exceptional parameters that are valuable to demonstrate surface energy regional environmental processes and the state of climate.

Ghahreman *et al.* (2010) demonstrated that soil temperatures fluctuate as a function of the occurrence of solar radiation, rainfall, local vegetation cover, soil properties, soil depth and seasonal movement in overlying air temperature. Variation in soil temperature occurs both daily and yearly and those changes are most apparent at or very close to the surface, where sunlight has most influence.

Lascalar and Pereira (2003) observed that solar radiation is a vital process governing the diurnal increase in soil temperature and water evaporation, regulating the rate of soil CO<sub>2</sub> production and CO<sub>2</sub> emission from soil to the atmosphere.

Lu and Cheng (2009) reported that soil CO<sub>2</sub> emission is determined by complex interfaces of temperature, soil properties, decomposition of organic substrates and moisture. During winter, global climate change have minute influences on soil CO<sub>2</sub> emissions, but during summer periods CO<sub>2</sub> emission increased considerably. Despite the variation in air temperature during winter, soil temperature declined as a result of snow and soil frost.

Zhang *et al.* (2005) argued that changes in soil temperature for lengthy periods may fluctuate from that of air temperature. These are due to the fact that modification of climatic variables, snow, vegetation and soil moisture (humidity, solar radiation and precipitation) generally control water and energy fluxes on the surface and within the soil. Hence, these processes transformed the connection between soil and air temperatures. Similarly, changes in precipitation and snow intensity are of much significance as changes in air temperature in assessing soil temperature changes.

In a review, Uvarov *et al.* (2011) observed that an array of temperature dynamics in soil considerably go beyond the changes predicted for future decades.

Similarly, global warming not only means levels but also series of temperature variations are likely to change. For instance, soil winter temperatures rapidly increased more than the mean temperature over recent years. Furthermore, Tang *et al.* (2010), argued that pattern of disparity in soil temperature with depth and time give insights into heat transfer development processes in soil. By nature, variation in soil temperature is constantly in reaction to the increasing shift in the meteorological regime acting on the soil-atmosphere boundary.

Lambert *et al.* (2005) noted that soil temperature frequently fluctuate. Yearly and diurnal soil temperature dynamics influenced many biotic and abiotic processes in soil systems. Lavigne *et al.* (2004) observed that soil temperature increased significantly from April and mid-June and then was comparatively stable between late June until late August. Thereafter, temperature decreased gradually from September and December. However, differences from the overall pattern caused yearly climatic data to fluctuate among the years. Small diurnal or weekly differences in soil temperature often occurred. Minimal diurnal and weekly temperature fluctuations occurred in winter, when snow cover was intact. Thus, notable diurnal and weekly differences of temperature occur intermittently between May and November.

Sierra *et al.* (2010) reported that many scholars demonstrated that seasonal fluctuation in soil temperature encouraged changes in the arrangement of microbial community related to soil organic carbon (SOC) turnover, (Fenner *et al.* 2005; cited in Sierra *et al.* 2010) noted that advanced thermal adaptation of soil micro-organisms can play a significant role in abating the positive response between the soil C cycle and warming climates.

Many researchers have explored the pattern of soil temperature fluctuation using different criteria. Correspondingly, an array of related studies were conducted for example Mila and Yang (2008), Wang *et al.* (2011), Gutknecht *et al.* (2012), and Schindlbacher *et al.* (2014) their contribution showed great extent of interest and knowledge in soil temperature regime.

There has not been any research on soil temperature fluctuation at Compton. Based on this background therefore, the aim of the study is to examine the mean monthly soil temperature fluctuation at the research site with a view to ascertain the typical behavior of this particular soil thermal environment at different depths. This will create awareness interest and knowledge on secular trends.

## Site Description

The current study was carried out at Compton Campus (University of Wolverhampton), West Midlands (UK). It is roughly 2.39 km from the University of Wolverhampton, City Campus, and is located at 52.58717<sup>0</sup>E and 2.163483<sup>0</sup>S, (URL:itouch map). The research site was established in 1970, also measurement of soil temperatures began in 1975 and has over the years been used for studies on soil temperature, soil conservation, plant experiment and meteorology. The meteorological station at the upper flat section has a short grass cover and elephant plant (*Miscanthus*) as part of long term meteorological observations.

Brandsma (1997) reported that the nature of the soil is sandy-loam of the Salwick series with a dark topsoil of 32 cm deep and a sandstone rock underneath. The texture of the soil consists of sandy silt loam 41.4% (2000-60  $\mu$ m), silt 51.3% (60-2  $\mu$ m), clay 7.3% (< 2  $\mu$ m) and soil organic matter content is 2.7% by weight. The pH level is 6.5 as (Vaz, 2001) demonstrated.

## II. METHODS AND MATERIAL

The materials for temperature measurement at Compton research site include: Slab minimum thermometer, The bare earth minimum thermometer, which is used to measure the temperature in open air on short grass, 60 cm deep thermometer, and rain gauge which is used to measure the amount of precipitation received (mm), a Stephenson's Screen (maximum and minimum wet and dry bulb) which is used to measure air temperature and relative humidity, the soil thermometer at depths of 5, 10, 20 and 30 cm.

An anemometer is used to measure wind direction and velocity.

Data for the present study was collected from Compton meteorological station. These soil temperature data were measured at different soil depths based on 37 years of observation 1975-2012. This study analyses the soil temperature data recorded between 1975-2008. Results are analyzed using statistical methods (Excel package). The statistical analysis involves descriptive statistics (mean, median, minimum and maximum temperature). Correlation and regression analysis were used to identify temporal trends in soil temperature.

## III. RESULT AND DISCUSSION

The findings of the study demonstrate that soil temperature at all depths increased considerably, although a dramatic fluctuation at 5 cm depth was apparent. The soil temperature decreased to around mid-April and then increased steadily, where it attained the maximum in August (Figure 1). Mean monthly soil temperature at all depths increased from 4.8<sup>0</sup>C, but soil temperature at 5 cm depth was relatively lower during this period. However, soil temperature at 30 cm was colder at the beginning of summer, compared to soil temperatures at 5, 10 and 20 cm depths and at 10 cm soil temperature was greater and increased continuously until October.

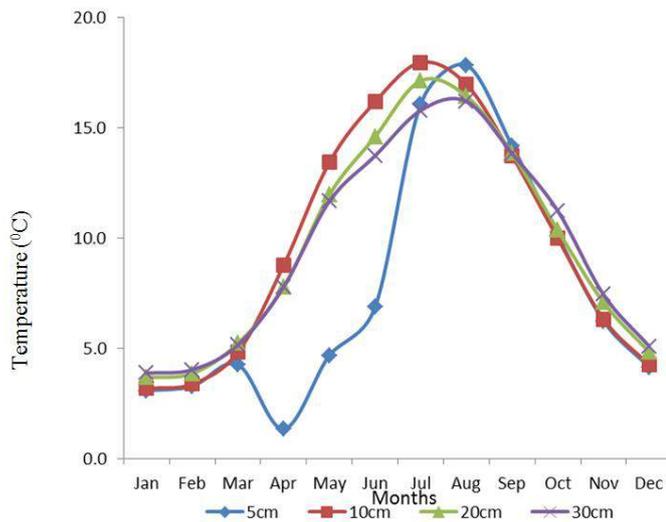
Uvarov *et al.* (2006) observed that variation in both seasonal and diurnal temperature significantly influenced the growth and population of earthworm species and dynamics of earthworm communities at different times. These variations in temperature also affect soil respiration, biomass and overall activity of soil micro-organisms. In general, decreased diurnal temperature variations resulted to an increase in CO<sub>2</sub> production. Temperature variation with season considerably alter the dynamics of carbon loss from the soil, as compared with consistent temperature systems.

## IV. CONCLUSION

The study illustrates that mean monthly soil temperature at all depths significantly increase, while a dramatic fluctuation at 5 cm depth was apparent. The soil temperature decreased to around mid-April and then increased steadily, where it attained the maximum in August. It has been observed that mean monthly soil temperature at all depths increased from 4.8°C. Soil temperatures fluctuate at 5 and 10 cm depths. The temperature range at 5 cm depth was 16.5°C and 10.5°C at 10 cm depth. Similarly, variation occurs at different soil depths and the pattern of fluctuation was in response to periodic shifts in meteorological variables acting on the soil system. Many scholars analyzed soil temperature fluctuation at different depths and the results of the investigation revealed a comparable trend. In addition, more detail analysis of soil temperature trend is still in progress at Compton with a view to provide a clear understanding on temporal temperature trends.

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**Figure 1:** Mean monthly soil temperature at different depths at Compton (1975-2008)

A periodic tendency of increased soil temperature at 10 cm depth revealed the distinctive attribute of this particular soil thermal environment, which indicates the degree of soil warming during summer (Table 1). Soil temperatures fluctuated at 5 and 10 cm depths. The temperature range at 5 cm depth was 16.5°C and 10.5°C at 10 cm depth. Tang *et al.* (2011) argued that variations in soil temperature occur at different soil depths and its importance at any given period and the pattern of fluctuations depend greatly on space and time.

**Table 1:** Descriptive statistics of mean monthly soil temperature at different depths at Compton (1975-2008).

Depths (cm)	Mean (°C)	Minimum (°C)	Maximum (°C)	Range (°C)	Standard Deviation	SE Mean	No. Measurements (months)
5	7.7	1.3	17.8	16.5	5.5	0.3	254
10	9.9	3.2	13.7	10.5	5.6	0.4	327
20	9.7	3.7	17.1	13.4	5.0	0.3	320
30	9.7	3.9	16.2	12.3	4.6	0.3	320

### Seasonal Trends

Soil temperature at 10 cm, was greater during the summer period and drastically declined with increased depth (Table 1). At 5 cm depth soil temperature was moderate and gradually decreased with increased depth. Uvarov *et al.* (2011) noted that recurring temperature variations considerably alter the dynamics of carbon loss from the soil system as in contrast with stable temperature system.

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# Microbiological Degradation of Starch Based Films: Preparation, Properties and Evaluation of Biodegradation

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## ABSTRACT

Discarded polymer materials are one of the causes of environmental pollution, leading to develop biodegradable materials, such as polymer composites. One commercial biodegradable polymer, called Ecobras<sup>TM</sup>, is claimed to be a good alternative in this respect, particularly because it is made with raw materials from renewable sources. Green coconut rush fiber is a lignocellulosic material, with low cost because it is a large scale waste. This article reports the preparation of new composites of Ecobras<sup>TM</sup> and green coconut rush fiber and the study of their biodegradation in simulated soil, revealing the microorganisms presence on the surface of the composites. The test consists in burying the samples in the soil for different periods, following the ASTM G 160-03 standard. After each interval, the samples were removed from the soil and analyzed by scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR). According to the results, Ecobras<sup>TM</sup> and its composites with green coconut rush fiber were considered biodegradable materials, and microorganisms presence on the material surface was observed. We expect these results will enable the development of biodegradable composites that will minimize the environmental impact generated by the inappropriate disposal of polymer materials.

**Keywords:** Ecobras<sup>TM</sup>, Green Coconut Fiber, Biodegradation, Polymer Composites, Polymer Morphology

## I. INTRODUCTION

Conventional synthetic polymers are used to produce a wide range of everyday products, such as clothing, plastic bags and bottles, among many others. However, these materials are very durable in the environment, causing them to accumulate both in landfills when properly disposed of and in open dumps and water bodies when improperly discarded. During the last decade, much attention has been focused on biodegradable polymers that can be produced from renewable resources, for example, developing polymer composites with insertion of natural fibers to produce materials with varying physical qualities that can be rapidly biodegraded by various types of microorganisms [1].

Ecobras<sup>TM</sup> is a biodegradable and compostable polymer, polyester Poly(butylene adipate-co-terephthalate) (Ecoflex) and starch based, obtained from renewable sources; produced by BASF in association with a Brazilian company CornProducts. Ecobras<sup>TM</sup> is economic viable for being highly compatible with

materials from renewable sources, which eases the use in composites and in polymer blends. Ecobras<sup>TM</sup> based products are widely used in tubes and plastic bags for reforestation, pens, injected packaging, films for food segments, plastic bags, among others [1-2].

Green coconut rush fiber is a lignocellulosic material, known for high strength and durability, because of its high lignin content compared to other natural fibers. The use of this fiber in Brazil is easy and has low cost because green coconut rush is large scale waste of coconut. Composites with a small amount of natural fiber can result in products with enhanced properties with a wide use in the polymers biodegradable industry. The advantages of this material include its renewable nature, biodegradability, enhanced mechanical properties and lower cost compared with synthetic fibers. [3-5].

This article reports the development of a new composite based on polyester and starch commercial polymer (Ecobras<sup>TM</sup>) with green coconut rush fiber. Besides, this work aims to evaluate the biodegradation of the polymer

and its composites in simulated soil, revealing the microorganism presence on the material.

## II. METHODS AND MATERIALS

### A. Materials

The following materials and devices were used in the experiments:

Ecobras, acquired from Corn Products Brazil; green coconut rush fiber, supplied by the *Coco Verde Project in Rio de Janeiro*; 4.0 kg of beach sand, collected in the Barra da Tijuca Beach (Rio de Janeiro); 4.0 kg of horse manure, collected in a private stable in the district of Imbatiê, Duque de Caxias, Rio de Janeiro State; 4.0 kg of fertile commercial soil with low clay content (Xaxim Furlan brand); cotton cloth with grammage of 445 g/m<sup>2</sup> and dimensions of 1.00 m x 0.75 m; glass beakers (600 mL); and sieves (35 and 40 mesh). Takemura soil moisture and pH meter; Nova Ética 410 DR forced-air chambers for bacteriological culture with refrigeration; Icamo model 3 sterilization chamber; Retsch sieve, IPAS (Perkin Elmer Pyris 1), Differential Scanning Calorimeter; Perkin Elmer Spectrum One Fourier-transform infrared spectrophotometer; JEOL Scanning Electron Microscope (JEOL 6510 JSMLV –SEM); Denton Desk Vacuum Sputter Coater; PH press 2 bar (350 x 350 x 1); and Torque Rheometer equipped with and mixing chamber (Haake PolyLab OS System).

### B. Methods

1. *Preparation of the Composites*: The polyester (Ecobras) was dried for 24 hours at 80 °C in a vacuum stove and the green coconut rush fiber was dried at 100°C drying chamber. The Ecobras<sup>TM</sup> and its composites with green coconut rush fiber were prepared at mixing chamber a temperature of 115°C, velocity of 60 rpm and processing time of 8 minutes. The test samples were prepared by compression molding at a temperature of 120°C for 10 minutes [6].

2. *Biodegradation test*: The biodegradation test in simulated soil was performed according to the ASTM G 160-03 standard, with control of soil moisture (variation from 20% to 30%) and temperature (30°C ± 2°C). This test lasted for 17 weeks, to evaluate the two composites with 5% and 10% of coconut fiber (ECO5% and ECO10%) as well as the Ecobras free green coconut fiber. The simulated soil used in the biodegradation test was prepared by mixing equal parts of the fertile soil with low clay content, dried beach sand and dried horse manure, following the ASTM G-160-03 standard. The viability of the soil for the biodegradation test was verified by burying a piece of cotton cloth for five days in the soil and then evaluating its mechanical resistance

lost according to the NBR 11912/1991 standard [7]. The test was carried out and the cotton cloth lost 75% of its mechanical resistance in the prepared soil, establishing the soil's suitability for the biodegradability test. After this, each sample was placed in a 500 mL beaker containing simulated soil to start the biodegradation test in a bacteriological chamber kept at a temperature of 30°C (± 2°C) and removed after periods of 2, 4, 7, 12 and 17 weeks. On being removed, the test samples were cleaned with a soft brush, dried in a desiccator and weighed to measure the mass loss. After each removal the samples were analyzed by Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC) and Fourier-transform Infrared Spectroscopy (FTIR).

3. *Mass loss*: After biodegradability tests, any materials attached to the surface of the specimens were removed they were thoroughly cleaned with a soft brush and then all movies are forwarded to a desiccator, to obtain to obtain a constant weight. To calculate the mass loss was taken into consideration the expression shown in Equation 1 that considers the relationship of the mass loss percentage of the dry sample. The weight loss percentage is calculated from the expression in Equation 1.

$$\text{Mass Loss (\%)} = (M_0 - M_i / M_0) \times 100 \text{ (Equation 1)}$$

4. *Morphology Analysis - Scanning Electron Microscopy (SEM)*: The samples of before and after burial test were sputtered with gold and their surfaces were analyzed under a scanning electron microscope at an acceleration voltage of 10 kV. The composites and Ecobras<sup>TM</sup> free of natural fiber samples were analyzed at scanning electron microscope (JEOL JSM - 6510LV model) and metalized by sputtering (Gressington 108) with a thin layer of gold to allow observation with an accelerating voltage of 15 kV. To observe the microorganisms present in the samples via SEM, the samples were prepared using a post-fixation technique with osmium tetroxide (OsO<sub>4</sub>) [8-9].

5. *Differential Scanning Calorimetry (DSC)*: The samples (mass of about 5 mg) were analyzed by DSC under a nitrogen atmosphere, at a flow of 20.0 mL/min and temperature range of 50 to 280°C, at a heating rate of 20°C/min [10].

6. *Fourier-Transform Infrared Spectrometry (FTIR)*: The samples were analyzed by FTIR using the attenuated total reflectance method (ATR). The structural analysis of films was performed using an FTIR spectrophotometer. The biofilms was placed on the sample holder and the spectra were recorded using attenuated the total reflectance technique [6].

### III. RESULTS AND DISCUSSION

#### A. Biodegradation Evaluation of the polyester and its composites by Mass Loss

The results of the samples mass loss according to time buried in the simulated soil are shown in Figure 1. All the samples lost mass during the period of 17 weeks of the burial test.

The polyester (Ecobras™) free of fiber has presented the greatest value of mass loss, around 55% and it was achieved very quickly, after 2 weeks of burial test and this mass loss remained constant until the end of the test. It is probably because of the 50% content of starch in Ecobras™ matrix [2]. The composites with green coconut rush fiber has lost maximum of 45% of mass and it was achieved after 7 weeks of burial and remained constant until the end of the test. These mass losses can be attributed to the biodegradation of both the polymer (Ecobras™) and the natural fiber.

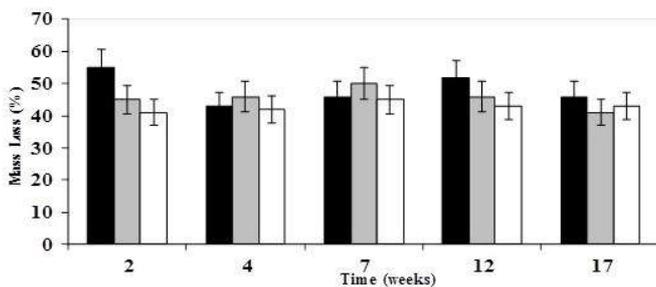


Figure 1: Results of the mass losses - biofilm Ecobras™ free from coconut fiber (black) and composite of film- grade Ecobras™ and coconut fiber ground in the proportions 95/5 (grey) and 90/10 (white)

These mass losses can be attributed to the degradation of Ecobras™ and the biodegradation of the natural fibers. The high content of starch in the Ecobras™ and its composites could have highly contributed to this initially higher degradation.

In a general way the presence of the fiber decrease the mass loss rate. This can be explained by the fact that the presence of the green coconut rush fiber makes more difficult the action of the microorganisms found in the simulated soil and also because of biodegradation rate of the fiber itself [10-11].

#### B. Thermal Analysis – Differential Scanning Calorimetry (DSC)

Ecobras™ is a semi-crystalline polymer and it is important to study the effect of the fiber in the final crystallinity of the prepared composites, and also the influence of biodegradation process in the crystalline phase of samples.

From the data obtained from DSC analysis it was possible to obtain the melting temperature (Tm) of the crystalline phase and the variation of enthalpy related to melting ( $\Delta H_f$ ) of the polyester Ecobras™ and its composites containing the different fiber concentrations (Table 1).

Table 1: Values of Tm,  $\Delta H_f$  of the samples Ecobras free of fiber (ECO), Ecobras/coconut fiber 95/5 (ECO/5%), and Ecobras/coconut fiber 90/10 (ECO10%F), during the biodegradation test

Samples	Fiber content (%)	Time (weeks)	Tm (°C)	$\Delta H_f$ (J/g)
ECO <sub>0</sub>	0	Zero	118	0.5
ECO <sub>2</sub>	0	2	119	0.5
ECO <sub>7</sub>	0	7	119	19.5
ECO <sub>17</sub>	0	17	122	10.6
ECO5% <sub>0</sub>	5	Zero	120	0.7
ECO5% <sub>2</sub>	5	2	120	0.6
ECO5% <sub>7</sub>	5	7	118	15.3
ECO5% <sub>17</sub>	5	17	125	21.5
ECO10% <sub>0</sub>	10	Zero	118	0.7
ECO10% <sub>2</sub>	10	2	118	0.7
ECO10% <sub>7</sub>	10	7	119	26.5
ECO10% <sub>17</sub>	10	17	121	33.2

The crystallinity degree could not be calculated. The  $\Delta H_f$  (enthalpy of fusion) obtained by DSC analysis was used as a parameter based on the fact the higher the  $\Delta H_f$  values the greater crystallinity content of the polymer. It can be observed at Table 1 that the fiber inclusion in polymer matrix did not change the polymer crystalline phase. During the biodegradation test an increase in the degree of crystallinity was observed for Ecobras™ samples as well as for the composites with green coconut rush fibers. This behavior can be assigned to changes of crystalline phase during the biodegradation process and to the preferable attack to the polymer amorphous phase by microbial consortium. In addition, from Table 1 it could be noticed that the higher the fibers content in the composite, the higher the increase in crystallinity through the biodegradation process.

Other authors have already reported an increase in crystallinity during biodegradation process in burial test [15].

### C. Morphology Analysis by Scanning Electron Microscopy (SEM)

The images obtained by SEM (Figure 2) show the surface morphology of Ecobras™ free of fiber and of the prepared composites with 5% and 10% green coconut rush fibers content. It can be observed the matrix-fiber adherence in the composites and also the changes in morphology of its samples surface during the biodegradation test in simulated soil. Observing the images of the samples before burial (ECO, ECO5% and ECO10%) it can be seen that the insertions of the fiber in Ecobras™ matrix was effective. There are no signs of unbounded fibers.

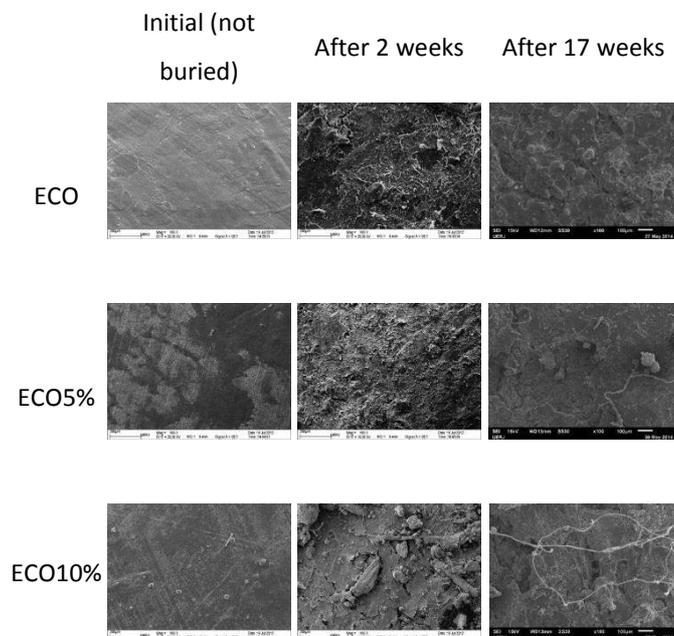


Figure 2: SEM photomicrographs of the Ecobras™/coconut fiber composite samples in the degradation test by burial in simulated soil, according to number of weeks. ECO: Ecobras™ free green coconut rush fiber samples; ECO5%: Ecobras™ composite samples with 5% coconut fiber; ECO10%: Ecobras™ composite samples with 10% coconut fiber

During the 17 weeks of burial a considerable change of the samples surface morphology can be noted namely the presence of holes and voids after the burial test. These surface modification has been caused by the microorganism attack to the samples and has originated the samples mass loss described before (section 3.1).

It can be also observed that the fibers turned to be less attached to the matrix after the burial because of the microorganism attack to the polyester during biodegradation process. These observations, related to different materials was reported by other authors [5,12].

After the 17 weeks test the samples were removed from the soil, treated as described in section 2.2.4 and analyzed by SEM in order to observe the biofilm at the surface samples. The Figure 3 shows the presence of microorganisms on the surface of Ecobras™ (ECO<sub>17</sub>) and the 10% fiber composite (ECO10%<sub>17</sub>) after the 17 weeks of simulated soil burial test. It is possible to observe the presence of bacteria with cocos cell morphology (single and grouped) on the surface of Ecobras™ free of fiber (ECO<sub>17</sub>) [13-14]. In Figure 3, one can also observe the presence of microorganisms (cocos and bacilli) adhered to the composite with 10% of fiber surface (ECO10%<sub>17</sub>). These observations can indicate the microorganisms participation in the degradation of the polymer, suggesting biodegradation process.

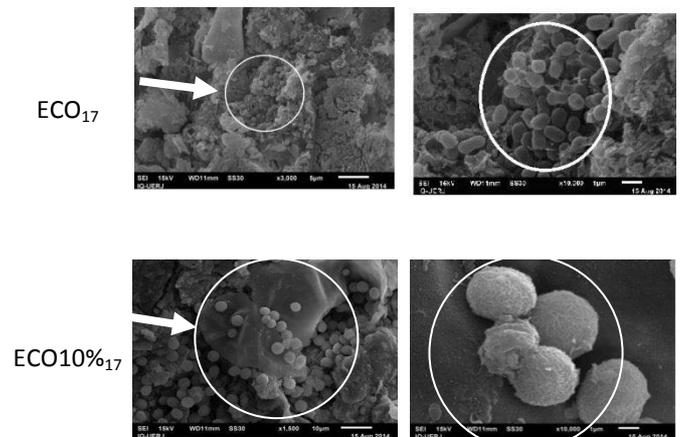


Figure 3: SEM micrographs of the Ecobras™ and ECO10% composites samples after 17 weeks of biodegradation burial test

### D. Analysis by Fourier-Transform Infrared Spectrometry (FTIR)

Figures 4 show the ATR spectra of Ecobras™ before burial test and at the various intervals (2,7 and 17weeks) of burial in the simulated soil in the biodegradation test (ECO<sub>0</sub>, ECO<sub>2</sub>, ECO<sub>7</sub> e ECO<sub>17</sub>).

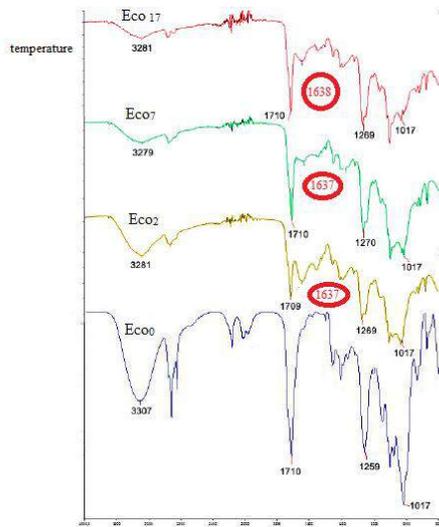


Figure 4: FTIR spectra of the polyester ECO before and during biodegradation test

Figure 5 show the ATR spectra of the prepared composite with 10% of fiber before burial test and at the various intervals (2,7 and 17weeks) of burial in the simulated soil in the biodegradation test (ECO10%<sub>0</sub>, ECO10%<sub>2</sub>, ECO10%<sub>7</sub> e ECO10%<sub>17</sub>).

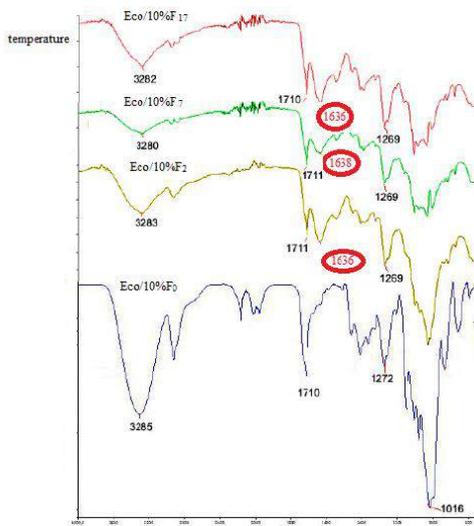


Figure 5: FTIR spectra of the composites ECO10% before and during biodegradation test

Observing Figures 4 and 5 it can be seen that before burial both Ecobras<sup>TM</sup> and its composite ECO10% presented axial deformations of carbonyl (C=O) at 1710 cm<sup>-1</sup>, and of C-O at 1160 cm<sup>-1</sup>, related to copolymer Poly(butyleneadipate-co-terephthalate (Ecoflex<sup>TM</sup>) which is a polyester [8]. It can be observed also the bands at 3280 and 3307 cm<sup>-1</sup>, which can be related to the hydroxyl groups present in the starch and with the

absorption of water. As related before Ecobras<sup>TM</sup> made from Ecoflex<sup>TM</sup> and starch.

Observing Figures 4 and 5 the FTIR spectra related to samples after all burial intervals, can be noticed that the samples suffered degradation during burial in the simulated soil, proved by the presence of bands at 3300 cm<sup>-1</sup> and 1659 cm<sup>-1</sup>, related to moisture (absorption of water) and the formation of acidic functional groups derived from the hydrolysis reaction of the polyester. According to the literature, chemical degradation can occur at the same time as microbiological degradation [5,16].

#### IV. CONCLUSION

The composites based on Ecobras<sup>TM</sup> and green coconut rush fiber were prepared and the presence of the fiber do not change the crystalline phase of the polymer matrix.

Ecobras<sup>TM</sup> and the prepared composites with green coconut rush fiber have lost mass during burial in simulated soil and these mass loss is related to microorganism attack, which was observed by SEM. Therefore Ecobras<sup>TM</sup> and the composite with green coconut rush fiber can be considered biodegradable materials according to the ASTM G160 – 03 standard. In addition, the microorganism presence was revealed on the surface of the material submitted to biodegradation test.

The biodegradation process can increase the crystallinity of both the Ecobras<sup>TM</sup> and the composites.

The formation of acid during the burial test and the presence of microorganisms confirmed that the samples were submitted to biodegradation and chemical degradation (hydrolysis reaction). During biodegradation process the attack of microorganism was probably facilitated by the breakdown of the chemical bonds in the molecules caused by hydrolysis due to absorption of water. The exposure of the samples in the simulated soil allows biodegradation to occur and allows the hydrolysis of chemical bonds.

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# Sustainable Supply Chain in Crises Management

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## ABSTRACT

During disaster, various organizations such as Red Cross or Red Crescent are often engaged with significant problems regarding to supply chain management. Sustainable supply chain management is defined as a collaboration between partners along supply chain to manage materials, information and capital flow for an achievement of sustainability along with environmental, economic and social dimensions. The aim of this paper is to develop a new framework for identifying the effect of sustainability concept on supply chain during emergency condition. Having a meticulous and thorough approach in this stage during the system averts technical and financial issues during the engagement with emergency condition and operational stages. In this paper, a VIKOR approach is utilized to find the best compromise solution. The most appropriate path way to improve the supply chain management towards sustainability regarding to ICS in emergency management was identified.

**Keywords:** Sustainable Supply Chain, disaster, Crisis Management, Emergency Management, ICS.

## I. INTRODUCTION

Sustainable supply chain management (SSCM) is the integration of two independent concepts: sustainability and supply chain management. Seuring and Muller (2008) have defined sustainable supply chain management as the collaboration between partners along supply chain to manage materials, information and capital flow for an achievement of sustainability along with environmental, economic and social dimensions. From the perspective of sustainability, the pressures tend to effect throughout the entire product lifecycle along the supply chains; from product design, sourcing, manufacturing, distribution, product use, to product end of life and recovery process (Linton et al. 2007, Halldórsson et al. 2009). On the other hand, one of the main reinforced arm during disasters is an agile supply chain during emergency management process.

The term “emergency”, means unexpected and potentially dangerous situation, requiring immediate action”, can describe a broad range of situations. These may vary from the most minor, which are dealt by persons without emergency services involvement, through “normal” emergencies, which involve response by one or more of the principal emergency services, to

major emergencies (Lindell et al. 2006). During disaster, various organizations such as Red Cross or Red Crescent are often engaged with significant problems of transporting large amounts of many different necessary material including food, clothing, medicine, medical supplies, machinery, and personnel from different locations to different destinations in the effected cordons. Sustainability in supply chain helps speed up the transportation of supplies and relief personnel to maximize the survival rate of the affected population and minimize the cost of such operations (Minner 2003). However, the main question is that “How it can become sustainable?”

It is clear that there are 3 major sustainability factors as environmental factors, economic factors and social factors (Mohamad et al. 2014). On the other hand, the main controlling center for managing the flow of resources and services is the incident command system (ICS). Incident Command System (ICS) has become a primary tool used to reduce the impact of disaster onset after making destruction to the infrastructures of the community (Rahman et al. 2015). It was developed to create a standardized approach for relief forces to use in order to conduct an efficient response effort (Zhang and She 2014). However, the traditional incident

management approach is a step-by-step approach, which is independently and largely performed with limited coordination among involved stockholders. It is essential that every engaged forces involved in the response operation work effectively and efficiently to minimize the incident response time (McGrath and Hall 2014).

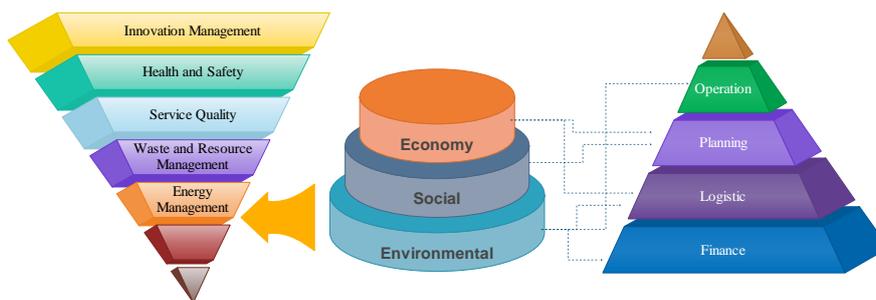
Different studies tries to transpose the concept of sustainability to the framework of ICS. Table 1 tries to define this path and help to identify the evaluating factors of sustainable ICS for implementation on supply chain management procedures.

**TABLE 1**  
MATRIX OF ICS VERSUS SUSTAINABILITY FACTORS

ICS	Environmental		Social			Economic		
	Environmental Concern	Waste Management	Collaboration	Respects	Capacity estimation	Mapping the talents	Economic consideration	Optimizations
Operations	Engaged	Engaged	Engaged	Engaged	x	x	x	x
	(Kapucu 2006, Kapucu <i>et al.</i> 2010)		(Parrillo <i>et al.</i> 2013, Singhry <i>et al.</i> 2014, Rahman <i>et al.</i> 2015)			(Kremers <i>et al.</i> 2010, Schreiber <i>et al.</i> 2012, Boersma <i>et al.</i> 2014)		
Planning	Engaged	x	Engaged	Engaged	Engaged	Engaged	Engaged	Engaged
	(McGrath and Hall 2014)		(Kapucu 2006, Kapucu <i>et al.</i> 2010)			(Kremers <i>et al.</i> 2010, Schreiber <i>et al.</i> 2012, Boersma <i>et al.</i> 2014)		
Logistic	Engaged	Engaged	Engaged	x	Engaged	x	Engaged	Engaged
	(McGrath and Hall 2014)		(Parrillo <i>et al.</i> 2013, Singhry <i>et al.</i> 2014, Rahman <i>et al.</i> 2015)			(Kremers <i>et al.</i> 2010, Schreiber <i>et al.</i> 2012, Boersma <i>et al.</i> 2014)		
Finance	x	x	Engaged	x	x	Engaged	Engaged	Engaged
	(Kapucu 2006, Kapucu <i>et al.</i> 2010, Rahman <i>et al.</i> 2015)		(Smith <i>et al.</i> 2012, Parrillo <i>et al.</i> 2013, Singhry <i>et al.</i> 2014, Rahman <i>et al.</i> 2015)			(Kapucu 2006, Kapucu <i>et al.</i> 2010)		

Thus it is very important to use this factors to integrate different parts of supply chain management system. Based on the previous studies there are different pathway to improve supply chain procedures. One of these path is accordingly energy beside saving resources (Adetunji 2008). Moreover waste management, service quality, health and safety and innovation management

are other paths to improve the supply chain management (Vrijhoef and Koskela 2000, Tah and Carr 2001, McCullen and Towill 2002, Halldórsson *et al.* 2009, Ho 2009, Sanayei *et al.* 2010). However, it is very important to identify the connectivity info-graph of these pathway with sustainability during emergency condition. Figure 1 illustrate this infographic concept.



**Figure 1:** info graphic concept

The aim of this paper is to develop a new framework for identifying the effect of sustainability concept on supply chain during emergency condition. It is clear that, the leading of flow in emergency management supply chain is in the hand of ICS. Thus, it is very important to improve the reliability of ICS sectors regarding to sustainability concept to develop a new sustainable supply chain framework.

## II. METHODS AND MATERIAL

In this research, the structured interview was employed to identify the weight of each sustainability criteria for improving supply chain management model. Furthermore, the VIKOR model was applied to evaluate the rate of effectiveness of each of the ICS sectors according to sustainability concept.

Rating and identifying the priority of pathway in sustainable supply chain is a MADM problem. For this problem, the decision is made from the courses of action (sorting and ranking) in presence of multiple, usually conflicting, attributes. There are several methods for solving the MCDM problems specified in literature (Jahan et al. 2010). However, previous methods were more focusing on simple comparative procedures between different criteria. This method was not reliable while the focus point of study was more on quantitative values and criteria such as hydrodynamic forces and structural stabilities. On the other hand, some criteria such as protection, flexibility and financial concerns were not considered in continues scales. The VIKOR method can solve this short come of previous methods.

VIKOR (ViseKriterijumska Optimizacija i Kompromisno Resenje) is a multi-attribute decision making technique with a simple computation procedure which considers the closeness to ideal alternative. In the literature, there are many studies, which have benefited from VIKOR method. Moreover, some studies conducted a comparative analysis of VIKOR and TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) methods with a numerical example (Opricovic and Tzeng 2004, Kaya and Kahraman 2010). This method focuses on ranking a set of alternatives for a problem with conflicting criteria, to determine a feasible solution which is the closest to the ideal solution, by taking into account the decision makers' mutual concessions (Opricovic and Tzeng 2002, Opricovic and Tzeng 2004, Opricovic and Tzeng 2007). The

mathematical formulation of the crisp VIKOR approach is starts with equation 1.

$$L_{pj} = \left[ \sum_{i=0}^n \left[ w_i \left( \frac{f_i^* - f_{ij}}{f_i^* - f_i^-} \right)^p \right] \right]^{\frac{1}{p}}, 1 \leq p \leq \infty; j = 1, 2, \dots$$

Where, the rating of the *i*th aspect is denoted by *f<sub>ij</sub>* for alternative *a<sub>j</sub>*. Furthermore, *w<sub>i</sub>* represents the weight of the *i*th indicator and *f<sup>\*</sup>* is the maximum number (best) and *f<sup>-</sup>* represents the minimum number (worst) values of all criterion functions for alternative *a<sub>j</sub>*. IN VIKOR method, the values of *Q<sub>i</sub>* were determined in the seventh step by the following relation (Eq.18).

$$Q_i = \nu \times (S_i - S^+) / (S^- - S^+) + (1 - \nu) \times (R_i - R^+) / (R^- - R^+)$$

Where *S<sup>+</sup> / R<sup>+</sup>* and *S<sup>-</sup> / R<sup>-</sup>* are the maximum and minimum values achieved in each category. *ν* is introduced as a weight for the strategy of maximum group utility. The value of *ν* lies in the range of 0 to 1. In Eq. 16 & 17, *ν* > 0.5 indicates that *S* is emphasized more than *R*, while for *ν* < 0.5, *R* is emphasized more. When *ν* is equal to 1, it represents a decision-making process that could use the strategy of maximizing group utility, as occurred in the traditional VIKOR approach. Whenever *ν* is equal to zero, it shows a process that could apply a minimum individual regret strategy that is found among maximum individual regrets/gaps of lower level criteria for each alternative.

## III. RESULT AND DISCUSSION

After evaluation of the responses achieved from survey, the first step was weighting of criteria. The results show that of the experts are approved that the “social factors” have the highest impact on supply flow. As it is illustrated in table 2 the economy factors follow social factors with 4 value. Third shows that the experts spend more concern on environmental aspects.

**Table 2**  
Linguistic Weighting Factors

No.	Criteria	Rated by Panel
1	Environmental	3.6
2	Economical	4
3	Social	4.5

However, 4 major pathways for improving the supply chain management was identified as Energy, Waste and Resource Management, service quality, health and safety and innovation management. Based on this selection the interviewer we asked about the impact of each of the sustainability major criteria on these factors. The mean score of the results were calculated and used in VIKOR method. Thus the normalized ratings were calculated as it is presented in table 4.

**Table 3**  
Normalized rating values

Weights	3.6	4	4.5
	Environmental	Economical	Social
Energy, Waste and Resource Management	0.566934	0.5356	0.45091
Service quality	0.496067	0.583209	0.515325
Health and safety	0.524414	0.333262	0.515325
Innovation management	0.396854	0.511796	0.515325

In the next step the weighted values for each factors were calculated accordingly (Figure 2). In this research, according to the second step of methodology, the mentioned performance weights collected from interviews as shown in previous Tables. Moreover, The best value and worst value performance score of alternatives with respect to each criterion were identified and denoted as  $x_j^+$  and  $x_j^-$ . Table 5 defines the amount of  $x_j^+$  and  $x_j^-$  for each criterion. According to the sixth step the amounts of  $S_i$  and  $R_i$  were calculated.

Energy, Waste and Resource Management	2.040963	2.142402	2.029093
service quality	1.785842	2.332837	2.318964
health and safety	1.88789	1.33305	2.318964
innovation management	1.428674	2.047184	2.318964

**Figure 2:** weighted variables



**Figure 3:** Comparative results for VIKOR method

However, for (as for the traditional VIKOR), service quality achieved the highest rank among the other technologies. This was the same result that had been advised by panel of experts during interview. Consequently, if a strategy of maximizing group utility was targeted by decision makers, then service quality was the best choice. However,  $Q(\text{service quality}) - Q(\text{Innovation}) > 0.25$  Thus, the service quality again should be assigned as the best solutions. The VIKOR approach reveals a more precise result and the result is also not so rigid. On the other hand, this approach prepares applicable ranking in contrast with conventional qualitative method. Therefore, based on the considered strategy for policy making process, one can decide which alternative is the best solution and which one have the most opportunity to be the best solution.

#### IV. CONCLUSION

Different categories of concepts are investigated as possible pathway to improve supply chain management towards sustainability by former. Focusing on ranking and selecting from a set of alternatives in the presence of conflicting criteria regarding to ICS in emergency management and sustainability criteria by a VIKOR approach achieves a more precise result rather than the conventional type applied in former research.

Selecting of a best strategy for improvement of supply chain management is often influenced by uncertainty in practice. Moreover, the fact that determining the exact values of the criteria is difficult or impossible suggests considering them in linguistic terms. By providing a maximum group utility for the “majority”, this approach determines a compromise solution that is approved by former results. However, If the consensus of a maximum group utility for the “majority”, and a minimum of individual regret for the “opponent” is required, then, also the service quality which is followed by innovation management could also be assigned as the appropriate concept for strategy of improvement. Flexibility is one of the significant characteristic of this proposed approach. This approach performs assessing and ranking alternatives to achieve a best framework for improving supply chain management towards sustainability regarding to ICS in emergency management.

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# A New Pharmacoscintigraphic Technique for the Evaluation of Pharmacokinetic Processes

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## ABSTRACT

In last few years for developing new chemical moiety or drug more time and more money is required. Also more energy is required to investigate the new molecule and to study its in-vitro analysis. A lot of time is been invested in study of pharmacokinetic parameters in the body. It includes study of absorption, distribution, metabolism and excretion. Dissolution study is important for in-vitro analysis of data or release of drug in the formulation and by using blood sampling and urine analysis in-vivo study is carried out. But this method requires much more time for analyzing data. Now we can save our time, money and energy by using a new pharmacoscintigraphic technique. A pharmacokinetic and pharmacodynamic study of newly investigated drug is carried out by using pharmacoscintigraphic technique. If any dosage form is administered by the body by any intended route then this body is scanned under gamma cameras and thus it can give whole information about rate, extent, site and mode of drug release. A new drug delivery systems are also evaluated by pharmacoscintigraphic technique.

**Keywords:** Pharmacokinetic study, *in-vitro*, *in-vivo* analysis, pharmacoscintigraphy

## I. INTRODUCTION

In last few years for developing new chemical moiety or drug more time and more money is required. Also more energy is required to investigate the new molecule and to study its in-vitro analysis. A lot of time is been invested in study of pharmacokinetic parameters in the body.[1]

Pharmacoscintigraphic, the application of Nuclear Medicine in drug development is a recent advancement. The safety, reproducibility, quantification and sensitivity of scintigraphy to detect pharmacological perturbation, together with wide choice of radiopharmaceuticals and flexibility of imaging procedures makes it an ideal modality to evaluate drugs / drug formulations. In India, a formal beginning of pharmacoscintigraphy has now been made at INMAS, DRDO, Delhi. Though presently its commercial use is limited, the scope is immense. Major advantages include high throughput screening at pre-clinical stage, objective *zero-phase* human trials and reduced size of other phases of clinical trial leading to significant reduction in developmental cost and time,

and evidence based comparison of the test drug / formulation with the conventional products in vitro, in animals and in humans.

Pharmacokinetic simulation models can be designed based on the information about the physiological environments around the delivery system and knowledge of its transit parameters in the GI tract (Grass and Sinko, 2002). The most convenient way to obtain the required in vivo transit data for the model is conducting an imaging study of the delivery system simultaneously with a common pharmacokinetic study. These kinds of studies are referred to as 'pharmaco-scintigraphy'. The combined data enables modelling of the dosage form behaviour and systemic pharmacokinetics of the drug simultaneously. Such physiologically-based models are useful in the analysis of the roles of the physiological factors and formulation parameters on inter-individual variance. Furthermore, they are useful in predicting in vivo behaviour of modified drug delivery systems. Implementation of scintigraphy-based pharmacokinetic modelling in the drug development processes may

reduce the rate of product attrition in the expensive clinical drug development phases.

## Gamma Scintigraphy

The first applied studies of gamma scintigraphy in the context of per oral pharmaceutical dosage forms were carried out in the 1970's (Casey et al., 1976; Alpsten et al., 1976). The technique had already been used for many years in studying the physiology of gastrointestinal (GI) tract (Griffiths et al., 1966). The idea was originally to gain information in relation to the anatomy and the physiology of the human body by using radio nuclides that localize in specific organs. When using high enough activity levels, also radiotherapy for treatment of e.g. tumours became possible. Soon after, it was discovered that the same basic procedure can be utilized in drug studies. Pharmaceutical gamma scintigraphy takes a step forward beyond the traditional anatomical imaging because the movements of drug molecules or delivery systems are monitored continuously. Therefore, it is called functional imaging.

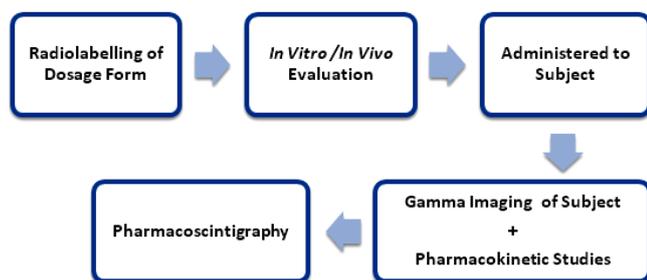


Figure 1: Fundamental principle of pharmacoscintigraphy

## Methods of Radiolabelling<sup>[2]</sup>

The use of compounds labelled with radionuclides has grown considerably in medical, biomedical and other related fields. In radiolabelled compounds, atoms or groups of atoms of a molecule are substituted by similar or different radioactive atoms or groups of atoms. In radiolabeling process, a variety of physicochemical conditions can be employed to achieve a specific kind of labelling. There are six major methods employed in the preparation of labelled compounds for clinical use:

1. Isotope exchange
2. Introduction of a foreign label
3. Labelling with bi-functional chelates
4. Biosynthesis
5. Recoil Labelling

6. Excitation labelling.

## Uses of pharmacoscintigraphy<sup>(3)</sup>

1. Formulation development & quality control
2. In vitro data generation
3. 3. Animal & human pharmacokinetic & pharmacodynamic data
4. 4. Formulation imaging

## II. METHODS AND MATERIAL

Gamma scintigraphy is an imaging technique that enables the direct visualisation and quantification of events occurring in vivo, in real time. Initially introduced as diagnostic tool, the potential of this method was quickly realised within the pharmaceutical industry. Gamma scintigraphy was first reported for the measurement of transit times in 1966 (gastric emptying) followed swiftly by the assessment of drug product performance in 1976 (capsule disintegration) 4, 5. Visualisation is achieved by the incorporation of short half-life gamma emitting radionuclides, eg technetium-99m (99mTc) and indium-111 (111In). The chosen radionuclide(s) is used to label the drug product or, for pharmacodynamic investigations, the component of interest (eg food or fluid for gastrointestinal transit; inhaled particles for mucociliary clearance). The radiation dose to the subject is minimal – often not exceeding that received from a single X-ray. A gamma camera is used to detect the gamma rays and record these as primary counts which are represented as an image (Figure 1).

Gamma scintigraphic investigations can be routinely incorporated into standard phase 1/2a studies alongside safety, pharmacokinetic and other biomarker assessments.

## III. APPLICATIONS IN DRUG PRODUCT DEVELOPMENT

### A. Oral products

The production of an oral product starts in the laboratory, where the pharmaceutical scientist is charged with developing a dosage form which meets a pre-determined specification for drug release. Release rate is measured by recognised methods, for example dissolution testing coupled with HPLC, to generate a

profile of drug release versus time. The primary use of these data is for the comparison/differentiation of prototype formulations, and for quality control. However, the results are also often intended as a representation of formulation performance in simulated in vivo conditions and are used as a first stage tool for formulation selection. However, an in vitro method cannot take into account all of the physiological factors that influence formulation performance and even if an in vitro-in vivo correlation (IVIVC) can be established, this is only confirmed after completion of a clinical study. Clinical studies designed to assess the performance of prototype formulations generate pharmacokinetic parameters. These data are at least onestep removed from formulation performance and so, when the pharmacokinetic profile is not as predicted, educated guesswork is needed to determine – and more importantly, fix – the cause.

Scintigraphic data provide the missing information, offering real-time visualisation and measurement of in vivo formulation performance. Key data are the rate of erosion of the dosage form – equating to release of drug (Case study 1)<sup>6</sup>. These data correspond to those obtained from in vitro dissolution, and assuming no other rate limiting factors may also parallel the appearance of drug in the systemic circulation. A further level of detail is obtained by tracking the transit of the dosage form through the gastrointestinal tract. How long does a gastroretentive formulation remain in the stomach? To which regions does an extended release formulation deliver? How rapidly does an enteric coated formulation deliver drug after gastric emptying? Does a colon targeting formulation reproducibly deliver to the target site?<sup>7-8</sup>.

## B. Oral inhaled products

The success of an orally inhaled product is a combination of the device, the formulation and the patient's technique<sup>9</sup>. As with oral formulations, development starts in the laboratory and the performance of prototypes is measured via particle size distribution (PSD) testing. While attempts continue to use PSD profiles as a predictor of in vivo deposition, the reality is that there is no direct correlation between individual or grouped stages and the anatomy of the lungs<sup>10</sup>. Consequently, while comparable in vitro performance can be used to support claims of

equivalence, de novo data cannot be relied upon to predict lung deposition. Products for oral inhalation can be radiolabelled by the addition of a radionuclide (eg <sup>99m</sup>Tc) to the formulation. In vitro testing is performed to confirm that the PSD of the radiolabel and the drug matches, ensuring that the deposition pattern of the radiolabel is representative of the drug molecule<sup>11</sup>. Scintigraphic data are most commonly used to quantify the initial deposition pattern providing a measure of how efficiently the device delivers the formulation, to which anatomical regions and the extent of lung penetration.

## C. Nasal products

Nasal administration is used for delivery to the systemic circulation (large surface area, non-invasive delivery) or for local delivery<sup>12</sup>. Delivery via the nasal cavity has been explored to deliver drug to the sinuses, and also to the olfactory region to achieve delivery to the brain. Consequently, drug products are often designed to target delivery to specific regions of the nasal cavity. Scintigraphic images co-registered with an MRI scan of the head can be used to quantify the amount of drug formulation delivered to target sites. Specific anatomical regions can be identified, or the cavity can be divided into zones such as upper:lower:inner:outer<sup>13</sup>. Further, scintigraphic imaging can provide evidence to support statements to the regulators that nasal delivery results in no deposition to the lungs.

## D. Locally acting drug molecules

The quantification of the availability of the active moiety at the site of action, ie the measurement of bioavailability, is a fundamental element of pharmaceutical development. For molecules which reach their site of action via the systemic circulation, pharmacokinetic parameters are an accepted surrogate measure and these data underpin the majority of safety, efficacy and bioequivalence assessments. However, for molecules that do not rely on systemic availability, this raft of assessments can be challenging to complete.

Bioavailability may be assessed by 'measurements intended to reflect the rate and extent to which the API becomes available at the site of action'<sup>14,15</sup>. Traditionally, for locally acting drugs these measurements have been limited to pharmacodynamic assessments, and large clinical trials to confirm efficacy.

For locally acting molecules delivered via the oral inhaled route, the use of in vitro assessments and the quantification of lung deposition via imaging are already recognised as supporting data – although pharmacokinetic data are still deemed to be advantageous<sup>16,17</sup>. The regulators now also recognise that the use of comparative clinical trials is inefficient and prohibitively expensive for locally acting molecules delivered to the gastrointestinal tract. As part of the FDA Critical Path Initiative, in vivo imaging has been suggested as a method to directly assess the rate of drug release at the target site<sup>18</sup>. Scintigraphic data provides a measure of both the location and rate of drug release, and comparative assessments of innovator versus test product can be performed.

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# Pharmacovigilance - A Review

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## ABSTRACT

Pharmacovigilance is an important and integral part of clinical research. Despite its 40 years history, pharmacovigilance remains a dynamic clinical and scientific discipline. It continues to play a crucial role in meeting the challenges posed by the ever increasing range and potency of medicines. When adverse effects and toxicity do appear especially, when previously unknown, it is essential that these are reported, analysed and their significance communicated effectively to an audience that has the knowledge to interpret the information, which carry an inevitable and some for all medicines there is a trade-off between the benefits and the potential for harm. The harm can be minimized by ensuring that medicines of good quality, safety and efficacy are used rationally and that the expectations and concerns of the patient are taken into account when therapeutic decisions are made. Taking medicines and prescribing them are among the commonest of activities of people who are unwell and of those who care for them. It makes sense that those medicines should be monitored to equally demanding standards as those evident in the development and evaluation of drugs and that prescribing habits and the extent of rational and cost-effective use should be reviewed. Responsibility for the holistic approach to drug safety that is encompassed in the science and practice of pharmacovigilance as reflected in this article has to be shared if ideal practice is to be achieved. The scientists, clinicians, pharmaceutical manufacturers, drug developers, regulators, public policy makers, patients and the general public all have their own complementary roles in achieving what is envisaged.

**Keywords:** Pharmacovigilance, National Pharmacovigilance Programme, Role of Pharmacovigilance, Pharmacovigilance Practice.

## I. INTRODUCTION

The World Health Organization (WHO) defines pharmacovigilance as the science and activities relating to the detection, evaluation, understanding, and prevention of adverse reactions to medicines or any other medicine-related problems. The definition and scope of pharmacovigilance have evolved to recognize the importance of a systems approach for monitoring and improving the safe use of medicines.<sup>1</sup>

Pharmacovigilance is a very important and inseparable part of clinical research. Both clinical trials safety and post-marketing pharmacovigilance (Popularly known as Post marketing studies or Phase IV clinical trials) are critical throughout the product life cycle. With a reasonably high number of recent high-profile drug

withdrawals, both the pharmaceutical industry as well as various regulatory agencies across the globe has raised the bar. Early detection of signals from the post-marketing surveillance studies and clinical trials in early phases have now been adapted by major pharmaceutical companies in order to identify the risks associated with their medicinal product/s as early as possible. If any such risk is present then effectively managing the risks by applying robust risk management plans throughout the life cycle of the product is adopted. These risk management plans are also widely known as Risk Minimisation Programmes/Strategies.<sup>2</sup>

## II. METHODS AND MATERIAL

### A. Story of Pharmacovigilance in India

It was not until 1986 that a formal adverse drug reaction (ADR) monitoring system consisting of 12 regional centres, each covering a population of 50 million, was proposed for India.<sup>2</sup> In 1997, India joined hands with the World Health Organization (WHO) Adverse Drug Reaction Monitoring Programme based in Uppsala, Sweden. Three centres for ADR monitoring were identified, mainly based in teaching hospitals : A National Pharmacovigilance Centre located in the Department of Pharmacology, All India Institute of Medical Sciences(AIIMS), New Delhi and two WHO special centres in Mumbai (KEM Hospital) and Aligarh (JLN Hospital, Aligarh Muslim University). These centres were to report ADRs to the drug regulatory authority of India. The major role of these centres was to monitor ADRs to medicines which are marketed in India. However, they hardly functioned as information about the need to report ADRs and about the functions of these monitoring centres were yet to reach the prescribers and there was lack of funding from the government. This attempt was unsuccessful and hence, again from the 1st of January 2005, the WHO- sponsored and World Bank-funded National Pharmacovigilance Program for India was made operational.<sup>3</sup>

The National Pharmacovigilance Program established in January 2005, was to be overseen by the National Pharmacovigilance Advisory Committee based in the Central Drugs Standard Control Organization (CDSCO), New Delhi. Two zonal centres-the South-West zonal centre (located in the Department of Clinical Pharmacology, Seth GS Medical College and KEM Hospital, Mumbai) and the North-East zonal centre (located in the Department of Pharmacology, AIIMS, New Delhi), were to collate information from all over the country and send it to the Committee as well as to the Uppsala Monitoring centre in Sweden. Three regional centers would report to the Mumbai centre and two to the New Delhi centre. Each regional centre in turn would have several peripheral centers reporting to it. Presently there are 24 peripheral centers.

### B. The Importance of Pharmacovigilance

Pharmacovigilance is an important and integral part of clinical research. Both clinical trials safety and post marketing pharmacovigilance are critical throughout the product lifecycle. Pharmacovigilance is still in its infancy in India and there exists very limited knowledge about the discipline. While major advancements of discipline of pharmacovigilance have taken place in the western countries not much has been achieved in India. There is an immense need to understand the importance of pharmacovigilance and how it impacts the life cycle of the product. This will enable integration of good pharmacovigilance practice in the process and procedures to help ensure regulatory compliance and enhance clinical trials safety and post marketing surveillance. Pharmacovigilance is not new to India and has in fact been going on from 1998, when India decided to join the Uppsala centre for adverse event monitoring. The importance of pharmacovigilance is withdrawals the regulatory agencies, media; consumers have become more aware about the benefit and risks of medicines.<sup>4</sup> Spontaneous reporting of adverse drug reaction and adverse events is an important tool for gathering the safety information for early detection. In recent years many Indian companies are increasing the investment in research and development and are enhancing their capacity to develop and market new drugs with their own research efforts. Further India is becoming a hub for clinical research activities due to its large population, high enrolment rate and low cost. Moreover, the lag period when a drug is placed for the first time on the market in USA, Europe, and Japan or somewhere in the world and its subsequent availability in India has decreased considerably. As a result, for such drugs the long term safety data is not available and the time of their marketing in India. This is clear by the fact that all the high profile drugs that have been recently withdrawn were available in Indian market. In such cases, the Indian regulatory agencies cannot count on the experience of other market to assess benefit risk balance of a drug.<sup>5</sup>

### C. Aim of Pharmacovigilance

1. Improve patient care and safety in relation to the use of medicines, all medical and Para medical interventions.
2. Research the efficacy of drug and by monitoring the adverse effects of drugs right from the lab to the pharmacy and then on for many years.

3. Pharmacovigilance keeps track of any drastic effects of drugs.
4. Improve public health and safety in relation to the use of medicines.
5. Contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use.
6. Promote understanding, education, clinical training in pharmacovigilance and its effective communication to the public. <sup>6</sup>

These processes involved in the clinical development of medicines. Once put onto the market, a medicine leaves the secure and protected scientific environment of clinical trials and is legally set free for consumption by the general population. At this point most medicines will only have been tested for short-term safety and efficacy on a limited number of carefully selected individuals. In some cases as few as 500 subjects, and rarely more than 5000, will have received the product prior to its release. <sup>7</sup>

#### **D. National Programme of Pharmacovigilance**

Before a product is marketed, experience of its safety and efficacy is limited to its use in clinical trials, which are not reflective of practice conditions as they are limited by the patient numbers and duration of trial as well as by the highly controlled conditions in which Clinical Trials are conducted. The conditions under which patients are studied during the pre-marketing phase do not necessarily reflect the way the medicine will be used in the hospital or in general practice once it is marketed. Information about rare but serious adverse drug reactions, chronic toxicity, use in special groups (e.g. pregnant women, children, elderly) and drug interactions is often incomplete or not available. Certain adverse drug reactions may not be detected until a very large number of people have received the medicine. Pharmacovigilance is therefore one of the important post-marketing tools in ensuring the safety of pharmaceutical and related health products.

- Assessing the risks and benefits of medicines in order to determine what action, if any, is necessary to improve their safe use.
- Providing information to users to optimize safe and effective use of medicines
- Monitoring the impact of any action taken

#### **E. Resources for Pharmacovigilance Centres**

The following books shall be provided to various centers as identified by the NPAC: Current editions of:

1. Meyler's Side Effects
2. 2.AHFS Drug Information hand book
3. 3. Martindale/online
4. 4.Davies Text Book of ADR
5. 5. Physician's Desk reference
6. British National Formulary

#### **F. The National Pharmacovigilance Centres**

At present, post-marketing surveillance of medicines is mainly co-ordinated by national pharmacovigilance centres. In collaboration with the Uppsala Monitoring Centre (UMC) the National Centres have achieved a great deal in:

1. Collecting and analysing case reports of ADRs
2. Distinguishing signals from background 'noise'
3. Making regulatory decisions based on strengthened signals
4. Alerting prescribers, manufacturers and the public to new risks of adverse reactions.
5. The number of National Centres participating in the WHO International Drug Monitoring Programme has increased from 10 in 1968 when the Programme started to 67 in 2002. The centres vary considerably in size, resources, support structure, and scope of activities. Collecting spontaneous reports of suspected ADRs remains their core activity.

#### **G. National Pharmacovigilance Centres are Responsible for:**

1. Promoting the reporting of adverse reactions.
2. Collecting case reports of adverse reactions.
3. Clinically evaluating case reports.
4. Collating, analyzing and evaluating patterns of adverse reactions.
5. Distinguishing signals of adverse reactions from "noise".
6. Recommending or taking regulatory action in response to findings supported by good evidence.
7. Initiating studies to investigate significant suspect reactions.
8. Alerting prescribers, manufacturers and the public to new risks of adverse reactions; and

9. Sharing their reports with the WHO Programme for International Drug Monitoring.

National centers have played a significant role in increasing public awareness of issues relevant to the safety of medicines. As a result, in some countries, pharmacovigilance is increasingly being seen as much more than a regulatory activity as it also has a major part to play in clinical practice and the development of public health policy. This development is partly attributable to the fact that many national and regional centres are housed within hospitals, medical schools or poison and medicine information centres and is in collaboration with a Medicines Regulatory Authority (MRA). The scope of activities of national centers has expanded to include communication of information about the benefits, harm and effectiveness of medicines to practitioners, patients and the public.<sup>8</sup>

### III. RESULT AND DISCUSSION

#### Current Problems in Pharmacovigilance<sup>10</sup>

1. Topical tacrolimus (Protopic) and pimecrolimus (Elidel): potential cancer risk.
2. Duloxetine (Yentreve, Cymbalta): need for monitoring.
3. Tenofovir (Viread): interactions and renal adverse effects.
4. Linezolid (Zyvox): severe optic neuropathy.
5. CosmoFer and high risk of anaphylactoid reactions.
6. Drotrecogin alfa (activated) (Xigris): risk benefit in the management of sepsis.
7. Rosuvastatin (Crestor): introduction of 5 mg starting dose<sup>11</sup>.
8. Osteonecrosis of the jaw with bisphosphonates.
9. High dose inhaled steroids: new advice on supply of steroid treatment cards<sup>12,13</sup>.
10. Local reactions associated with pre-school d/DTap-IPV boosters.
11. Salmeterol (Serevent) and formoterol (Oxis,Foradil) in asthma management<sup>14</sup>.
12. Risk of QT interval prolongation with methadone
13. Tamsulosin (Flomax) and Intraoperative Floppy Iris Syndrome (IFIS)<sup>15</sup>.
14. Cardiovascular safety of NSAIDs and selective COX-2 inhibitors.
15. Erythromycin and other macrolides: focus on interactions

16. Glucosamine adverse reactions and interactions
17. Isotretinoin (Roaccutane): psychiatric adverse reactions.
18. Cardiac arrhythmias associated with antipsychotic drugs.
19. HRT and tibolone (Livial): update on the risk of endometrial cancer.
20. Hypoglycaemia unawareness on transferring insulins.
21. Withdrawal of co-proxamol .
22. Intravenous human normal immunoglobulin (IVIg) and thromboembolic adverse reactions.
23. NSAIDs and infertility.
24. Patients across the UK may report suspected adverse reactions.

### IV. CONCLUSION

Pharmacovigilance and risk management are an essential part of pharmaceutical product development and commercialization, the activities of which are highly regulated in many part of the world. Rare adverse events may not be identified until large numbers of patients receive the product, so pharmacovigilance and risk management must extend throughout the product's life cycle. Benefit and risk must be continually assessed as more is learned about the product through its use. Building pharmacovigilance and risk management capacity requires a systematic approach to ensure that all safety aspects are monitored and addressed properly. Since capacity building takes time and resources, outsourcing of certain activities may enable capacity building to proceed before all capabilities can be done in-house. The use of a limited number of safety centers is a viable and cost-effective option, provided there are good processes, good tools, and good communication of responsibilities and events.

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# Influence of Boron and Zinc on Nitrate and Nitrite Reductase Activity in Roots and Leaves, and Sulfur Containing Amino Acids, Protein and Oil Content in Seeds of Soybean [Glycine Max (L.) Merr.]

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## ABSTRACT

Field Studies were conducted over two years on sandy loam soils, on the influence of boron (B) and zinc (Zn), on nitrate reductase (NR), nitrite reductase (NiR) activities in roots and leaves, and sulfur containing amino acids, viz., cysteine, cystine and methionine; protein, and oil content in seeds of soybean. Enzymatic activities were measured at 30, 60 and 90 days after emergence (DAE). Amino acids, protein and oil content were estimated in seeds after harvest. In absence of B and Zn applications, B and Zn symptoms such as distorted, chlorotic and puckered appearance of leaves, dwarfed plants, impaired flower development and seed set were noted. B and Zn applications were effective in overcoming the B and Zn deficiency symptoms. A significant increase in the nitrate reductase (NR) and nitrite reductase (NiR) activity in roots and leaves were noted. Sulphur containing amino acids (Cysteine, cystine and methionine), protein and oil content in seeds also significantly increased by B and Zn treatments. This work, in part, gives insight in mitigating environmental stresses through applications of micronutrients B and Zn

**Keywords:** Soybean seed, Boron, Zinc, Enzyme activity, Amino acids, Oil content

## I. INTRODUCTION

Grain legumes like soybean [Glycine max (L.) Merr.] provide a rich and cheap source of protein as well as oil. In legume proteins the relative proportions of essential amino acids differ from crop to crop. The quality of a protein depends on its amino acid composition. The soybean protein is rich in sulphur containing amino acids, viz., cysteine, cystine and methionine. Although, nitrogen is present in atmosphere in plenty yet its availability in a form, which can be utilised by plants, is essential. Life on earth is dependent on the transformation of atmospheric nitrogen to a form in which it can be absorbed from the soil by plant for protein synthesis. The process can be accomplished industrially but at a very high-energy cost. Legume crops have unusual ability to be self-sufficient in nitrogen supply. Biological nitrogen fixation by symbiotic associations of legume plant with microorganisms is economically sounder and

environmentally more acceptable than use of nitrogen fertilizer in agriculture. Both roots and shoots require organic nitrogen compounds but in which of these organs is nitrate reduced and incorporated into organic compounds can be estimated by activity of enzymes involved. Nitrate reduction occurs in two distinct reactions catalyzed by two enzymes, i.e., nitrate reductase (NR) and nitrite reductase (NiR). In some plants roots reduce almost no nitrate. So they depend upon amino acids translocated in the phloem from the leaves. In other plants, nearly all nitrates are absorbed and converted into amino acids and amides in the roots. Much more research is needed with the plants of different ages. Nutritional factor also plays an important role in altering the activity of different enzymes and manipulating the composition of seeds. With these ideas in mind this investigation was carried out to understand the influence of B and Zn on NR and NiR activities in leaves and roots as well as S-containing amino acids, protein and oil contents in seeds of soybean.

## II. METHODS AND MATERIAL

Field studies were conducted at Agricultural Research Farm, Banaras Hindu University, Varanasi, in two successive years on a sandy loam soil. Soil test values prior to the establishment of experiments are given in Table 1. Seeds of soybean [*Glycine max* (L.) Merr.] Var. PK-327 treated with thiram @ 4.58 g kg<sup>-1</sup> seed inoculated with (Brady) *Rhizobium japonicum* @ 0.5 kg ha<sup>-1</sup> seed rate were sown during *kharif* (first week of July). Application of boron as boric acid @ 1.5 and 3.0 mg kg<sup>-1</sup> soil and zinc as zinc sulphate @ 5.0 and 10.0 mg kg<sup>-1</sup> soil were made with two modes of application, i.e., basal and foliar, in order to receive a timely and adequate amount of these micronutrients. The plants were treated in two split doses. The first half of the dose of B and Zn was applied at the time of sowing as basal application at the pre-flowering stage [i.e., 30 days after emergence (DAE)]. Basal application of N: P: K: S @ 40:60:40:20 kg ha<sup>-1</sup> as N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and S respectively was made uniformly in all plots irrespective of B and Zn application. A control lot of plants were also maintained where no additional supply of B and Zn was made. Weeds were constantly removed from experimental site to keep the crop free from unwanted plants.

### Assay of nitrate reductase (NR) and nitrite reductase (NiR) activity in vivo in roots and leaves:

The NR and NiR activities were measured in fresh roots and fresh leaves at 30, 60 and 90 DAE. The third leaf from the top was taken for use in the assay of enzymatic activities. The third leaf from the top represents both the mature and young leaves with respect to growth and age. Moreover, it was also convenient to keep sample of leaf position on the plant constant throughout the study.

NR and NiR activities in the root and leaf samples were determined by *in vivo* method as described by Nicholas *et al.* (1976) and Ferari and Varner (1971), respectively. Determination of sulphur containing amino acids:

Sulphur containing amino acids, viz., cysteine, cystine and methionine in seeds were determined by the method of Bielecki and Turner (1966). Samples (50 –100 mg) were extracted with methanol: chloroform: water (MCW) 12: 5: 3 (by vol.) overnight at – 20°C, then stirred for 1 min using an Ultra-Turrax. The extract was centrifuged at 10000 g for 15 min and the supernatant (MCW extract)

collected. The chloroform layer of the MCW extract was separated from the aqueous layer and discarded. The pellet was re-extracted with cold 80% (v/v) ethanol for 4 min in an Ultra-Turrax, and centrifuged as above. The supernatant of the ethanol extract was combined with the aqueous layer of the MCW extract and evaporated to dryness at 40 – 50°C. After dissolving the residue in 5.0 cm<sup>3</sup> 100 mol m<sup>-3</sup> HCl, analysis of free amino acids was performed using a HPLC system consisting of two pumps, a controller for gradient programming and a modified auto-injector with a 20 mm<sup>3</sup> filling loop for automatic on-line derivatization. Fluorescence was monitored with a spectromonitor at an excitation wavelength of 330 nm and an emission wavelength of 450 nm. The derivatization was performed as described by Graser *et al.* (1985). For the separation of amino acids a Hyperchrome HPLC column (125 x 4.6 mm i.d.) and a guard column (10 x 4.6 mm i.d.) were used, both packed with Spherisorb ODS II, 3.0 μm. A two-component gradient system was used (Solvent A: 1% tetrahydrofuran + 5% methanol in 12.5 mol m<sup>-3</sup> sodium phosphate buffer, pH 7.2; Solvent B: 4.5% tetrahydrofuran + 35% acetonitrile + 14.5% methanol in sodium phosphate buffer, pH 7.2). The solvent programme used was: 0 min 85% A; 12 min 73% A; 16 min 60% A; 21 min 55% A; 38 min 0% A; 40 min 70% A. The flow rate was 1.2cm<sup>3</sup> min<sup>-1</sup> and the separation was performed at room temperature.

### Protein estimation:

500-mg seed sample was homogenised with 5 ml of 80% ethanol and centrifuged at 4000 r.p.m. for 20 min. Supernatant was kept aside and the residue was re-extracted twice with 5 ml ethanol (80%) each and centrifuged. Supernatants were discarded. The residue was left after 80% ethanol extraction was hydrolysed in 5 ml of 1 N NaOH for overnight and centrifuged at 4000 r.p.m. for 20 min. Supernatant was kept aside and residue was again extracted with 5 ml of 1 N NaOH for 1 hr. and centrifuged. Both supernatants were pooled and volume was made to 10 ml. The total protein was determined in this supernatant by folin ciocalteau reagent by the method of Lowry *et al.* (1951). To the sample (1.0 ml) was added 10 ml of 2% Na<sub>2</sub>CO<sub>3</sub> in 1 N NaOH + 0.5% CuSO<sub>4</sub> in 1% in sodium potassium tartarate (50:1 v/v) and was kept for 10 min. 0.5 ml of 1 N folin ciocalteau reagent was added and it was immediately shaken vigorously. It was kept for 30

minutes and thereafter absorbance reading was taken at 750 nm on spectrophotometer. Protein content was determined from a standard curve drawn with the help of bovine serum albumen (BSA).

#### **Oil Extraction:**

Oil was extracted by the cold percolation method of Kartha and Sethi (1957). A small glass percolator (20 cm long and 0.5 cm in diameter) was prepared by drawing a taper on one end of a glass tube; a perforated glass plate was inserted just above the taper. A thin wad of glass wool was adjusted over the glass plate. A layer of coarsely powdered anhydrous sodium sulphate (0.25 – 0.31 inches thick) was packed over the glass wool wad. 0.3g of seed material was transferred to a porcelain mortar. 2.0g of each of glass powder (Pyrex glass washed with concentrated hydrochloric acid) and anhydrous sodium sulphate were added and the mixture was reduced to fine powder. The mixture was transferred to percolator. The mortar and pestle were washed twice with 0.5g of anhydrous sodium sulphate and the washings were also packed over the seed powder. Finally the mortar and pestle were washed with 3-4 cc of freshly distilled petroleum ether, B.P. 70 – 90°C and this was transferred to the packed meal powder. This initial 3 – 4 cc of distilled water solvent served to wet the mixture. This was allowed to remain as such for 5 min. and the percolation started by adding measured quantity of solvent on the top of the percolator. The oil present in the seed powder is soluble in the solvent. The percolated solvent was collected below the percolator in a weighed dish containing four; 1 inch square strips of filter paper. The percolated solvent collected in the dish brings with it oil content present in the seed powder. Keeping the dish in an oven at 90 – 100°C for half an hour evaporated the solvent. The constant weight of the dish was obtained after the complete evaporation of the solvent. The difference in the initial and final weight of the dish is equal to the amount of oil present in the seed sample.

#### **Statistical Analysis:**

The experimental design was a randomised complete block with three replications. The treatments were a factorial combination of the levels of B and Zn. The results were statistically processed for calculating Least

Significant Difference (LSD) at P=0.05 (Gomez and Gomez, 1984).

### **III. RESULT AND DISCUSSION**

The chemical analyses of soil, as presented in Table 1, indicate inadequate B and Zn for optimum growth and development of soybean plants, which were found to be improved considerably by the addition of these two micronutrients at two different levels and their combinations. B and Zn at the threshold individual levels of 1.5 and 10.0 mg kg<sup>-1</sup>, respectively and threshold combined level (B + Zn) of 1.5 + 10.0 mg kg<sup>-1</sup> soil significantly increased NR and NiR activities in roots and leaves (Tables 2, 3, 4 & 5). This was followed by enhancement in amino acids cysteine, cystine and methionine percentage in seeds (Table 6 and Figure 1), and protein and oil content in seeds (Table 6 and Figure 2) of soybean.

In a comparison of nitrogen metabolism of cocklebur (*Xanthium pennsylvanicum*) with that of field pea Wallace and Pate (1967) concluded that each exemplified a group of plants with a characteristic distribution of nitrate reducing capacity. Whereas both the roots and leaves of field pea displayed vigorous NR activity, such activity in *Xanthium* was restricted wholly to the leaves. Soybean appears to resemble the field pea in that the present (Table 2 and 3) and previous studies (Evans and Nason, 1953; Weissman, 1972) give evidence of active nitrate reduction centres in both roots and leaves. Significant increase in NR activity by B and Zn application indicates the involvement of these two micronutrients in the activity of this enzyme either directly or indirectly. Maximum activity recorded in coincidence with the pod setting stage, a crucial phase for improving yield of this proteinaceous crop implicitly indicates the utility of B and Zn for nitrogen metabolism. Previously individual foliar spray of B to B-deficient sorghum at reproductive stage increased the nitrogen content and NR activity (Misra *et al.*, 1991) as found in the present study by splitting the dose. Similarly Zn also exerts a beneficial effect on the N-assimilation indirectly through its influence on NR activity (Rossel and Ulrich, 1964; Garg *et al.*, 1986). In B-deficient sugar beet and tomato plants a decrease in the NR activity (Bonilla *et al.*, 1980; Ramon *et al.*, 1989) is in conformity with the present findings.

The pattern of NiR activity in the roots and leaves follows closely the distribution of NR in these tissues. In roots and leaves an obvious enzymatic activity is present (Table 4 and 5). Beevers and Hageman (1969) have noted the limited demonstration of NiR in the roots, but in the present study increased NiR activity was obtained which might be characteristic of genotype taken as well as influence of treatments applied. Indirect evidence for the presence in soybean roots of a system capable of converting nitrite to ammonia comes from past studies (Woodhouse and Hardwick, 1966) indicating an increase in the ammonia content in the root exudes of plants provided with nitrate. In the present study B and Zn application found that enzymatic system seems to be in active form and an increase in activity. The decrease in activities of both the enzymes by higher level of B might be due to toxic effect at higher concentration.

In absence of Zn, protein content decreases with a simultaneous increase in the total free amino acids (Cakmak *et al.*, 1989), but in the present study only three sulphur containing amino acids, viz., cysteine, cystine and methionine were estimated and were found to be increased by B and Zn treatment (Table 6), of which methionine content was greater than cysteine and cystine. There might be a simultaneous decrease in other free amino acids. In wheat, boron application improved amino acid composition and protein synthesis (Iqtidar and Rehman, 1984). Boron has a role in the process of atmospheric nitrogen fixation (Bolanos *et al.*, 1994). Increased nitrogen metabolism in turn helps in the process of protein synthesis. Zinc is also required for protein synthesis because in higher plants under conditions of Zn deficiency several metabolic processes are impaired such as RNA metabolism and protein synthesis (Sharma *et al.*, 1981; Kitagishi and Obata, 1986). Therefore, fairly high Zn concentrations are required in plant tissues for extensive synthesis of proteins (Cakmak and Marschner, 1993). This specific role of these micronutrients is also evident in present study (Table 6) in the form of increased protein content of seeds. Generally at given latitude, variations in protein and fat contents are inversely proportional to about two weights of proteins equivalent to one of fat (Hanson *et al.*, 1961). Increase in both protein and oil

content (Table 6) might be due to efficient protein synthesis as well as fat synthesis in the presence of required nutrients.

On the basis of overall findings, as discussed in the preceding pages, it may also be assumed that B and Zn help better growth of bacteroids in root nodules. Through the chemical analyses of experimental soil presented in Table 1, it is apparent that Zn and B contents are below the required level for the growth of pulse crop, in general. The same may be limiting to provide a congenial environment to the N<sub>2</sub>-fixing microbial partner inside the root nodules, which require the needful supply of the micronutrients, including B and Zn for their optimal activities of N<sub>2</sub>-fixation and release of growth promoting substances. These micronutrients help a higher rate of N<sub>2</sub>-fixation not especially because of their direct role in N<sub>2</sub>-fixation but because of their possible role in producing a sufficient bacteroid mass. Consequently, the total fixed N output expected to be more by the increased bacterial population and this may be one of the good reasons why the total N content of plant is significantly enhanced. Similarly requisite supply of micronutrients including B and Zn has a positive impact on the activities of nitrate reductase and nitrate reductase enzyme systems, which collectively add to the assimilable N-status of the plant. An increase in the total plant N content through the above processes facilitates better amino acid synthesis and protein assembly. The latter is well reflected through the increased protein content of the treated plants (Table 6; Figure 2). A better amino acid synthesis under B and Zn treatment is apparent from the higher methionine, cysteine and cystine contents in the treated plants. May be that the synthesis of other amino acids has been also similarly increased, although the same was not tested in this series of experiments. However, the increase in the amount of these three amino acids is a reflection of the qualitative increase in the amino acid contents of plants under B and Zn treatments. The findings provide enough scope, therefore, for detail biochemical analyses of the qualitative and quantitative status of various other amino acids in plants with graded application of B and Zn.

**TABLE 1.** Soil Test Values Prior to the Study

1.	Soil Extractable Nutrients (mg kg <sup>-1</sup> soil)				
(a)	Nitrogen	84.50	88.50	Alkaline Permanganate	Subbiah and Asija (1956)
(b)	Phosphorous	11.00	10.75	0.5 N NaHCO <sub>3</sub> extractable	Oslen <i>et al.</i> (1954)
(c)	Potassium	104.00	102.00	Ammonium acetate extractable flame Photometer	Jackson (1973)
(d)	Sulfur	8.50	8.25	Turbidity	Chesnin and Yien (1950)
(e)	Boron	0.89	0.80 boron	Hot water soluble	Sippola and Ervco (1977)
(f)	Zinc	1.20	1.00	DTPA	Lindsay and Norvell (1978)
2.	Soil pH (1: 2.5 soil water ratio)	7.48	7.50	Glass electrode pH meter	Jackson (1973)
3.	Electrical Conductivity d Sm <sup>-2</sup> (1:2.5 soil water Ratio)	0.15	0.16	Systronics electrical conductivity meter	Jackson (1973)

**TABLE 2.** Effects of Boron (B) and Zinc (Zn) Levels on Nitrate Reductase (NR) Activity ( $\mu$  mol.NO<sub>2</sub>.hr<sup>-1</sup>.g<sup>-1</sup>) in Fresh Roots of Soybean [*Glycine max*(L.) Merr] at different growth stages

Treatments B Zn (mg kg <sup>-1</sup> soil)	First Year Age of plants (DAE)			Second Year Age of plants (DAE)		
	30	60	90	30	60	90
0.0 0.0	0.830	1.590	0.976	0.940	1.670	1.010
1.5 0.0	0.930	1.740	0.990	0.990	1.810	1.070
3.0 0.0	0.620	1.310	0.580	0.710	1.370	0.630
0.0 5.0	1.130	1.950	1.140	1.200	1.970	1.210
0.0 10.0	1.190	2.010	1.210	1.220	2.086	1.350
1.5 5.0	1.270	2.173	1.273	1.340	2.170	1.430
1.5 10.0	1.330	2.260	1.390	1.430	2.256	1.490
3.0 5.0	0.740	1.520	0.730	0.830	1.510	0.830
3.0 10.0	0.753	1.660	0.810	0.890	1.570	0.910
LSD (P=0.05) for B or Zn	0.043	0.010	0.020	0.045	0.032	0.035
LSD (P=0.05) for B x Zn	0.075	0.018	0.035	0.078	0.055	0.062

**TABLE 3.** Effects of Boron (B) and Zinc (Zn) Levels on Nitrate Reductase (NR) Activity ( $\mu\text{ mol.NO}_2.\text{hr}^{-1}.\text{g}^{-1}$ ) in Fresh Leaves of Soybean [*Glycine max*(L.) Merr] at different growth stages

Treatments B Zn (mg kg <sup>-1</sup> soil)		First Year Age of plants (DAE)			Second Year Age of plants (DAE)		
		30	60	90	30	60	90
0.0	0.0	1.620	3.090	1.610	1.690	3.190	1.750
1.5	0.0	2.810	3.506	1.790	1.870	3.430	1.910
3.0	0.0	1.200	2.560	1.070	1.270	2.660	1.200
0.0	5.0	2.220	3.770	2.190	1.280	3.840	2.280
0.0	10.0	2.320	3.920	2.330	2.370	4.010	3.460
1.5	5.0	2.473	4.120	2.530	2.530	4.220	2.690
1.5	10.0	2.620	4.486	2.743	2.690	4.426	2.870
3.0	5.0	1.410	2.790	1.310	1.490	2.930	1.470
3.0	10.0	1.480	2.910	1.420	1.560	3.030	1.570
LSD (P=0.05) for B or Zn		0.020	0.144	0.030	0.034	0.011	0.030

**TABLE 4.** Effects of Boron (B) and Zinc (Zn) Levels on Nitrite Reductase (NiR) Activity ( $\mu\text{ mol.NO}_2.\text{hr}^{-1}.\text{g}^{-1}$ ) in Fresh Roots of Soybean [*Glycine max*(L.) Merr] at different growth stages

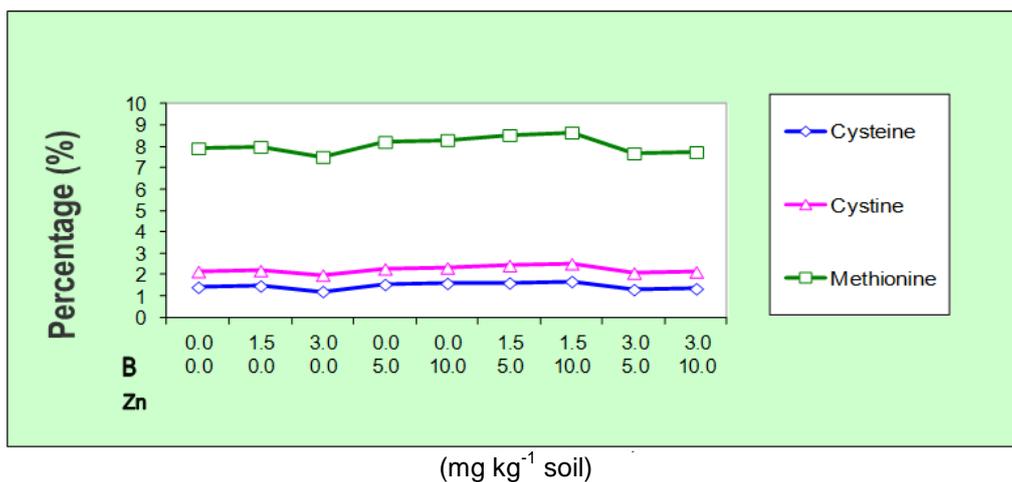
Treatments B Zn (mg kg <sup>-1</sup> soil)		First Year Age of plants (DAE)			Second Year Age of plants (DAE)		
		30	60	90	30	60	90
0.0	0.0	1.770	3.720	1.750	2.100	4.090	2.050
1.5	0.0	1.990	4.080	1.920	2.220	4.430	2.190
3.0	0.0	1.300	3.020	1.100	1.560	3.310	1.260
0.0	5.0	2.440	4.600	2.220	2.710	3.840	2.490
0.0	10.0	2.580	4.760	2.370	2.760	5.160	2.790
1.5	5.0	2.760	5.180	3.703	3.180	5.357	2.970
1.5	10.0	2.910	5.400	3.750	3.270	5.700	3.110
3.0	5.0	1.560	3.520	1.390	1.840	2.660	1.670
3.0	10.0	1.610	3.860	1.550	1.980	3.830	1.840
LSD (P=0.05) for B or Zn		0.048	0.040	0.651	0.021	0.032	0.035
LSD (P=0.05) for B x Zn		0.082	0.069	1.127	0.036	0.056	0.060

**TABLE 5.** Effects of Boron (B) and Zinc (Zn) Levels on Nitrite Reductase (NiR) Activity ( $\mu \text{ mol. NO}_2 \cdot \text{hr}^{-1} \cdot \text{g}^{-1}$ ) in Fresh Leaves of Soybean [*Glycine max*(L.) Merr] at different growth stages

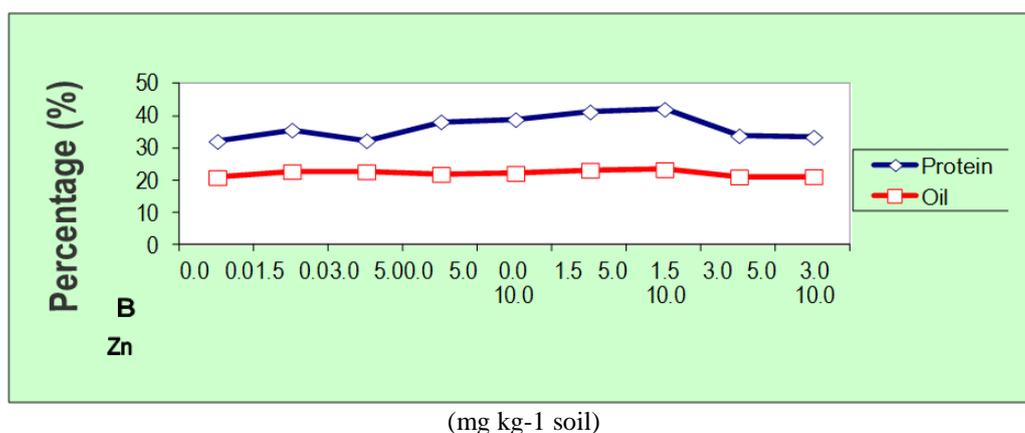
Treatments		First Year			Second Year		
B	Zn	Age of plants (DAE)			Age of plants (DAE)		
(mg kg <sup>-1</sup> soil)		30	60	90	30	60	90
0.0	0.0	2.340	5.060	1.980	2.600	5.510	2.320
1.5	0.0	2.640	5.510	2.210	2.890	5.960	2.550
3.0	0.0	1.690	4.120	1.290	1.910	4.520	1.560
0.0	5.0	3.260	6.250	2.730	3.550	6.720	3.070
0.0	10.0	3.430	6.540	2.930	3.720	7.050	4.700
1.5	5.0	3.660	6.920	3.210	3.657	7.460	3.780
1.5	10.0	3.930	7.300	3.460	4.270	7.880	3.960
3.0	5.0	2.000	4.510	1.590	2.260	5.010	1.920
3.0	10.0	2.110	4.740	1.740	2.440	5.210	2.070
LSD (P=0.05) for B or Zn		0.037	0.027	0.032	0.184	0.022	0.059
LSD (P=0.05) for B x Zn		0.064	0.048	0.056	0.318	0.038	0.103

**TABLE 6.** Effects of Boron (B) and Zinc (Zn) Levels on Sulfur containing Amino Acids, Protein and Oil Content (per cent) in Seeds of Soybean [*Glycine max* (L.) Merr.]

Treatments		First Year					Second Year				
B	Zn	Age of Plants (DAE)					Age of Plants (DAE)				
(mg kg <sup>-1</sup> soil)		Cysteine	Cystine	Methionine	Protein	Oil	Cysteine	Cystine	Methionine	Protein	Oil
0.0	0.0	1.410	2.150	7.910	32.097	20.900	1.480	2.230	8.010	32.483	20.970
1.5	0.0	1.470	2.190	7.980	35.600	22.750	1.540	2.290	8.100	39.083	23.000
3.0	0.0	1.210	1.980	7.510	32.287	22.670	1.250	2.050	7.650	32.607	22.850
0.0	5.0	1.540	2.270	8.210	38.160	21.900	1.630	2.370	6.663	35.296	21.650
0.0	10.0	1.590	2.320	8.300	38.847	22.200	1.690	2.430	8.420	35.523	21.900
1.5	5.0	1.610	2.430	8.530	41.223	23.150	1.720	2.540	8.610	41.603	23.200
1.5	10.0	1.680	2.510	8.650	42.037	23.350	1.800	2.620	8.750	42.356	23.450
3.0	5.0	1.300	2.080	7.680	33.830	21.100	1.360	2.130	7.830	33.413	21.260
3.0	10.0	1.340	2.130	7.740	33.415	21.210	1.420	2.180	7.873	33.790	21.350
LSD (P=0.05) for B and Zn		0.037	0.029	0.037	0.823	0.176	0.027	0.028	0.965	0.242	0.201
LSD (p=0.05) for B x Zn		0.065	0.051	0.065	1.350	0.305	0.047	0.049	1.672	0.419	0.349



**Figure 1.** Effects of Boron (B) and Zinc (Zn) Levels on Sulfur Containing Amino Acids (Cysteine, Cystine and Methionine in per cent) in Seeds of Soybean [*Glycine max* (L.) Merr]



**Figure 2.** Effects of Boron (B) and Zinc (Zn) Levels on Protein (%) and Oil (%) in Seeds of [*Glycine max* (L.) Merr]

#### IV. CONCLUSION

Physiological significance of B and Zn application in relation to nitrate and nitrite reduction, protein and fat synthesis in this particular crop is explicit. Nitrate and nitrite reduction in accordance with the need of plant depends on the activity of enzymes involved, which in turn are regulated by genetic as well as nutritional factors. Improvement in sulphur containing amino acids, protein and oil content, which are the quality parameters of soybean seeds, by B and Zn treatments expounds the importance of these micronutrients for seed quality. Further, this work gives insight and keeps relevance in mitigating environmental stresses through applications of micronutrients B and Zn.

#### V. ACKNOWLEDGEMENTS

The study is gratefully acknowledged to the UGC, New Delhi for providing financial support and Institute of Agricultural Sciences, Banaras Hindu University for field facilities in Research Farm.

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# A Brief Survey on the Practical Applications of Sebera Equation for Predicting Permanence Index During Transfer of Cultural Heritage to Distinct Environmental Conditions

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## ABSTRACT

This work provides information about the use of Sebera Equation in the calculation of permanence index of paper documents based on simulations that include relative humidity and temperature changes, being an useful tool to predict the long-term life of paper documents in libraries, museums and archives.

**Keywords:** Sebera Equation, Temperature, Relative Humidity, Permanence Index, Museum, Archive

## I. INTRODUCTION

Arrhenius Equation (Equation 1) is widely used in the calculation of the variation of the velocity constant of a chemical reaction with temperature. It is an equation widely used to determine chemical kinetics, and also widely used to determine the energy of activation reactions.

$$k = Ae^{-E/RT} \quad (1)$$

Where:  $k$  = Reaction constant;  $A$  = Pre-exponential constant, dependent on the área of contact, among other factors;  $E$  = Activation energy;  $R$  = Constant of perfect gases;  $T$  = Temperature.

This equation can be used to calculate the activation energy of a series of reactions, mainly chemical reactions, such as the energy of activation for the thermal destruction of a microbial cell, deterioration of foods, degradation of paper, wood. This equation can also be used according to Wilson [1] as described in a Technical Report from NISO.

According to the description contained in the NISO Technical Report, this equation can also be used to study the degradation of paper, introduced with the objective of giving technical support to librarians, personnel from

archives, engineers, architects and staff involved in the conception, construction, renovation and maintenance of buildings used as storage of collections.

The present article aims to provide technical orientation about the environmental parameters that affect deterioration of paper, if these paper documents are stored in a deposit designed for this purpose. This orientation will be based on simulations of typical situations including temperature and relative humidity changes, indicating what would be the consequence in the permanence of paper, based on calculations from Sebera Equation.

In the present paper it is intended to suggested minimum and maximum values for temperature and relative humidity, with the purpose of achieving a suitable preventive conservation. However, even though the Arrhenius Equation clearly can be used for these purposes, isoperms are being preferentially used, due to its characteristic relation between temperature and relative humidity in a single mathematical expression. The basis for the development of Sebera isoperm can be found in the combination of these two parameters, producing as a result of this combination, the measurement of the Permanence, the inverse of the deterioration rate.

The limits that can be simulated with this equation depend on the particular use intended. For instance, depending on the local climate conditions, considerations about human comfort and modern technology to control environmental standards must be considered. According to the actual practice, and also according to isoperm equations, some considerations can be reached, particularly considerations on the chemical stability of a document paper, where it is widely stated that the smaller the temperature the paper is stored, the best. However, not always conditions predicted can be achieved and not all institutions present climate control available to reach desired standards. For instance, it is widely spread the for the proper storage of paper documents the use of temperature in the range of 0 to 18°C is suitable; Variations in the temperature markedly affect the degradation of paper, in comparison to a previously stated condition; The smaller the relative humidity of the air, the best for paper documents; For daily use, paper documents must be stored at 20% relative humidity; In order to prevent fungal growth, paper must be kept at 55% relative humidity. However, these figures do not constitute the goal of some institutions, and proper simulations need to be conducted in order to reach specific targets for conservation.

In reality, the options for preservation are well known, however, the lack of studies on the matter, brings some hesitation to the use of chemical procedures to reduce fungal growth or to improve climate conditions. In that matter, the use of Arrhenius Equation with its theoretical predictions about the storage conditions of paper documents can be useful to establish suitable environmental conditions. So, this is clearly an important tool for the development of preservation techniques and policies and also to develop strategies for long-term storage, and also, prediction of lifetime of documents depending on climate changes and seasonal variations.

The relatively simple Arrhenius equation can be used as a basis to correlate relative humidity and temperature values to predict stabilization levels in relation to the stability of paper documents. In that case, Sebera Equation (Equation 2) can be used [2].

This expression correlates the permanence index (P) of a material (paper, for instance) in a particular room with the permanence index predicted during transfer of this

same material for another space at distinct temperature and relative humidity levels.

$$\frac{P_2}{P_1} = \left( \frac{RH_1}{RH_2} \right) \left( \frac{T_1 + 460}{T_2 + 460} \right)^{394 \Delta H \left( \frac{1}{T_2 + 460} - \frac{1}{T_1 + 460} \right)} \quad (2)$$

Where P1 = Permanence index in the present conditions; P2 = Permanence index in the estimated desired conditions; RH = Relative humidity; ΔH = Activation enthalpy; T = Temperature.

Strang e Grattan [2] revised Equation 2, taking into account the fact that the intrinsic humidity and not the relative humidity must be considered in the evaluation of permanence of an organic material. This new approach could bring more realistic information about the process of deterioration.

This new model (Equation 3), that introduces information about the cellulose and water content of the materials, that means, the intrinsic humidity) considers the concept of isotherms and can be combined with the Sebera isoperm.

$$M = \frac{M_0 K C A_w}{(1 - K A_w)(1 - K A_w + C K A_w)} \quad (3)$$

Where: M = Cellulose and water content on a dry basis; M<sub>0</sub> = Content of the mixture on the surface monolayer of paper; K = Status difference between the pure liquid (water) and surface layers of the paper; C = Status difference between the monolayer and surface layers of the papers; A<sub>w</sub> = Water activity.

This expression can be easily combined with Sebera Equation, giving place to a new expression, with a high number of parameters to be solved (Equation 4).

$$\frac{P_2}{P_1} = \frac{\frac{M_{01} K_1 C_1 A_{w1}}{(1 - K_1 A_{w1})(1 - K_1 A_{w1} + C_1 K_1 A_{w1})}}{\frac{M_{02} K_2 C_2 A_{w2}}{(1 - K_2 A_{w2})(1 - K_2 A_{w2} + C_2 K_2 A_{w2})}} \cdot \exp \left( \frac{\epsilon}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right) \right) \quad (4)$$

Suggestions on the use of the present system in the prediction of permanence index based on different T and RH simulations can be presented (Table 1)

TABLE 1

SIMULATIONS OF THE PRACTICAL APPLICATIONS OF SEBERA EQUATION FOR MUSEOLOGICAL USE

Simulation	Temperature Range (°C)	Relative Humidity Range (%)
A	Range $T_1-T_2$ (Smaller range of T between Year A and Year B) e Range $T_3-T_4$ (Greater range of T between Year A – Year B)	$RH_1-RH_2$ (Average RH between Year A and Year B)
B	$T_1-T_2$ and $T_3-T_4$ (Summer) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Autumn) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Winter) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Spring) <sup>1</sup>	$RH_1-RH_2$
C	From T1 to T2 at different reference T and RH	
D	$T_1-T_2$ and $T_3-T_4$ (Summer) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Autumn) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Winter) <sup>1</sup>	$RH_1-RH_2$
	$T_1-T_2$ and $T_3-T_4$ (Spring) <sup>1</sup>	$RH_1-RH_2$
E	Similar To Simulation D, considering $\Delta H = 25$ kcal	

<sup>1</sup>Reference conditions:  $\Delta H = 35$  kcal,  $T = 23$  °C or  $25$  °C and  $RH = 60$  % or  $65$ %.

From Table 1 it can be estimated the effects of the transfer of paper documents from different climate conditions, predicted by the equation.

For example, in Simulation A, the use of the lowest or highest temperature range in a certain period, can bring useful information about the effect on the transfer of paper documents from one place to another, at distinct environmental conditions. It can also be useful to predict permanence index between hot and cold seasons. This can be predicted based on the permanence index, bringing information about what can be expected between extreme conditions all over the year.

In the case of Simulation B, the use of the equation can serve to predict climate changes due to different seasons of the year. In this case, it is important to consider minimum and maximum observed temperature and relative humidity values, in order to characterize what is expected from the permanence index, under extreme cold and hot weather conditions.

It is known that environmental conditions do not change abruptly during the transition of a season to another. It is a gradual process, being also gradual the daily changes in the permanence index, as temperature and humidity gradually change. This way, simulations that can predict the effects from growing temperatures, for instance from  $30$  a  $40$ °C, in comparison to pre-determined reference conditions, such as  $23$ °C/ $60$ %;  $25$  °C/ $60$ %;  $23$ °C/ $65$ % e  $25$ °C/ $65$  %, typical of tropical countries are useful during changes in the cycles of temperature control in indoor spaces.

In simulation D it can be expected to know permanence index in conditions where it is possible to evaluate the effect of growing RH considering small and high T ranges between one specific year and another, or between a range of years can be easily performed based on the equation. Similar conclusions can be reached by changing  $\Delta H$  value from  $25$  to  $35$  kcal, as predicted by some literature sources (Simulation E).

In fact, this brief survey includes just simulations of some climate conditions, opening the possibility of performing infinite simulations, to calculate permanence index for a wide range of T, RH and not only paper, but any other museological piece amenable to suffer from unsuitable storage conditions.

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# Design of Classical and State Space Controller Using a Moving Coil Machine

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## ABSTRACT

This paper looks at two methods of designing controllers among numerous approaches used in controller designs; the root locus method using the SISOTOOL in MATLAB and the state space method. The root locus is a classical graphical method, of all the classical methods this was chosen because it helps to understand fundamental concept of controller design, and a graphical understanding of what is going on in the system. This paper analyse, design, compare and contrast a classical method, PID and a more recent approach; state space to controller design. The state space approach is more modern and used to highlight the short comings of the classical approach. A laboratory moving coil meter was used for the design of the controller. The utilisation of the system identification toolbox in MATLAB was employed for the system modelling obtained and analysed. Finally the paper compares the performance of both designed controllers based on root locus and state space methods at the second order moving coil system and previous work done were subjected to due comparison.

**Keywords:** Controller, Root Loci, State Space, MATLAB, Model and Moving coil meter.

## I. INTRODUCTION

The paper tends to make the difference between the classical and a more modern approach in the design of controllers. When we have a system, we want that system to behave in a desired manner. But that is often not the case as external signals often known as noise interferes with the system and therefore an output less than the desired.

The need for a method to make the system behave in the desired manner leads to the development of control engineering. Control engineering involves the design of an engineering product or system where a requirement is to accurately control some quantity (Dutton, K 1997). The system used in this paper is the moving coil machine. The moving coil machine is used to measure current. Whenever electrons flow through a conductor, a magnetic field proportional to the current is created. This effect is useful for measuring current and is employed in many practical meters (Kim, 2006). To use a permanent –magnet moving coil device as a meter two problems must be solved. First, a way must be found to

return the coil to its original position when there is no current through the coil. Second, a method is needed to indicate the amount of coil movement. The first problem is solved by the use of hairsprings attached to each end of the coil. With the use of the hairsprings, the coil will return to its initial position when there is no current. The other problem is solved by attaching a pointer to the coil and extended out to a scale (Kim, 2006). The accuracy and the efficiency can be improved by incorporating a controller. A controller is a device which can sense information from linear or nonlinear system to improve the systems performance (Cheng et al, 2008). There are several methods to the design of controllers. We have the classical methods and more modern methods. To avoid the problem of open loop controller, control theory introduces feedback which forms a closed loop controller. Common closed loop controller architecture is the Proportional Integral Derivative (PID) controller. The historical development of the classical field started with Stability Criterion (Nyquist, 1932), Analysis of the Feedback Amplifier (Black 1934), Frequency Domain Analysis (Bode, 1940) and Root Locus Method (Evans, 1948). There are also the lead, lag, and the lead-lag

compensation method. The root locus method is adopted in this paper without any specific reason.

After the introduction we have the methodology where the root locus and the state space methods are discussed followed by the discussion of results obtained and finally the conclusion.

## II. METHODS AND MATERIAL

The methods employed in the design of controllers for the moving coil machine is the root locus approach and the state space method. For certain systems, their properties are described using graphical models which use numerical tables and plots. That means for linear systems, they are describing by their step or impulse responses and or by their frequency function. For more advanced applications, models that describe the relationship among the system variables in mathematical expressions like differential equations are used which could be time continuous or time discrete, lumped or distributed, deterministic or stochastic, linear or non-linear as well as the graphical method. The third method uses the experimental approach. Here experiments are carried out on the system and a mathematical model of the system can be found. This is known as system identification and it includes the following; Experimental planning, Selection of model structure, parameter estimation and Validation (Dutton 1997). This method is used for the purpose of this paper. The experimental design involves several factors. The choice of the input signal and choice of sampling interval is of great importance. In a system identification experiment, the input signal applied to the system can have great influence on the resulting parameter estimates. The major types of input signal used in system identification experiment are; Step function, Steady input (static test), Impulse input and Random input-Pseudorandom binary sequence. A pseudo-random-binary-sequence (PRBS) is a two state signal of logic 1 levels and logic 0 levels. Logic 1 will be a positive voltage and logic 0 an equally negative voltage. It can be generated by using a feedback shift register. It cannot be said to be truly random since it repeats itself every  $2^n-1$  bit interval for an n-bit shift register (Soderström and Stoica, 1989).

Inappropriate model structure is the most common cause of problem in system identification. Optimum model structures which have just sufficient parameters are

needed to model the process. There are numerous time series model but for the purpose of this paper the (ARMA) model is used. Auto Regressive model is one where the current value of a time variable is a function of its past values only. In other words the current output is a weighted sum of previous output values. It is auto regressive because its form shows a regression of a time variable with itself at different time instants (Tham, 1999).

It is generally represented as:

$$y_n = a_1y_{n-1} + a_2y_{n-2} + a_3y_{n-3} + a_{NA}y_{n-NA} + \dots \quad \dots\dots\dots 1$$

$N_A$  defines the oldest output value that has a significant influence on the current output. The Moving average model believes that its output is dependent upon the current state of the input and what that input was doing for some time in the past represented as:

$$y_n = b_0u_n + b_1u_{n-1} + b_2u_{n-2} + b_3u_{n-3} + b_{NA}u_{n-NA} \dots \quad \dots\dots\dots 2$$

A more realistic model would have been to consider both the input and output because practically the output of the system is a function of both the input and outputs.

$$y_n = a_1y_{n-1} + a_2y_{n-2} + a_3y_{n-3} + a_{NA}y_{n-NA} + AR + b_0u_n + b_1u_{n-1} + b_2u_{n-2} + b_3u_{n-3} + b_{NA}u_{n-NA} \dots \quad \dots\dots\dots 3$$

MA  
ARMA

The background knowledge of the system made it easy to make decisions on the model and to estimate its parameters. The system is a linear second order under damped system, and because a continuous transfer function is preferred as the system representation, the choice of process model in system identification of MATLAB is not a difficult choice to make. Having specified the characteristics, the mathematical representation is obtained:

$$\frac{-0.4201}{0.0259s^2 + 0.02649s + 1} \quad \dots\dots\dots 4$$

To design the controller using state space the mathematical model of the moving coil meter is converted into a difference equation or its non-minimal

state space description to enable manipulation. A new mathematical model is obtained in discrete form to avoid converting from the continuous form to discrete form. This is to avoid the unnecessary uncertainties in the obtained result.

Using the same process used in obtaining the mathematical model for the system in the earlier section of identification. Using linear parametric models, specifying ARX as the structure, using 2,2,1 as the order and instrumental variables as the method we obtain

$$G(z) = \frac{-0.01789z^{-1}-0.01986z^{-2}}{1-1.866z^{-1}+0.9533z^{-2}} \dots\dots\dots 5$$

Verification shows that the transfer function truly represents the system.

The controller must be able to do the following: correct the steady state error, improve the speed of response that is rise time and settling time, stability and better oscillation i.e. the percentage overshoot and damping ratio. Proportional action takes corrective action based on magnitude of the error and integral takes corrective action based on the area under the error curve. This means that when error rapidly changes when the magnitude has not changed much then P+I will not give much corrective action. The inclusion of the integral action only is not good enough so we need a third term that will make control signal proportional to the time derivative (rate of change) of the error signal

$$u(t) = K_d \frac{de(t)}{dt} \dots\dots\dots 6$$

Where  $K_d$  is the derivative gain.

### Root Loci Design

The root locus plot is the plot of the s zero values and the s poles on a graph with real and imaginary coordinates. The root locus is a trace of the spots of the poles of a transfer function as the gain K is varied.

The locus of the roots of the characteristic equation of the closed loop system as the gain varies from zero to infinity gives the name of the method. Such a plot shows clearly the contribution of each open loop pole or zero to the locations of the closed loop poles. This method is a very powerful graphical technique for investigating the effects of the variation of a system parameter on the locations of the closed loop

poles. Sketching root loci is simple if the general rules for constructing is followed. “The closed loop poles are the roots of the characteristic equation of the system. From the design viewpoint, in some systems simple gain adjustment can move the closed loop poles to the desired locations. Root loci are completed to select the best parameter value for stability. A normal interpretation of improving stability is when the real part of a pole is further left of the imaginary axis”. (Roy 2010).

### State Space Design

The state space approach is a more modern approach to controller design. It is a unified method for modelling, analysing, and designing a wide range of systems. In the recent past there have been a number of developments and papers written about true digital control (TDC) design, in which discrete time is used in the design of control systems. TDC is actually based on simplified refined instrumental variable identification and estimation algorithms for data based modelling (Young, 2004) and later the design of Proportional Integral Plus (PIP) control algorithms (Young et al 1987).

A proportional integral plus (PIP) controller is a full discrete state variable feedback controller based on non-minimal state space description (NMSS). NMSS follows methodological approach from earlier research. Hesketh (1982), Young, Behzadi, Wang & Chotai (1987), in which NMSS models are formulated so that, in the deterministic situation, full state feedback control can be implemented directly from the measured input and output signals of the controlled process, without resort to the design and implementation of a state re-structor (or observer). This yields a PIP design that is naturally robust to uncertainty and eliminates the need for measures such as loop transfer recovery.

By considering the difference equation obtained from system identification we can get a non-minimal state space description. The method used in calculating the controller gains for this machine is the Linear Quadratic (or Optimal) regulator design which involves seeking a control that will maintain the system at the set point when subjected to external influences or driving the system to its zero state in the shortest possible time with respect to the system’s constraints.

The mathematical editor on which along with text you can also write

### III. RESULT AND DISCUSSION

Using SISOTOOL on MATLAB to achieve the design requirements we add a complex zero and adjust the gain. Reading off the compensator values from the SISOTOOL we obtain equation 7

$$G_{cs}=4.59351s+0.65+0.412s \quad \dots\dots\dots 7$$

Rearranging and writing in form of a PID transfer function, that is;

$$G_{cs}=K_p1+1sT_i+sT_d \quad \dots\dots\dots 8$$

We have

$$G_{cs}=2.9861+10.65s+0.2586s \quad \dots\dots\dots 9$$

Having;

$$K_p=2.986;$$

$$T_i=0.65;$$

$$T_d=0.2586.$$

Where the state vector  $x$  is a column vector of length  $n$ ; Input vector  $u$  is a column vector of length  $r$ ;  $A$  is an  $n \times n$  square matrix of constant coefficients;  $B$  is an  $n \times r$  matrix;  $Y$  is a column vector of the output variables;  $C$  is an  $m \times n$  matrix of the constant coefficients that weight the state variables and  $D$  is an  $m \times r$  matrix of the constant coefficient that weights the system inputs.

And to obtain our non-minimal state space representation,

$$PP(n+1) = 1.866pp_n - 0.9533PP_{n-1} + 0.01789UU_n + 0.01986UU_{n-1} \quad \dots\dots\dots 13$$

$$\begin{bmatrix} PP_{n+1} \\ UU_n \\ Mn+1 \end{bmatrix} = \begin{bmatrix} 1.866 & -0.9533 & -0.01986 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -1.866 & 0.9533 & 0.01986 & 1 \end{bmatrix} \begin{bmatrix} PP_n \\ PP_{n-1} \\ UU_{n-1} \\ Mn \end{bmatrix} + \begin{bmatrix} -0.01789 \\ 0 \\ 1 \\ 0.01789 \end{bmatrix} UU_n \quad \dots\dots\dots 14$$

the above matrix include an integral of the error state. Comparing the above expression to equation 9 we can say that;

$$A = \begin{bmatrix} 1.866 & -0.9533 & -0.01986 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -1.866 & 0.9533 & 0.01986 & 1 \end{bmatrix}$$

$$B = \begin{bmatrix} -0.01789 \\ 0 \\ 1 \\ 0.01789 \end{bmatrix}$$

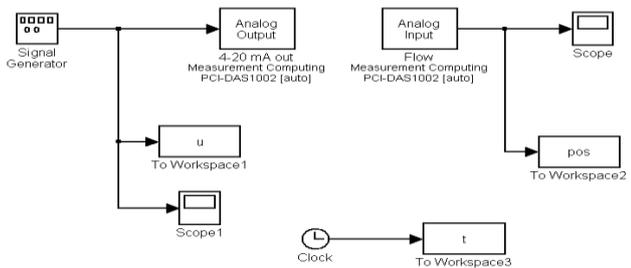


Figure 1: Simulink model used to obtain system data

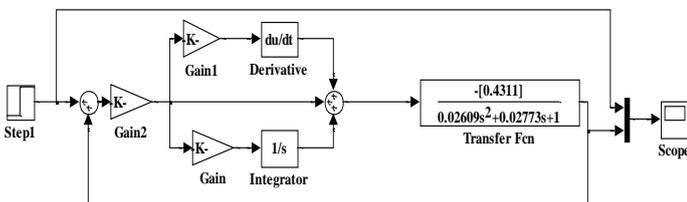


Figure 2: Obtained PID being tested on the model and system

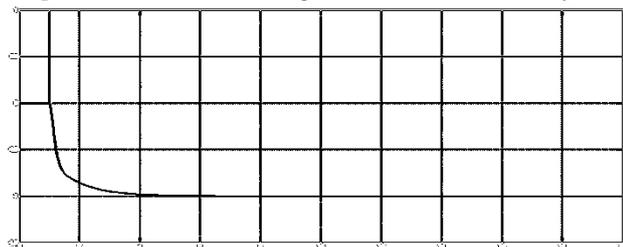


Figure 3: Response to a step input. Downward because of the negative transfer function

The difference equation gives for equation 5 gives:

$$PP(n) = 1.866pp_{n-1} - 0.9533PP_{n-2} - 0.01789UU_{n-1} - 0.01986UU_{n-2} \quad \dots\dots\dots 10$$

Considering the State Space equation;

$$\dot{x} = Ax + Bu \quad (X_{k+1} = AX_k + BU_k) \quad \dots\dots\dots 11$$

$$Y = C^T + Du \quad \dots\dots\dots 12$$

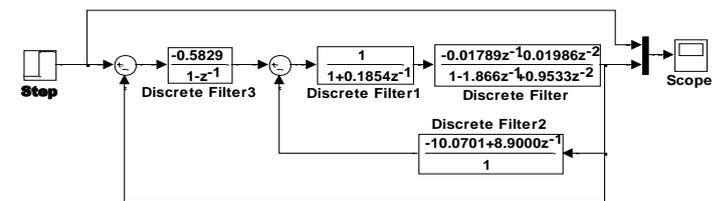


Figure 4: Implementation of PIP controller on the model and real system.

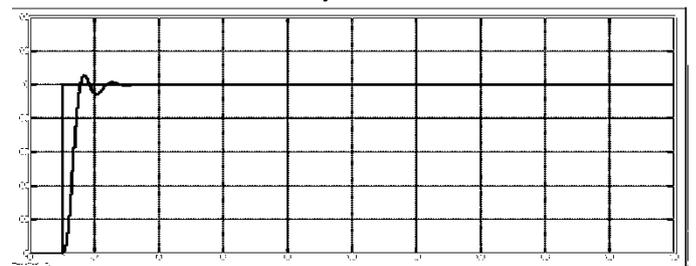


Figure 5: response to a step input

The design carried out to obtain Fig.3 using root locus considered steady state error and transient response. The design method was iterative. Iterating around the design specifications changing the design and checking the response until the result is deemed acceptable. The approach has the difficulty in detecting if the system is controllable or not while the designer labour over a long period of time before coming to such conclusion.

The design of state variable systems feedback (SVF) is a very different approach. The desired closed loop performances are specified in advance and together with the state space model of the open loop plant are fed into an algorithm (an m.file in MATLAB) the algorithm then produces the details of the required controller.

To be able to design the necessary a controller for our dynamic system the system must be controllable. In other words it must be possible to move all of the system open loop poles by state variable feedback to any arbitrary closed loop locations.

#### IV. CONCLUSION

This paper has looked at two approaches to analysis and design of feedback control system. The first part looked at the classical approach. This method uses root locus and the MATLAB SISOTOOL, by obtaining a model of the system and analysing its poles and zeroes.

The main advantage of the method is that they readily provide the stability and transient response information so it is easy to see the effect of adjusting the poles and zeroes until an acceptable design is met.

However the main disadvantage lies in the fact that its usability is limited. It can only be useful for linear time invariant systems or system that can be approximated as such.

The second method used a more recent approach to system control. This method cannot only be used for same class of systems as classical method but also analyse non-linear systems that have backlash, saturation and dead zone. It can also model systems with non - zero initial systems as well as multiple input multiple output (MIMO) systems.

One of the draw backs of frequency domain method of design using either root locus or frequency domain is that after designing the location of the dominant second order pair of poles , we keep our fingers crossed, hoping that the higher order poles do not affect the second order approximations. Frequency domains methods do not allow us to specify all poles in the systems of order higher than 2 because they do not allow for a sufficient number of unknown parameters to place all of the closed loop poles uniquely. One gain to adjust, or compensator pole and zero to select, does not yield a sufficient number of parameters to place all the closed loop poles at desired location. To place n unknown quantities, you need n adjustable parameters.

State space method solves this problem by introducing into the system

- (1) Other adjustable parameters and
- (2) The technique for finding these parameter values, so that we can properly place all poles of the closed loop system.

On the other hand, state space methods do not allow the specification of closed loop zero location, which frequency domain method do allow through placement of the lead compensator zero. This is a disadvantage of the state space methods, since the location of the zero does not affect the transient response. Also, a state space design may prove to be very sensitive to parameter changes.

Finally there is a wide range of computational support for state space methods; many software packages support the matrix algebra required by the design process. However as mentioned before the advantages of computer support are balanced by the loss of graphic insight into a design problem that the frequency domain methods yield.

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# Replacement of Mandibular Anterior Tooth with “Riding Pontic” A Case-Report

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## ABSTRACT

With the ever changing and ever growing developments in the field of materials and techniques, new vistas are open to us to provide our patients with better treatment modalities. A new and simple technique for the patients who desire an esthetic tooth replacement before any definitive Prosthodontic and/or orthodontic treatment is described in this case-report. In this technique, a provisional prosthesis is fabricated, using an acrylic tooth, resin cement and an orthodontic wire which is also cost effective and less time consuming. This provisional prosthesis may be used where aesthetics is a prime concern and a definitive treatment has to be delayed for some period of time.

**Keywords:** Riding Pontic, Provisional Pontic, Resin Cement, An Orthodontic wire.

## I. INTRODUCTION

A beautiful smile at the end of the prosthetic treatment is a primary concern for all the patients, but most are also concerned with appearance while undergoing treatment. Many conditions like agenesis, extractions or trauma may lead to loss of anterior teeth and compromises esthetics.

A chair-side temporary prosthesis made an easy option for the treatment of a missing anterior tooth while maintaining the space for the definitive procedure<sup>1</sup>. To fulfil the need of esthetics and to boost up the morale of the patient, a simple method of fabrication of a provisional fixed prosthesis is presented in this case report.

## II. METHODS AND MATERIAL

### CASE HISTORY

A 20 year old female patient reported to the Department of Prosthodontics, Rama dental college and Hospital & Research Centre Kanpur, with a chief complaint of a gap between her lower front teeth. She had no history of any tooth extraction and/or orthodontic treatment (Fig-1).

On clinical and radiographic examination, it was revealed that both her mandibular central incisors were congenitally missing, leaving a gap of a single tooth in between and rotated lateral incisors (Fig-2).

Keeping in mind the esthetics of the patient, the orthodontic treatment was preferred over the fixed prosthetic treatment, which included the correction of rotated lateral incisors and closing the gap while maintaining the midline in place. As per the patient's convenience, the orthodontic treatment was planned after 6 months by the decisions of the concerned orthodontists. So, a provisional prosthesis using an acrylic tooth, fiber reinforced composite and an orthodontic wire of 21 gauges, was given to the patient.

### PROCEDURE:

1. The acrylic tooth was selected according to the colour of the adjacent natural teeth.
2. The mesio distal width and the height of the tooth was determined and adjusted.
3. The stainless steel orthodontic wire of 21 gauges was intertwined.
4. Cotton rolls were placed on the lingual and labial vestibule to keep the area dry.
5. The intertwined stainless steel orthodontic wire was bonded on the lingual aspect of 32, 33 and 42, 43 incisal to the cingulum using fiber reinforced composite resin(Fig-5).
6. Then the acrylic tooth was stabilized and bonded to the wire using the same composite (Fig-3), (Fig-4).

### III. RESULT AND DISCUSSION

The introduction of the chair-side provisional prosthesis using an acrylic tooth, composite resin, and a stainless steel orthodontic wire of gauges 21 has provided the dental profession with the opportunity to fabricate and deliver adhesive, esthetic and metal free tooth replacements.<sup>2</sup>

This technique has also provided the benefit of dependable space maintenance for anterior teeth prior to the orthodontic treatment. Other indications for such type of service include: a fixed replacement following tooth loss from trauma until complete healing occurs, a fixed tooth replacement

in medically compromised patients who cannot sit for extended period of time, patients who cannot be administered local anaesthesia, periodontally compromised abutments, a fixed space maintainer following orthodontic movement, and a fixed provisional during the post implant healing prior to loading.<sup>3</sup>



Figure-1 (Pretreatment-Extra Oral)



Figure-2 (Pretreatment- Intra Oral)

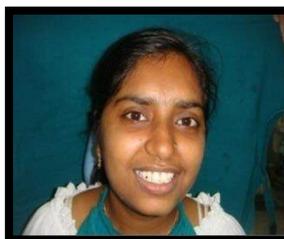


Figure-3 (Post Treatment-Extra Oral)



Figure-4 (Post Treatment-Intra Oral)



Figure-5 (Post Treatment-Lingual View)

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# A Study on Production Performance of Restructured or Revived SLPEs in Kerala

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## ABSTRACT

This paper is an attempt to see whether there has been any significant change in the production performance of the selected State Level Public Enterprises subsequent to the implementation of the revival and restructuring package. The analysis mainly involves the comparison of the performance prior to and post revival with the help of Production Performance Analysis. Three public sector enterprises located in the state of Kerala were selected and their financial soundness is assessed with the help of Production Performance Analysis. The conclusions drawn could provide effective guidelines to the management of selected public sector enterprises in Kerala and their stakeholders.

**Keywords :** Public Sector Enterprises, Production Performance analysis, Value of production, Closing Stock, Opening Stock and Finished Goods

## I. INTRODUCTION

The Public Sector Enterprises have played a significant role in the economic and social development of our country since independence. In pursuit of these objectives, massive investment has been made over the past five decades to build a strong Public Sector. Besides acting as a wheel of economic development, Public Enterprises are entrusted with the task of contributing to ensure social justice, poverty eradication, employment generation, achieving balanced regional development and accelerating the growth of agriculture and industry.

The performance of Public Sector Enterprises during the past decades reveals a wide gap between the aims and achievements. The poor performance of the Public Sector Enterprises has been a great concern to the society in general and for planners and policy makers in particular.

The aim of industrial and economic development cannot be achieved by merely having an ideological bias. It has to be supplemented with concrete and

positive performance. Unfortunately most of the Public Sector Undertakings have failed in developing positive image in terms of financial performance. Some of them have even turned 'sick'. The sick firms are either closed down or rehabilitated. Many Public sector enterprises have been restructured or revived pumping in large sums of public money. Such rehabilitation programmes have often been made on non-economic grounds. Since tax payers' and state financial institution's money is spent on such revival programmes, it merits a bit of research to analyse the objectives of such revival and restructuring programmes and how much these have actually been met.

## II. METHODS AND MATERIAL

### Statement of the Problem

The Government of Kerala has spent large amounts of money year after year on rehabilitating or restructuring state public sector enterprises. Such programmes have been implemented at the cost of new enterprises or welfare spending. It is in public interest to evaluate the outcomes of such large

spending from the state's exchequer, often justified on saving hundreds of jobs.

It is required to look into the objectives of such state spending and how much of it has helped in reviving the enterprises involved. If it has not served the purpose, why the effort and money went waste? Whether such revival or restructuring programmes need any monitoring in the future?

The study will attempt to find answers to a few important questions like: whether such state spending could have been saved? Does the revival or restructuring meet the objectives set for such revival or restructuring?

### Objectives of study

The study centers on the following objectives.

1. To assess the Production Performance of the selected public sector enterprises.
2. To compare the Production Performance before and after implementation of revival and restructuring package.
3. To suggest appropriate measures for improving the performance of the selected public sector enterprises and restore them to sound health.

### Methodology

The study is designed as a descriptive one based on secondary data. It primarily covers state enterprises engaged in the manufacturing and marketing activities. There are 104 state enterprises in Kerala

of which nine are statutory corporations. Of the remaining, 63 are working under Industries Department out of which 17 are closed down for long periods. The study identified the list of state public sector enterprises which were restructured in the past ten years to evaluate the effectiveness of such interventions. For the purpose of the study, three companies were selected about which data were available.

The secondary data required for the study were collected from the rehabilitated or restructured state public sector enterprises in Kerala and from the Industries Department of the state and also from various journals, magazines and related websites etc. To understand the veracity of data, Production Performance Analysis was used.

## III. RESULT AND DISCUSSION

### Production Performance analysis of First Enterprise

The company is engaged in the production of a variety of steel products such as Mild, Medium Carbon and Spring Steel billets of 100 mm. As part of the revival package, it undertook an expansion scheme by adding the third electric arc furnace in 2008 by which the production capacity was raised to 55000 tonnes per annum. The production performances are given below.

**Table 1**  
**The Value of Production of First Enterprise**

(Rs. Lakh)

	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09
Sales	1727.57	990.83	1735.05	730.49	491.20	435.26	835.05	2061.09	3591.57
Add CS of FG	272.13	170.41	193.59	193.47	298.35	368.63	446.26	271.23	1224.52
Less OS of FG	468.98	272.13	170.41	193.59	193.47	298.35	368.63	446.26	271.23
VP	1530.72	889.11	1757.23	730.37	596.08	505.54	912.68	1886.06	4544.86
% of VP to TA	24.54	12.86	26.51	11.39	8.58	7.10	12.06	24.48	58.19

Source: Annual Reports of First Enterprise

VP=Value of production, CS=Closing Stock, OS=Opening Stock, FG=Finished Goods, TA=Total Assets, NW=Net Worth

Table 1 indicates that during the initial years prior to implementation of revival and restructuring package, the value of production was showing a declining trend. The value of production in 2006 was Rs. 505.54 lakh which has increased to Rs. 4544.86 lakh by 2009. From this, it can be inferred that there has been a big change in production of the company after implementation of expansion scheme.

## Production Performance Analysis of Second Enterprise

The company is engaged in the production of a variety of alloy steel, super alloys, aluminium and titanium. After the implementation of revival package, its diverse product mix caters to a wide range of sectors. These include complex and high precision aerospace forgings, specialised forgings for defence, heavy forgings for commercial vehicle, railways and other components for automobile etc.

**Table 2**  
**The Value of Production of Second Enterprise**

(Rs. Lakh)

	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99	1999-00
Sales	995.30	820.95	948.86	1382.92	1704.90	1544.39	1505.41	1542.77	1522.47
Add CS of FG	146.10	158.26	122.48	125.94	160.22	187.36	210.37	338.90	333.32
Less OS of FG	134.16	146.10	158.26	122.48	125.94	126.7	187.36	210.37	338.90
VP	967.24	833.11	913.08	1386.38	1739.18	1604.99	1528.42	1671.30	1516.89
% of VP to TA	36.72	30.20	30.3	43.45	68.40	72.65	75.41	76.17	68.44

Source: Annual Reports of Second Enterprise

From Table 2 the percentage of value of production to total assets was Rs.36.72 lakh in 1991-92 and after the implementation of revival package in 1994-95, it increased to Rs.68.44 lakh in 1999-00. This shows a marked improvement in the value of production of the revival package was implemented in the company after the implementation of revival package. during the year 2007-08.

**Table 3**  
**The Value of Production of Third Enterprise**

(Rs. Lakh)

	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09
Sales	5130.67	4302.62	4314.85	3630.80	3623.57	3545.55	3694.92	3723.07	3161.68
Add CS of FG	378.45	403.68	517.80	296.93	242.70	411.37	462.36	273.66	223.80
Less OS of FG	363.44	378.45	403.68	517.80	296.93	242.70	411.37	462.36	273.66
VP	5145.68	4327.85	4428.27	3409.92	3569.34	3714.22	3745.91	3534.37	3111.82
% of VP to TA	102.74	81.95	76.35	51.85	47.97	49.50	45.85	43.33	33.47

Source: Annual reports of Third Enterprise.

VP=Value of production, CS=Closing Stock, OS=Opening Stock, FG=Finished Goods, TA=Total Assets, NW=Net Worth

The Table 3 shows that during the years prior to implementation of revival and restructuring package, the value of production was showing a fluctuating trend. The value of production in 2003-04 was Rs. 3409.92 lakh which marginally increased to Rs. 3534.37 lakh by 2007-08. This is due to the improvement in production of the company after implementation of revival package in the year 2007-

08. After that the value of production decreased in 2008-09. Hence, there is no permanent improvement in the value of production.

#### Cost Trends of First Enterprise

The particulars of sales, cost of sales and percentage of cost of sales to sales for the nine years up to 2009-10 are tabulated below.

**Table 4**  
**Cost Trends of First Enterprise**

(Rs. Lakh)

	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10
Sales	990.83	1734.05	730.49	491.2	435.26	835.05	2061.09	3165.	3150.91
Less Profit / Add loss	500.05	286.33	333.03	263.17	157.55	131.53	27.98	163.80	-5768.66
Cost of sales	1490.88	2020.38	1063.52	7554.37	592.81	966.58	2089.07	3328.8	-
% of cost of sales to sales	150.47	116.51	145.59	155.58	136.2	115.75	101.36	105.18	-

Source: Annual Reports of First Enterprise

From Table 4, it can be observed that percentage of Cost of sales to sales for the period 2001-02 to 2008-09 is more than 100%. After implementation of revival package in 2007-08, the percentage of cost of sales to sales has decreased in 2009-10. From this, it can be inferred that the implementation of revival

and restructuring package is successful. But, the trend must continue to confirm revival.

#### Cost Trends of Second Enterprise

The particulars of sales, cost of sales and percentage of cost of sales to sales for the nine years up to 1999-2000 are tabulated below.

**Table 4.38**  
**Cost Trends of Second Enterprise**

(Rs. Lakh)

	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99	1999-00
Sales	955.3	820.95	948.86	1382.92	1704.9	1544.39	1505.41	1542.77	1522.47
Less Profit / Add loss	190.91	212.97	294.95	-8.74	-98.52	-195.04	-183.4	-131.56	13.37
Cost of sales	1146.21	1033.92	1243.81	1374.18	1606.38	1349.35	1322.01	1411.21	1535.84
% of cost of sales to sales	119.98	125.9	131.08	99.37	94.22	87.37	87.82	91.47	100.88

Source: Annual Reports of Second Enterprise

The Table 4.38 shows an increasing trend in the percentage of cost of sales to sales up to 1993 - 94. After implementation of revival package during the

year 1994-05, the percentage of cost of sales to sales has decreased in 1997 - 98. But from the year 1998 - 99 the cost of sales of the company shows an increasing trend. From this, it can be inferred that the

implementation of revival and restructuring package was not successful up to 1999 - 2000. But, at present, company started a new unit.

### Cost Trends of Third Enterprise

The particulars of sales, cost of sales and percentage of cost of sales to sales for the ten years up to 2009-10 are tabulated below.

**Table 4.39**  
**Cost Trends of Third Enterprise**

	(Rs. Lakh)									
	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10
Sales	5130.67	4302.62	4314.85	3630.80	3623.57	3545.55	3694.92	3723.07	3161.68	4124.39
Less profit /Add loss	115.31	629.69	569.46	1168.37	324.94	191.36	-83.04	370.54	483.18	-48.35
Cost of sales	5245.98	4932.31	4884.31	4799.17	3948.51	3736.91	3611.88	4093.61	3644.86	4076.04
% of cost of sales to Sales	102.25	114.64	113.20	132.18	108.97	105.39	97.75	109.95	115.28	98.83

Source: Annual reports of KSTC Ltd.

From Table 4.39, it can be observed that percentage of cost of sales to sales for the period 2000 - 01 to 2005 - 06 is more than 100%. In 2006 - 07 the company made profit of Rs. 83.04 lakh. The revival package was implemented on 31<sup>st</sup> March 2007, but in the next two years again the company went into loss. After that, the percentage of cost of sales to sales has decreased to 98.83% in 2009 - 10. From this, it can be inferred that the implementation of revival and restructuring package is successful. But, the trend must continue to confirm revival.

### Suggestions

To increase the effectiveness of the revival package a few suggestions are made on the bases of forgoing study.

- The state has to review its policy on revival or restructuring of public enterprises.
- A detailed study is required to set the state's policy on revival or restructuring.
- The analysis of sales performance shows improvement in sales health of the companies under the study except Third Enterprise. How much of that is due to fresh capital infusion, how much due to debt write off etc. have not been analysed due to non-availability of data. Along with finance, appropriate management

talent must also be made available to these companies so that the revival is long-standing in nature.

### IV. CONCLUSION

The sales analysis of the selected firms reveals a mixed result after the implementation of the revival and restructuring programme. In First Enterprise, all the above analyse shows that there is improvement in the performance of the company after the implementation of revival package in 2007-08. In Second Enterprise, it has been able to write off its losses with the help of the revival package. In 2010 it started a new unit of the company. Now the company is earning profits and it is hoped that it can stand on its own in future. In Third Enterprise, It has not been able to control its losses through the implementation of revival and restructuring package.

In short most of the units have improved their performance after implementation of the revival package. The sales analysis of the selected public sector enterprises shows a creeping improvement after the implementation of the revival programme.

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# Reproductive performance of Red Sokoto Goats from a semi-intensive management system in semi-arid zone, Nigeria

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## ABSTRACT

On-farm data were collected to evaluate reproductive performance of Red Sokoto does reared under small-holder agro-pastoral production system within metropolitan Kano, semi-arid, Nigeria. The effects of age of dams, parity, litter size(s) and sex of kid(s) on pre-weaning growth rate were investigated. Data was obtained from semi-intensively managed herds of twenty four households for a period of six months. Pregnant does were ear tagged and age determined through dentition. Upon kidding, litter size, parity of dam and sex of kid(s) were recorded. Subsequently, daily liveweight changes of kids was monitored and recorded. Results obtained revealed average weight at birth to be 3.18 kg and 2.87 kg for female and male kids with average daily weight gain of 0.11 and 0.13 kg, respectively. Result also showed that male kids gained higher liveweight from 21<sup>st</sup> day to weaning and single or twin births had higher liveweight changes relative to triplets. Does at third parity produced kids with higher weight gain. From the results of this study, it is concluded that male kids at 21 days of age (single or twin) or dam at third parity or three years of age be selected for a sound breeding programme.

**Keywords:** *agro-pastoral, goats, parity, reproductive, semi-intensive*

## I. INTRODUCTION

Small ruminants form an integral part of the economic and ecological niche in small-holder farming systems due to their high prolificacy and ability to thrive on a wide range of feed resources. They have potential capacity for higher levels of production but currently low that is dismal and not commensurate with their potential production (Devendra, 2001). Numerous studies on small ruminants in developing countries have also buttressed their importance to the livelihood of farmers (Braker, Udo and Webb 2002; Solomon *et al.* 2005). In the tropics however, these specie of livestock have low productivity partly due to slow growth rate which had been attributed to poor nutrition, managerial factors and non-genetic factors such as age and parity of dam, sex and type of birth as reported by Gbangboche *et al.* (2006). Selection of genetically superior individuals

as parent stock to future generation is hindered by non-genetic factors which tend to mask the actual breeding values of the selected individuals. It therefore becomes imperative to identify those non-genetic factors with a view to seeking appropriate ways for accurate estimation of breeding values (Dadi *et al.* 2008). Thus, the aim of this study was to evaluate the effects of some non-genetic factors on pre-weaning growth and reproductive performances of Red Sokoto goats managed semi-intensively in the semi-arid zone of Nigeria.

## II. METHODS AND MATERIAL

### Sampling Technique

Twenty four households were selected purposively based upon possession of minimum of five pregnant

does per household and willingness of farmers to participate in monitoring activities. Pilot testing was undertaken prior to commencement of the study using trained enumerators.

### Data Collection

Data was collected with the aid of the structured questionnaires. Variables that were monitored and recorded included reproductive performance (parity, litter size and sex of kids), productive (age of dam, birth weight of kid, weaning weight and live-weight changes). Age of the dam was determined by dentition. Within 24 hours of kidding, the date of birth and birth weight were recorded. Also, litter size, sex of kids and parity of dam was recorded. Weights were measured and recorded weekly using spring balance scale (50 kg capacity) and weaning weight was recorded on the 90<sup>th</sup> day after birth (3months).

### Statistical Analysis

Data collected were analyzed using least square procedures of the General Linear Model procedure of Statistical Analysis System (SAS, 2000).

The model used for reproductive performances was:

$$Ywt = \mu + Pi + Lt + Sx + Et; \quad (1)$$

where:

Pi = parity (i= 1, 2, 3, 4)

Lt = litter size ((Single -1, Twins - 2, Triplets - 3)

Sx = sex of kids (Male - 1, Female - 2)

Et = error term.

While productive performance was measured using:

$$Ywt = \mu + Ad + Bwt + Wwt + Lwt + Et \quad (2)$$

where:

Ad = age of dam (years)

Bwt = birth weight of kid(s), (kg)

Wwt = weaning weight (Kg)

Lwt = live weight changes (Kg)

Et = error term.

There was no interaction between fixed effects and thus, dropped from the model. Trend of the variables were computed using linear regression (SAS, 2000).

## III. RESULTS AND DISCUSSION

### Influence of Sex on Pre-weaning Liveweight Changes

Liveweight changes of kids as influenced by sex is presented in Figure 1. Female kids recorded higher weight at birth relative to the males (3.18kg against 2.87kg). However, at 21<sup>st</sup> day of birth, the males gained and maintained higher weight throughout the study period. The increase in weight gain was linear in both sexes which are described by regression equation in Figure 1: Male (y) =0.938x + 2.367 (r<sup>2</sup> = 0.996); Female (y) =0.837x + 2.550 (r<sup>2</sup> = 0.997). Their daily weight gain were 0.13kg and 0.11kg for male and female kids respectively.

### Influence of Doe's Parity on Liveweight Changes of Red Sokoto Kids

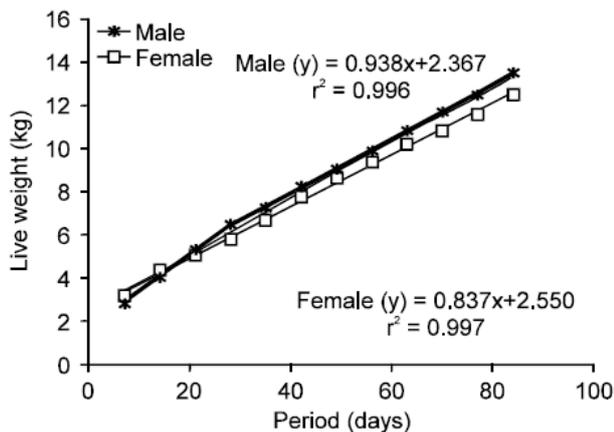
The relationship between liveweight and age of kids as influenced by parity is presented in Figure 2. Result revealed that kids obtained from does at third parity had significantly higher birth weight (3.41 kg), this was followed by kids obtained at second parity (3.10 kg) then from the first and fourth. This trend was maintained throughout the study period. The average daily weight gains observed were 0.09, 0.13, 0.14 and 0.06 kg from the first parity to the fourth respectively. However at 84 days of age, kids obtained at third parity attained 15.03 kg in weight. Kids obtained at first and fourth parity had significantly (P< 0.05) lower birth weight and weight gain throughout the study period.

### Influence of Age of Does on Liveweight Changes of Red Sokoto Kids

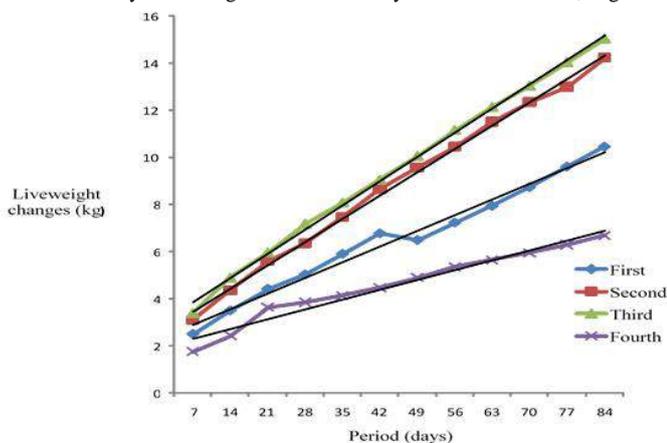
Liveweight changes of kids as influenced by age of does is presented in Figure 3. Results obtained showed does that kid at three years of age produced kids with significantly (P<0.05) higher liveweight (3.22 kg). The liveweight changes showed similar increasing trend for all ages of does from the 7<sup>th</sup> day to the 84<sup>th</sup> day. However, kids obtained from does that were one and five years of age recorded significantly (P<0.05) lower liveweight changes.

## Influence of Litter Size on Liveweight Changes of Red Sokoto kids

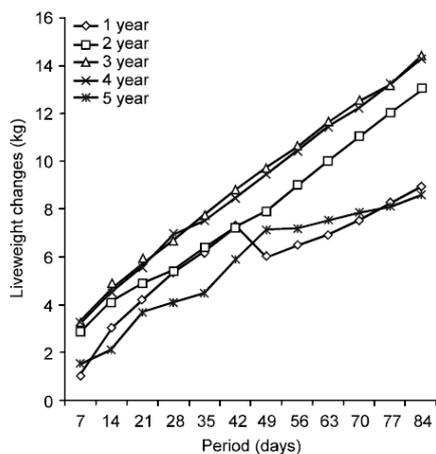
Figure 4 presents the influence of litter size on liveweight changes of kids. Result revealed that kids obtained from single and twin birth had significantly ( $P < 0.05$ ) higher liveweight changes. Kids from triple births gave lower liveweight changes (Figure 4).



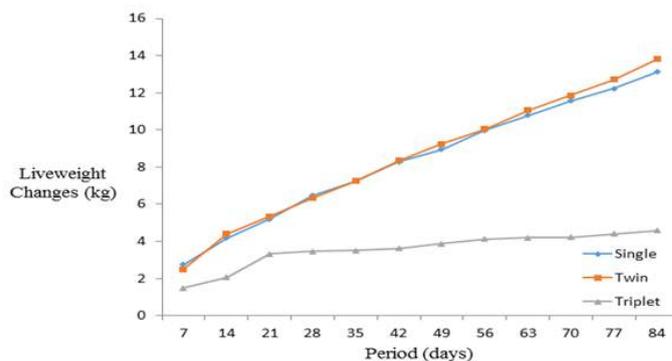
**Figure 1:** Pre-weaning Liveweight Changes of Red Sokoto Kids as influenced by sex managed semi-intensively in a semi-arid Zone, Nigeria



**Figure 2:** Changes in Liveweight of Red Sokoto Kids as influenced by parity of Does managed semi-intensively in a semi-arid Zone, Nigeria



**Figure 3:** Weekly Liveweight Changes of Red Sokoto Kids as influenced by age of does managed semi-intensively in a semi-arid Zone, Nigeria.



**Figure 4:** Influence of litter size on liveweight changes of Red Sokoto Kids managed semi-intensively in a semi-arid Zone, Nigeria.

## DISCUSSION

Findings from the present study revealed that weight of kids (male or female) were statistically comparable. This observation is contrary to literature report by Nkungu, Kifaro and Mtenga (1995) and Zahraddeen, Butswat and Mbap (2008). The authors had observed male kids were superior to their female counterparts at birth. However, in the present study, the male kids gained consistently higher weight from the 21<sup>st</sup> day of birth till 84<sup>th</sup> day of age, which is in agreement with the report by Adamu and Arowolo (2002). The observed superiority in weight by male kids as against their female counterparts could be attributed to the presence of the hormone androgens implicated in high competitive ability and aggression at feeding according to Kiango (1989); Nkungu *et al.* (1995) and Hirschenhauser and Oliveira (2006). These attributes in male could translate to the consumption of more milk and feed and might explain why the males gained weight faster from the 21<sup>st</sup> day. Results of the present study is in conformity with Bemji *et al.* (2006) who reported that liveweight of kids increased with parity of does. High parity effect observed in the study with respect to daily weight gain of kids could be attributed to the corresponding increase in liveweight of does as parity increases to three before decline. Similar increases were noted by Bemji *et al.* (2006) which they attributed to the increase in weight of does and corresponding increase in weight of dam as parity increases. Daily weight gains of kids were in favour of both single and twin litter size irrespective of sex from birth to 84 days of age. This finding is in disagreement with an earlier study by Adamu and Arowolo (2002) that single male, single female, twin male and twin female kids were comparable in terms of daily weight gain from birth to 100 days of age. It is also contrary with the reports by Nkungu *et al.* (1995) that kids born single

gain weight faster relative to their twin counter parts from birth to 12 months of age. There was consistent daily weight gain in both single and twin kids which may be linked to pre-and-early post-natal nutrient availability from the dam. Akanno and Ibe (2006) stated that regression equation could be valuable in some rural African farm communities where sensitive weighing scales are not readily available or if available are expensive beyond the reach of the farmers. Thus, findings of this work is the development of equation that had high  $r^2$  values between 81 to 99 for predicting kid liveweight changes based on sex of kid, parity of kid, age of dam and type of birth of the kids for semi-intensively managed goats in the semi-arid region of Nigeria.

#### IV. CONCLUSION

It is concluded that selection for stock improvement could be targeted at male kids for higher liveweight gain from 21<sup>st</sup> day to weaning; kids obtained from single and twin births had higher liveweight changes relative to triplets. It is thus recommended that does at third parity or of three years of age should be selected to give fast multiplier effect.

#### V. ACKNOWLEDGEMENT

The support granted by the management of Bayero University Kano Nigeria and the Centre for Dryland Agriculture by way of research costs via grant No. (BUK/CDA/ANS/RLM/PHD/2012/13/001) is acknowledged.

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# Role of Aquaporins in Diseases and Drug Discovery

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## ABSTRACT

Aquaporins as water channels in transportation of water in and out of the cells due to their water permeability property play an important role in maintaining water's constancy which result in normal human physiology. Any mutation of the genes encoding them result in causing many diseases which are life threatening and dangerous like hyperinsulinemia, Sjogren's syndrome, vasogenic brain edema, glaucoma, nephrogenic diabetes insipidus, carcinoma, lymph node metastatic carcinoma and tumor growth, etc. Recent studies and discoveries has shown that aquaporins have fundamental role in drug discovery and they serves as attractive targets for different diseases. Several types of aquaporins are discovered which play important role in different types of diseases and drug discovery. In this paper we focused on role of aquaporins, structure- function relationship, types of aquaporins and their related diseases and strategies for identification of modulators of these drug targets for discovery of novel therapies and recent discoveries on different types of aquaporins and introduction of them as attractive targets. These paper showed significant role of aquaporins in normal human physiology and pathophysiology and give insights for deserving attention for them to effectively treat some of life threatening diseases.

**Keywords:** Aaquaporins, Diseases, Drug Discovery, Target, Physiology and Pathophysiology

## I. INTRODUCTION

Water as an essential molecule of the body forms major components of body cells and tissues. It is fundamental, supportive basis of all physiological activities of the cells and tissues such as transportation, body temperature regulation, urine control, digestion control, lubrication and cell communication. Circulation and supplement of water as vital life substance should be done using essential elements of the body. Among these elements aquaporins are very important in maintaining water's constancy and any disturbance in their function causes a pathophysiological state. [1,3] Aquaporins are integral membrane proteins serve as water channels for selectively conducting water in and out of the cells and prevent the passage of ions and other solutes. Aquaporins are formed by intrinsic membrane proteins that forms pores in cells. Aquaporins located in plasma membrane and membrane of intracellular organelles. Aquaporins are mostly present in lens, brain, kidney, lungs, skin, vascular endothelium, gastrointestinal tract, sweat glands, liver, WBC, adipose tissue, salivary, lachrymal and etc. [1,2,3] Aquaporins are formed of six

transmembrane alpha helices and their arrangement is in a right-handed pattern, with amino acid and COOH termini present on the cytoplasmic surface of the membrane. The amino acid s and COOH divisions of the arrangement show resemblance to each other like tandem repeat. Extracellular and cytoplasmic vestibules of the aquaporins are formed by five interhelical loop regions (A-E), out of which loops B-E are hydrophobic and consist of highly conserved Asn-Pro-Ala(NPA) motif, which flap the center of lipid bilayer of the membrane, forming a 3-D "sand clock" or "hour-glass" structure where water molecules flow through. This overlap forms one of the two channel constriction sites in the peptide, the first one is Asn-Pro-Ala(NPA) motif and second constriction site which is narrower is called ar/R selectivity filter. Aquaporins by formation of tetramers in the cell membrane, facilitate water transportation and also other small solutes such as CO<sub>2</sub>, glycerol and urea (uncharged molecules) across the membrane. [1,3]. Size of pores in aquaporins are different and depend upon the type of molecule that are passed through the pore. These pores are not permeable to charged molecules like protons. The movement of

water molecules through the narrow channel is in a single align by orientation themselves in the local electrical field generated by the atoms of the channel wall. Upon entering, the water molecules face with their oxygen atom down the water channel. At the middle time, they reversely oriented facing with the oxygen atom up. This rotation of molecules of water in the pore is due to the interaction of H-bonds between the oxygen of water and the asparagines in the two NPA motifs. This mode of movement for entering and leaving of the water molecules during passing through the channel means entering face down and leaving face up is normal passage of the molecules and essential in normal physiology of the body and any disruption in this normal transportation mechanism result in development of diseases. [3,2]. So far aquaporins are classified into 13 types which are implicated in different diseases, such as brain oedema, cataract, cancer, nephrogenic diabetes insipidus and gallostone, obesity development and polycystic kidney diseases. Most of aquaporins are exclusively water channels and will not permit other small molecules and ions pass through. Some of them are called as aquaglyceroporins which perform transportation of water and glycerol, and a few other small molecules. Aquaporins 1,2,4, and 5 are selectively passage channel of water, whereas AQPs,3,8 and 9 perform transportation of glycerine and larger solutes. AQP3,7 and 9 have permeability property towards urea and glycerol. Cloning of human genes encoding aquaporins identified associated disturbances with abnormal functioning of them. So they have played important role in several related disorders and drug discovery. In this paper we listed types of aquaporins and their contributed tissues where they distributed and their association diseases. [1,2,3].

## II. METHODS AND MATERIAL

### **Aquaporins's association with diseases and their selection as potential drug targets**

*Aquaporin-0* (AQP0), also called as major intrinsic protein, is a type of the ubiquitous aquaporin family. This type of AQPs is highly expressed in the fiber cells of lens. Main function of AQP0 is lens clarity. This protein consist of 4 identical monomer so is tetrameric protein and each monomer has its own water pore but under specific condition these pores function cooperatively. Any mutation in the coding genes and

disturbance result in development of hereditary cataract. So this protein can be a potential target in drug discovery projects. [3,4].

*Aquaporin 1* is an integral membrane proteins serve as water channels whose main function in body physiology has been characterized in the kidney. It is also distributed in gastrointestinal tract, sweat glands, red blood cells, vascular endothelium and lungs. Any disturbance in their functioning leads in development of following disease: thickening of cornea, defect in the ability for concentration of urine, tumour growth, conjunctival ailment, glaucoma and cotton-null blood antigen transfusion incompatibility. The role of AQP1 in mentioned disorders emphasizes its role as effective drug target for their treatment. [3,5]

*AQP2* report released in 1994 about its mutation in chromosome 12q13 which causes nephrogenic diabetes insipidus, a non -x linked diseases. This mutation is very rare. Since this report, more than 25 mutations in human AQP2 have been identified. The main function of AQP 2 is to reabsorb water from urine during removing it from the blood by the kidney so facilitate production of concentrated urine by the kidney. [3,6]. Mutation of arginine vasopressin receptor type 2 (AVPR2) gene required for translocation of AQP2 water channel to the membrane result in Congenital nephrogenic diabetes insipidus (NDI). arginine vasopressin (AVP), antidiuretic hormone (ADH) regulates body's water retention by increasing the water permeability of the renal collecting duct. The AQP 2 has been shown to be the target for this action. Recent studies has been reported that AQP2 is a attractive target for treatment of nephrogenic diabetes insipidus (NDI). [7]

*Aquaporin 3* is another member of ubiquitous aquaporin family. This AQP is expressed in the basal lateral cell membrane of collecting duct cells of the kidney and facilitate a route for water to exit these cells. Any disturbance in its function can be result in polyurea. [3]. Recently, scientists discovered the role of AQP3 in Nonmelanoma Skin Cancer (NMSC). In this study using immunohistochemical expression, the skin biopsies of nonmelanoma Skin Cancer, normal and psoriasis samples which were 60,40, and 30 in number respectively, were evaluated and result has demonstrated that AQP3 was expressed in 93.3% of squamous cell carcinoma (SCC) cases and 66.7% of basal cell

carcinoma (BCC) cases . SO AQP3 may play a role in NMSC pathogenesis. This study help in unrevealing g the mechanism involved in development of this cancer type and facilitate conduction of new target discovery projects with focus on AQP3 as new target for treatment of NMSC. [8]. The role of AQP3 also identified in development of the pathogenesis of psoriasis via nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling .[9].

*AQP4* is another type of the aquaporin family of integral membrane proteins for conduction of water through the cell membrane. The protein is distributed in kidney, brain,gastrointestinal tract,lungs and muscles . The related diseases to this proteins are vasogenic brain edema, seizures, hydration of stratum corneum in skin,Devic's autoimmune diseases and glaucoma . [2,3]. Aquaporin 4 also plays important role in cerebral ischemia in association with MicroRNA-29b a therapeutic target. Scientists concluded that miR-29b could potentially anticipate stroke outcomes as a novel biomarker, and overexpression of miR-29b decreased blood-brain barrier disruption after ischemic stroke through downregulation of AQP-4. These studies support the potentiality of AQP4 as therapeutic target for treatment of related diseases. [10] .

*AQP5* is another class of aquaporin family of water channel proteins. This protein has significant role in production of tears, saliva and pulmonary secretions .The disturbances associated with its function cause Sjogren's syndrome thickening of cornea and primary carcinoma and lymph node metastatic carcinoma of on-small cell lung cancer (NSCLC) .[1,3]. Regarding its role in primary and lymph node metastatic NSCLCs, scientists analyzed the AQP5 expression using an immunohistochemical labeled streptavidin-biotin method which determined AQP5 expression in 94 NSCLC cases primary carcinoma including 51 cases associated with lymph node metastasis. The results demonstrated that AQP5 expression was notably higher in adenocarcinomas compared with squamous cell carcinomas (P=0.002). Additionally AQP5 in the primary carcinomas with lymph node metastasis significantly showed higher percentage compared with percentage of those without lymph node metastasis (P=0.024). So AQP5 is a potential drug target for treatment of the related diseases.[11].

*AQP6* is another type of integral membrane protein which functions as water channel. This type of AQPs are specific for the kidney .The diseases related to this protein are Hyperinsulinemia and decreased plasma glycerol.[3].Recent study on AQP6 demonstrated role of AQP6 in the Mercury-sensitive osmotic lysis of rat parotid secretory granules. The scientists used  $Hg^{2+}$  for activation of AQP6 to investigate the properties and characteristics of permeability of solute in rat parotid secretory granule lysis. The result showed permeation of halide group anions which serves as a  $Hg^{2+}$ -sensitive anion channel in parotid secretory granule of rat by AQP6. AQP6 can be investigated as drug target for better treatment of kidney diseases. [12,13]

*AQP7* is another type of aquaporin family encoded by the AQP7 gene. This protein has significant role in sperm function, facilitation of transportation of water, urea and glycerol. The protein shows similarity in sequence with AQP3 and AQP9 so suggested to be a subfamily. AQP7 and AQP3 located at the same chromosomal locus, The main disease associated with its disturbance is Hyperinsulinemia. [1,3,14].

*Aquaporin 8* is water conducting channel expressed in pancreas and colon. The Studies by RT-PCR demonstrated detection of AQP8 mRNA in proximal jejunum, duodenum,, rectum, pancreas, proximal colon and liver and, to a few degree, in stomach and distal colon. Distribution of AQP8 suggested its significant role in the water absorption in the intestine, bile secretion in liver and pancreatic juice in pancreas. Its cytoplasmic localization may also link its participation in intracellular osmoregulation process. So this protein association and function can be promising support for their role in drug discovery as drug target.[3,15]

*AQP 9* is another member of aquaporins belonging to the aquaglyceroporin subfamily of aquaporins. The main function of the protein is transportation of water, urea,glycerol, purines and pyrimidines . [3].It plays a role in metabolism of glycerol and differentiation of osteoclast. It also has some roles in immunological response and bactericidal activity.Recent studies has demonstrated association of decreased hepatic AQP9 and glycerol permeability with insulin resistance in non-alcoholic fatty liver disease . In this analysis, scientists observed downregulation of APQ9 together with subsequent decrease in hepatic glycerol permeability in

insulin –resistant cases. Using real-time PCR, western blotting and immunohistochemistry. These studies are fundamental support for their role in drug discovery as drug target for new treatment of related diseases.[1,3,16] *AQP10* is another type of aquaporins family with binary functional characteristics as a channel/carrier for transportation of solute. The studies also showed representation of AQP10 as an alternative pathway for glycerol efflux from human adipocytes. These finding help in providing a new insight into its performance mechanism, which would help further illustrate its physiological role.[17,18].

*AQP11* is another type of aquaporins family which shows functional distinction from other proteins of subfamily of aquaporin. The studies have shown its role in the brain but still further studies is required. [19].The studies also demonstrated disruption in AQP11 result in polycystic kidneys following vacuolization of the proximal tubule. This study is done using generation of AQP11-null mice .The mice expressed cyst formation of the proximal tubule and vacuolization .These finding demonstrate that AQP11 has essential role in proximal tubular function.[20]

*AQP12* is a novel aquaporin member which expressed in pancreatic acinar cells. Scientists identified this type of protein using BLAST program search. They applied northern blot analysis for revealing expression of AQP12 in pancreas .using other techniques such as in situ hybridization and RT-PCR, selectively localization of AQP12 in the acinar cells of pancreas identified .Additional investigation using expression of AQP12 in *Xenopus* oocytes, cultured mammalian cells and Immunocytochemistry suggested a role of AQP12 in secretion of digestive enzyme such as exocytosis of secretory granules and maturation. More investigation and analysis is required for supporting selection of these proteins as drug targets for treatment of life threatening disorders.[21].

### III. CONCLUSION

Because of significant role of aquaporins in selectively transportation of water and solutes, considerable medical focus has been made in human aquaporins as potential drug targets. Also due to their important role in physiological process such as wound healing, angiogenesis, migration of cells during tumour

development and regeneration, these proteins are suggested to be attractive drug targets. The drugs target aquaporins in such a way that can control their role by activation and deactivation of them. Theses activation and deactivations mechanisms of drugs on aquaporins are such as monitoring urine formation for fluid imbalance, cancer treatment by inhibition of tumour growth and prevention of its metastasis, prevention of brain injury, controlling energy production to fight weight gain for obesity, maintaining moisture in dry skin, controlling formation of polycystic kidney, prevention of polyurea and so on. So association of aquaporins with development of different pathological conditions in human, emphases their role as therapeutic targets .Although discovery of drugs that target aquaporins is still in the young stage but this area of research demands attention in order to successfully treat some of these aquaporins related diseases .Although available conventional technologies and methods in biological sciences are used for screening ion channel but these technologies cannot be effectively used for screening of aquaporins. However, some technologies are available for indirectly measuring aquaporins such as confocal and internal reflectance fluorescent microscopy for measurement of quantitative changes of volume of water, radiolabelled auaporins for study of permeability, fluorescent indicators for measurement of water permeability in the cells and so on. Recently application of several recent biological tools such as real-time PCR, western blotting, generation of transgenic aquaporin knockout mice like null mice for AQP1,3,4,5, and 8, in situ hybridization, culturing of the mammalian cells including the tissues having aquaporins like AQP12 and immunohistochemistry and so on helped in understanding the mechanisms, association role and identification of more Aquaporins types. Recent discoveries on aquaporins demonstrated generated medical interest in aquaporins as attractive drug targets. So This attention deserves towards aquaporins conduction projects for selection of them as drug target for treating life threatening disorders. Identification of pharmacologically effective aquaporins modulators is a challenging area as they have significant role in human physiology and pathophysiology. But this research area is still at nascent stage and demand scientists to focus on the area for generation of new therapy for decreasing suffering and death percentage related to aquaporins disruptions.

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# Ion Channels Association with Diseases and their Role as Therapeutic Targets in Drug Discovery

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## ABSTRACT

Ion channels as potential drug targets has been widely designated in pharmaceutical industries for treatment of life threatening diseases. This targeting is a challenging area in drug development and discovery because of availability of extensive knowledge of structures and functions about ion channels such as sodium and calcium voltage-gated ion channels found in mammals. This knowledge mostly obtained on the basis of 3-D crystallographic studies along with their computational analysis. Analysis and modulation of Ion channels provide us with understanding of various properties, such as channel opening, pore function, voltage sensing and ion selectivity. Any disruption and deregulation in the mentioned functions leads to pathological states thus supporting the idea of their choice as valid targets in drug discovery projects. In this paper we focused on importance of ion channels in drug discovery and reviewed their structures and functions and their role in disease and drug discovery and their modulation technologies in drug discovery for their targeting purpose.

**Keywords:** Ion Channel, Target, Drug Discovery, Pathology. Modulation of Ion Channels

## I. INTRODUCTION

Ion channels are membrane proteins which form potential drug targets. Therapeutic drugs with ion channels as their targets has been formed the third bestselling prescribed drugs so far. In this paper we survey information on various types of ion channels that play an important role in pathophysiological conditions, providing designation of target specific drugs that either cause direct block of channel or modulation of ion channel functions. Attempts are required for exploration of new drug gable ion channel targets with a strong focus on the following aspects: Exploration of technology of channel discovery at the molecular level using high-throughput screening (HTS), Identification of the disorders accompanied with ion channel dysregulation, Exploration of therapeutic scope for ion channel modulation and Identification of strategies for ion channel modulators.[1,2]. Although ion channels with high therapeutic potential has taken a great place in drug discovery but only 7% of them have been commercially exploited as drug targets. However, only,

ligand or voltage –gated channels in excitable tissues has been targeted so far and non-voltage-gated cation channels present in non-excitabile tissues like inflammatory cells have been remained unexplored and commercially unexploited as drug target. In the history of ion channels targeting drugs, Sulphonylureas were used as blockers of the L type voltage –gated calcium channels (L-VOCCs) for treatment of cardiovascular diseases. These drugs were strongly investigated on observation in human patients and animal models. The molecular targets for sulphonylureas were, however, detected as the K ATP channels in B cells of pancreases. Later on after identification of K ATP channels molecular compositions of these channels was worked out, and since then ion channels became more attractive targets in pharmacological industries. With the appearance of patch-clamp instrumentation technology, which facilitate investigation of opening, closing and ion conduction, voltage –gated ion channel research area has become a promising filed in drug target discovery projects. Non-voltage gated ion channels also show attractive interest in development of novel drugs for

CBS diseases due to their potential role. During 1980s, ion channels research area was expanded and a large number of ligand-gated ion channels were investigated and categorized. Among these, the prominent ones are 5HT<sub>3</sub> receptors and nAChRs along with their appropriate ligands identified. For identification of blockers antagonists of 5HT<sub>3</sub> channels, animal models were used to treat certain neuronal diseases such as migraine and anxiety which were not so much successful. In case of other receptors (nAChR) blockers as muscle relaxant have been discovered that were quite effective. The difficulties are present in ion channel discovery, in spite of wide availability of technologies, are lack of appropriate high-throughput assay technologies and pharmacological tools for assessment of their physiological roles, their role in development of pathophysiological conditions and verification of them as targets. [1,2,3,4]

## II. METHODS AND MATERIAL

### A. Molecular Structures and Properties of Ion Channels

Ion channels are membrane proteins which are pore forming and their functions are such as establishment of resting membrane potential, configuration of action potential, conduction of electrical signals by gating the ions flow across the cell membrane, regulation of cell volume and controlling ion flow across secretory and epithelial cells. Ion channels are classified into four divisions such as:

*Voltage gated* : These type of channels opens while changing membrane potential of the channel. Examples are K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>+</sup> channels distributed in cardiac muscles

*Ligand-gated*: includes three types :

*External ligand-gated* these type of channels opens due to their induction by a specific extracellular ligand molecule.

*Internal ligand-gated* these type of channels open or close when an intracellular molecule like ATP or cyclic nucleotide stimulate them.

*Mechanically-gated*. These open by exertion of some mechanical pressure (there is no involvement of membrane potential)

Voltage gated channels consist of two transmembrane helices which form the basic building block and separated by a loop which is called as P-loop and form an inverted pore. In voltage-gated cation channels, more transmembrane helices form a basic block which contains six transmembrane helices and the P-loop. For a functional voltage-gated channel, four basic blocks are required. The location of amino and carboxyl terminals of the six alpha helical transmembrane proteins is in the cytoplasm. The transmembrane domains contain intracellular and extracellular loops. In calcium and sodium channels, these blocks are associated with one large polypeptide, forming the alpha-1 subunit in calcium channels and alpha subunit in sodium channels. Except to these subunits which form central pore forming subunits, sodium channels contain two auxiliary beta subunits, and calcium channels consist of large complexes of alpha1, alpha2 subunits. Formation of actual pores in channels is done by subunits apposition. The pore forming subunits are named the alpha subunits, while the complementary subunits are symbolized as  $\alpha$   $\beta$   $\gamma$   $\delta$  and so on. While some ion channels allow the passage of ions based on their carrying charge, the archetypal pore is only one or two atoms wide at its narrowest point. It transports specific ions like Na<sup>+</sup> or K<sup>+</sup> and transfer them through the membrane. Passage process through the membrane in some ion channels is regulated by "gate" which is opened and closed by external factors such as temperature, chemicals, electrical signals and mechanical forces, depending on the channel variety. Channels are gated because they open transiently and these opening and closing of gates precisely controlled. [2, 5, 6]. Voltage gated channels are mainly consisting of four types such as:

*Ca<sup>++</sup> channel*: It is calcium channel which shows selective permeability to calcium ions. It consist of five types : L-type which is mostly distributed in Skeletal muscle, smooth muscle, bone (osteoblasts), dendrites and dendritic spines of cortical neurons and ventricular myocytes, P/Q type present in Purkinje neurons in the cerebellum / Cerebellar granule cells, N type distributed throughout the brain and peripheral nervous system, R type found in Cerebella granule cells, other neurons and T type found in neurons, cells that have pacemaker activity, bone (osteocytes), thalamus (thalamus).

*K<sup>+</sup> channel* these are potassium channels that are found most widely in all living organism. They consist of potassium pores which are selective to potassium. They consist of following classes: Calcium-activated potassium channel, Tandem pore domain potassium channel, Voltage-gated potassium channel and Inwardly rectifying potassium channel.

$Na^+$  channel are another type of integral membrane proteins that transporting sodium through plasma membrane they are grouped into voltage-change ("Voltage-gated", "voltage-sensitive and voltage-dependent" sodium channel.

$Cl^-$  channel are another type of ion channels which consist of 13 members.[2]

## B. Role of Ion Channels in Diseases and Drug Discovery

As the importance and role of every molecule in drug discovery and its targeting is understood by analysis its participation in emergence of diseases and its linkage with pathophysiological states. Here we focus on diseases associated with ion channels to provide extensive knowledge to support their role as attractive target in drug discovery. Ion channels are various types and distributed in variety of tissues and they have several functions which have vital role in physiology of the body. Any mutation in the genes encoding them can cause specific diseases. Ion channels functions are regulated by several factors and any dysregulation of their function leads to pathophysiological state. A number of human diseases have been detected that are caused by ion channels gene mutation. As these diseases are due to mutation of their genes so they are inherited and known as channelopathies. Some specific types of channelopathies are mentioned below: [2].Sodium – channel diseases: some specific diseases are associated to dysfunction of sodium channel which is due to its gene mutation. These diseases are such as certain types of muscle spasms, Liddle's syndrome which is due to inadequate sodium transport out of the kidney. Other diseases which is result of defection and disruption in function of sodium channels are Blockage of hERG, Showing prolonged QT interval in ECG, Hyperkalemia, periodic paralysis of skeletal muscles, Long QT syndromes and Familial generalized epilepsy. [2,8,9].Potassium channel diseases: The most inheritable life threatening disorders in the heartbeats are caused by defection in  $K^+$  channels. Other important diseases associated to this ion channel are Episodic ataxia type 1 and Benign infantile epilepsy [10-14].Chloride –channel diseases : Dysregulation of  $Cl^-$  channels caused cystic fibrosis and this defect is a inheritable disorders and causes kidney stones . Other disorders related to this ion channel are Osteoporosis, Epilepsia, Myotonic congenital[1,2]. Calcium channel diseases: The main disease caused by dysfunction of calcium channels are Migraine,Spinocerebellar ataxia type6,Congenital night blindness, central core disease and Hypokalemic paralysis of skeletal muscles .[1,2] .Due to distribution of ion channels in different body cells ,tissues and organs ,several diseases caused because of dysfunction and dysregulation of these distributed ion channels

.These diseases mainly are Hypertension due to dysregulation of voltage gated  $Ca^{++}$  channels in cardiovascular system. Epilepsia due to dysregulation of voltage gated  $Na^+$  channels in central nervous system. Stroke and inflammatory bowel syndrome due to dysregulation of voltage gated  $Ca^+$  channels in nervous system .Neuropathic pain due to dysregulation in voltage gated  $Na^+$  channel in nervous system. Cystic fibrosis and Myotonia congenita due to dysregulation in  $Cl^-$  channels in lungs, pancreas and cardiac muscles respectively and Paralysis due to dysregulation in voltage gated  $K^+$  channels in skeletal muscles. [2, 15] Association of ion channels with mentioned diseases has demonstrated their significance in drug discovery as drug targets. Studies on ion channels regulation and expression helped us in understanding their properties such as voltage sensing, channel opening and closing, pore function and ion selectively. Any dysregulation of these functions result in development of pathological states thus support their role as novel drug targets. The ion channels consist of several interaction sites that can be investigated and studied for development of potential drugs. Expression and regulation of ion channels' function are complicated phenomena. In addition to transmembrane proteins, some complementary regulatory proteins are also involved in their functioning and this point adds another aspect to modulation strategy of ion channels. Highly excitable ion channels found in nerve and muscle cells can be stimulated by passing a current, so specific channel can be targeted by appropriate drugs. Their excitability can either be increased or decreased for better study and analysis. Ion channel modulation has been a fortunate area for drug discovery. [2,16-18]Modulation of ion channels helps in development of novel therapeutic drugs without extensive knowledge of their structures. This challenging area of ion modulation provides new opportunities for discovery of new drugs and designation of drugs for targeting specific ion channels is done by mechanisms of direct channel block or ion channel modulation for functional expression. [19,20].In drug discovery process via ion channel modulation following methods is used:

*Voltage –dependent block.* This direct blocking method of channel. In this method ion channel is blocked by a charged drug molecule.

*Tonic-block.* It is also called state –independent block and in this strategy, no current change is experienced by the channel and the channel may be in inactive or active state. In this strategy a tonic blocker or the drugs is bounded to the channel whether it is open or close .In this strategy, several pulses fail to unblock the channels, thus result in state –independent block.

*State –dependent block* .In this strategy the channel can be in three states namely resting, activated and inactivated states. During resting state ,the channel remains open and blocked by high affinity drug molecules towards the channel. This is called open-state block strategy. During inactivated state , binding of a drug molecule to the channel without alternation the channels open time occur as result of decreasing the ion flow .This strategy called inactivated –state block.

*Phasic block*. In this strategy , Irrespective of channel's states such as open, activated, inactivated, a drug molecule with high affinity towards the channel is applied and repetitive pulses enhance the block .[2,15-17]

Role of accessory proteins in the expression of ion channel is notable .Some drugs functioning as blockers which inhibit specific accessory proteins to regulate ion channel functioning .Most channels are studied and analyzed as drug target so far belong to voltage-gated and ligand gated classes found in excitable tissues where blockers are detected for clinical purposes .However , discovery of ion channels in non –excitable cell types are basically unexplored . 5HT<sub>3</sub> and nicotinic acetylcholine receptors are the best known examples of ligand –gated channels and their blockers have been identified that treat effectively many neural diseases . These studies mainly involved animal models.[2,19,20] The technologies for modulation of ion channels play essential role in their modulation. Different types of assay technologies are available for researchers to modulate ion channels . Although due to lack of high throughput technology, drug discovery process of ion channel is very slow but still using available technologies and their combination with other related technology is helpful for modulation of ion channels. The most known technologies are fluorescent technology, Non fluorescent technology and electrophysiology.

*Fluorescent technology* is used for determination ion concentration with the help of florescent indicators .This technology has been successfully used for studying Ca<sup>++</sup> and P2X channels and Fluo-3 and 4 ,Fura and Indo-1 used as indicators .

*Non –fluorescent technology* is a direct method of measurement of ion flux through a channel with radiotracers such as <sup>86</sup>Rb<sup>+</sup> for potassium channels and <sup>22</sup>Na<sup>+/14</sup>C for sodium channels or by using atomic absorption spectrophotometer .

*Electrophysiology*. This technology is a high throughput technology and used for screening ion channel function

and also enables investigation of opening, closing and ion transfer of channels. It demonstrated that a single assay technology may not be sufficient to address all the problems regarding ion channels function and a combination of these technologies may be useful and more effective to achieve the desirable results.[2]

### III. CONCLUSION

Ion channels as significance attractive targets for treatment of various types of life –threatening diseases such as Hyperkalemia periodic paralysis of skeletal muscles ,Long QT syndromes and Familial generalized epilepsy, disorders in the heartbeats ,Episodic ataxia type 1 and Benign infantile epilepsy ,Migraine, Spinocerebellar ataxia type6,Congenital night blindness, central core disease and Hypokalemic paralysis of skeletal muscles ,cystic fibrosis ,Osteoporosis,Epilepsia and Myotonic congenital, has been attracted drug discovery attention .Studies of regulation and expression of ion channels provided scientists with extensive knowledge about ion channels and their role in drug discovery , these knowledge widely are about opening and closing of the pore, orientation of pore, structure of pore, voltage sensing, gating process, ion selectivity and pore function. These basic and essential knowledge have been supported the idea of choice of ion channels as therapeutic targets .By studying of above mentioned functions and structures , scientist enable to identify associate pathological states which emerged as result of dysregulation of these functions. In drug discovery, one of the most challenging areas for targeting ion channel is their modulation. Modulation of ion channels has advantage of not requirement of extensive knowledge of the molecular structure of ion channels, their subtypes and their full regulation of their expression. Most of the drugs developed by modulation methods marketed are without extensive knowledge of above mentioned characters and they are therapeutically effective. Generally development of ion channel drug is very slow due to inadequate availability of high-throughput technologies. Electrophysiological measurement technologies have not achieved successful results because of their disadvantages such as low speed, time consuming and deficiency in sensitivity .However, exploration and synthesis of novel drug molecules as ion channel's ligands has been possible via advances in patch clamp instrumentation technology. Now days due

to advancement in *in silico* drug designing methods used in bioinformatics projects, several companies undergo such methods for benefitting ligand discovery. Although ion channels has been started for analysis and new discovery but as compared with some important clinical drugs, as a class, they are poorly explored and underexploited in drug discovery projects. This review considered ion channels as attractive drug targets in challenging area with high potential ability of being targets, and encourage scientists to focus on this challenging area with promising results for development of high desirable drugs and more successful treatment of life threatening disease.

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# Applied GIS in Assessment Water Quality Modeling in the Malacca River.

## Case Study: Introduction to Research Study

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### ABSTRACT

A research study documents the process of examination, using experimentation or investigation to discover and interpret on certain topic for the purpose of increasing the understanding of an issue. The main purposes of research study are to help people to understand and solve problems, communicate ideas and information to the public, help researchers to make decisions through data collection, and develop new knowledge for humankind. Research may be divided into the first stage (problem statement, research questions, hypothesis or objectives), second stage (literature review, research design, instrumentation, preliminary study), and third stage (data collection, data analysis or research findings, preparation of reports). The problem statement of this study involves river water pollution, while the objective of the study is to assess river water quality in the Malacca River, to determine major source and the factors contributing to river pollution, and to determine a spatial decision support system (SDSS) for minimizing water pollution in the Malacca River. The research design involves a quantitative approach (experimental methods), which collects primary data (water sample from Malacca River and GPS data information) and secondary data (water sample from government, GIS map-based data, and RS data). This data will be grouped together and undergo the analysis process of GIS and RS to develop SDSS. Information and results provided will become answers to the objective and determination of achievement of the research study. Therefore, this study provides new information for other researchers to perform more in depth research according to their field of study.

**Keywords:** Research study, water quality, GIS, RS, SDSS.

### I. INTRODUCTION

A research study may be defined as ‘gathering data, information and facts for the advancement of knowledge’ [16]; ‘a process to collect and analyze information in systematic steps with purposely to increase the understanding of an issue or topic’ [4]. In more detail, a study is ‘hard work in examination especially towards experimentation or investigation with the aimed to discover and interpret the facts, revision of accepted theories or laws in accordance to new facts, or practically application of new or revised theories or laws’ [17]. The definition for a research study may be described by various definitions in accordance with the specific field of study, but the main purpose for carrying out a research study is to help people to understand and solve problems, communicate ideas and information to

the public, help researchers to make decisions through data collection, and develop new knowledge for humankind. Conducting a research study should undergo in a systematic process, which may be divided into three stages namely first stage (problem statement, research questions, hypothesis or objectives), second stage (literature review, research design, instrumentation, preliminary study), and third stage (data collection, data analysis or research findings, preparation of reports). The probability of success for a research study can only be increased by following systematic methods such as the three phases stated previously.

According to the world statistics, a majority of developing countries (70 percent) are dumped the industrial wastes with untreated into water especially river and polluting the usable supply [14]. One of the

factors that causes these issues to occur is about 99 million pounds or 45 million kilograms of fertilizers and chemicals, and 2 million tons or 1.8 billion kilograms of human waste disposed into the waterways around the world every day [14]. The world's most polluted rivers are the Ganger River (India), Jian River (China), Jakarta River (Indonesia), Pasig River (Philippines), Tiete River (Brazil), and Yamuna River (India) [2]. River pollution is not exceptional to Malaysia. According to the report from Department of Environment in 2012, 41 percent or 195 rivers out of 278 are considered polluted, including the Malacca River in Malacca state. According to the report of Department of Environment [5], recorded 15,740 are water pollution that arises from variety of sources namely wastewater plants (63%), manufacturing industry (29%), animal farms (5%), and agricultural activities (3%). Hence, the problem of water pollution should be dealt as soon as possible by investigating the causes and contributing factors of pollution, and conducting a research study to find a solution for water pollution.

Malacca is a popular and well-known as World Heritage Site recognized by UNESCO in July 7, 2008 [6], leading the state become as a tourist destination based on historical tourism industry with the famous of A' Famosa City, Portuguese village, Bukit St John, Christ Church, Cheng Hoon Teng temple, etc. [12]. Apart from the historical value, the tourism industry is also focused on recreational activities such as the Melaka River Cruise, Zoo Melaka, Taman Buaya Melaka, Taming Sari Tower, Taman Botanikal Melaka, and so on [13]. The development of Malacca state is at an advanced stage and has provided job opportunities, services, and a comfortable life to the local residents while attracting the attention of various communities to concentrate in Malacca. Centralization or concentrated at Malacca city has affected the quality of environment such as water pollution in the Malacca River [15] [11] [7]. These issues and problems have had a negative impact on the local residents. An observation research had done by Hua & Kusin [10] stated that there are various human activities that carried out along the Malacca River such as agriculture, livestock, factories, commercial activities, and settlements. This situation does not only affect the tourism industry of Malacca state, but also brings harm to human and animals. Therefore, a research study should be carried out to solve this problem from continuously disrupting human

life (for example daily activities and health) and harming animals (for example extinction or poisoning).

## II. METHODS AND MATERIAL

The methodology used to carry out a research study involves the stages process, namely stage one, stage two, and stage three. These stages are further depicted in figure 1 and figure 2.

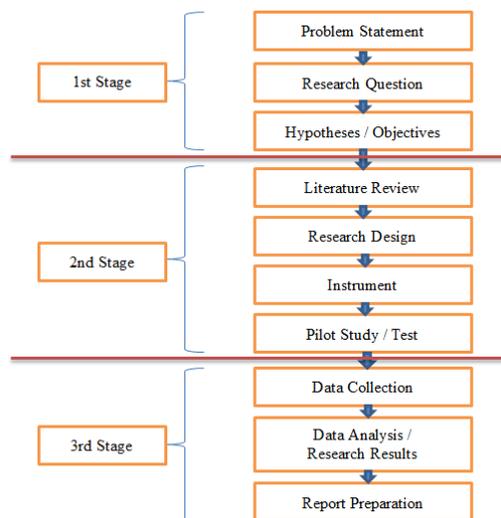


Figure 1: Stages process for research study.

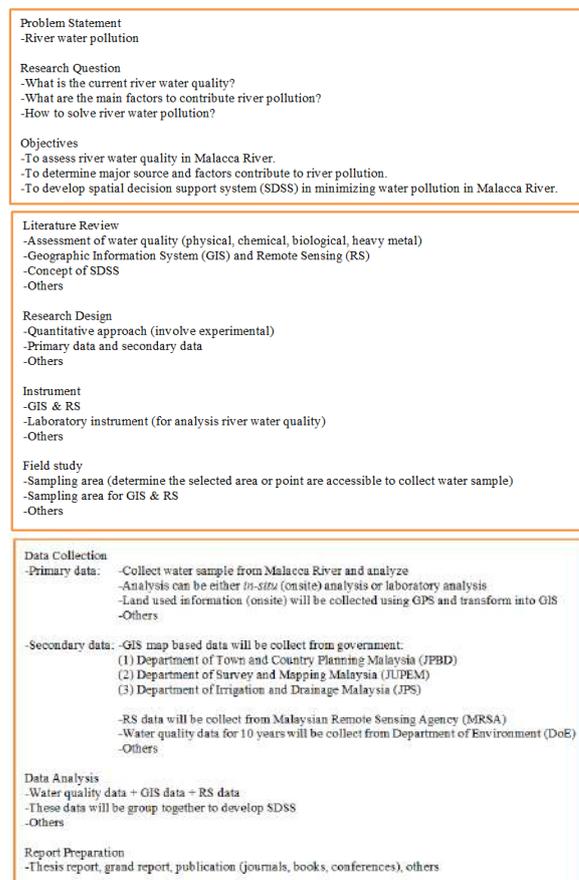


Figure 2: Stages of the process to assess water quality

### III. RESULT AND DISCUSSION

Since water quality in the Malacca River is affected by water pollution due to certain activities, a research study should be carried out to prevent and seeking solution to the river pollution from being continuously polluted. The problem statement in this research study is the river water pollution, which becomes the first step to be involved in the first stage. Since the researcher needs to find the answer for river water pollution, the first questions will be 'what is the current river water quality?' 'Is the water quality in river are slightly polluted or polluted?' 'What is the value used to determine water pollution?' 'Did the pollution value is based on physical parameters, chemical parameters, biological parameters, and heavy metal?' Next, the second questions that come to mind are 'what are the main factors to contribute river pollution?' 'Did river pollution happen naturally?' or 'Did the river pollution that happen has connection with human?' Lastly, researcher will apply critical thinking by asking 'How to solve the river water pollution?' 'Can humans stop the contributing factors of pollution?' and 'How much are the percentage of successful to implement the idea of reducing the river pollution?' Therefore, the objective of this research study is to assess river water quality (which involve physical parameters, chemical parameters, biological parameters, and heavy metals) in the Malacca River, to determine major source (refer to point source and non-point source pollution) and the factors (human activities, animals activities, natural activities) that contribute to river pollution, and to develop a spatial decision support system (SDSS) in minimizing water pollution in the Malacca River.

After the first stage is defined, the second stage will be carried out for the literature review, research design, instrument, and field study. Literature review can be explained as making references with critically and systematically on documents containing information, ideas, data and methods of obtaining information, which is relevant to the topic of research study [3]. In this research study, researcher will do refer to references of methods to assess water quality, which involve physical parameters, chemical parameters, biological parameters, and heavy metals. Generally, these four parameters will have different methods to analyze the raw water from river. For example, there are some physical parameters and chemical parameters can be analysed onsite or *in-*

*situ*, while others will need to be analyzed in the laboratory. Next, since the researcher will use tools and computer systems like GIS to help analyze large quantity of data, the researcher should know the concepts, functions, and advantages of GIS before starting the next process. It is important for the researcher to be familiar with GIS to prevent any mistakes, errors, or carelessness in collect and analyzing data, and presenting the results. At the same time, the researcher is needs to pay attention on RS, where it is a tool to help in analyzes the data that exist in image form from satellite. There are slight differences between GIS and RS, especially in collecting data, as GIS data can be in primary (researcher collect data by its own) and secondary (researcher collect data from institution) data, while RS data is only available in secondary (researcher collect data from institution) data. Also, researchers should know and understand the concept of SDSS before applying it together by using GIS and RS to seek solution and answer for minimize the river pollution. So overall, a researcher needs to study more in the literature review, especially the methods of water quality assessment, GIS and RS, and the concept of SDSS, so that the results and information provided can help achieve the objective of research study.

Typically, literature reviews are related to the research design, where the process of collecting and gathering data will be decided. This research study is involved with an experimental process, which means the data that collected and analyzed will be quantitative approach. In other words, the water sample will be collected from the site and analyzed in laboratory with a selected process (which refer as the repeating process of analysis) to provide raw data. In some cases, the raw data from the laboratory analysis for certain parameters are not considered as complete and will need to undergo calculations to provide a result before further analysis. So, the research design will use a quantitative method to collect and gather the data, which exist in primary and secondary data. Secondary data will only be collected from government, privates, or other sectors, while primary data will require researcher to collect sample from the site and analyze it to provide result data. This data will exist in large quantities and require a tool to help synchronize the data systematically. Instruments involved in this research study are GIS and RS. GIS has the ability to convert any information from hardcopy (include spatial data, water quality data, humanities data)

into softcopy or digital data in the computer and represent it in the form of map-based information. If there are any changes such as land use, the GIS are able to discover and manage the data so that the information are updated for analyze and produce new information [8]. RS has the ability to observe information about ground surface and water surface by using satellite technology and interpret it into images or pictures using electromagnetic spectrum through electromagnetic radiation [9]. Therefore, RS can be used to help determine the current situation, factors, and impacts of water pollution that occurred in the Malacca River. Also, the researcher also needs to be concerned with the laboratory instrument that require analysis of river water quality. Basically, the standard procedure and instrument used to test the water quality is based on the American Public Health Association (APHA) – Standard methods for the examination of water and wastewater [1].

Once the research design and instruments are decided, researcher has to conduct a field studies in order to collect raw data, for example the Malacca River. The selected sampling area to collect water samples from the Malacca River should be accessible and will not bring any harm and dangerous situation to researcher. Information for land use near to Malacca River will also be collect using GPS (geographical coordinates, land use profile) and information will be keyed-in GIS as soft copy. When everything is ready, the researcher can start to collect data. Secondary data can be divided into two types, which is GIS, RS, and water quality. GIS map-based data can be collected from the government sector, involving with Department of Town and Country Planning (JPBD), Department of Survey and Mapping (JUPEM), and Department of Irrigation and Drainage (JPS). Meanwhile, RS data can be collected from Malaysian Remote Sensing Agency (MRSA), and water quality data for 10 years can be collected from Department of Environment (DoE). For primary data, researcher have to collect water sample from Malacca River and analyze the sample either onsite analysis or laboratory analysis. There is some information of land use that will be collected using GPS and transform into GIS for analysis. If the map-based that receive from government department are not up-to-date, then this will be a good opportunity to re-correct the information before undergo for analysis to provide new information. Basically, the application of GIS and RS in this research study is to determine the water quality status, the factors

that contribute water pollution, impact of water pollution, and solution towards water pollution. So, the water quality data will be input into GIS and RS to assess and solve the river pollution problem. There are various analyses that can be used, for example GIS for buffering analysis, proximity analysis, overlay analysis, and reclassification analysis, and RS for spectral image analysis, decision tree image analysis, spatial image analysis, integrated image analysis, and so on. After the data are group together, the SDSS can be develop to form a new information and result, which become an answer to minimize the river pollution and achieve the research objective. The last step in third stage is to prepare the report, which can be a thesis report, grand reports, publications (in term of journals, books, and conference), and so. New information from this research study is important to other researchers to continue further research in advances for this particular field.

#### IV. CONCLUSION

A research study may be considered successful when the objective of the study is achieved with the aim to solve the problem statement. In order to achieve the objective of the study, the research should undergoes a systematic methods and steps to reduce mistake, incorrect, confusing, and carelessly, involving several stages namely first stage, second stage, and third stage. Basically, the first stage involves the problem statement, research question, and objective study; while the second stage involves the literature review, research design, instrument, and pilot study or pilot test. The third stage will involve data collection, data analysis or research results, and report preparation. It is important to conduct a pilot test or pilot study because this method will determine whether the whole process will either be correct or incorrect way to carry out the study. If there is any mistake in the process of the pilot test, changes can be made to reduce the mistakes so that they will not affect the analysis and results of the research study. Therefore, the research study entitled ‘Applied GIS in Assessment Water Quality Modeling in the Malacca River’ may be successful when the researchers follow the stages and processes systematically and answer all the objectives suggested. So, this study will become a starting point of new information for other researchers to do more deep-further research according to field of study.

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# Comparative Study of Some House-Hold Surface Active Substances

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## ABSTRACT

Present study describes how surface tension changes of some house-hold substances that we have use in our daily life? Here we use four different surface active substances such as Face wash (Neem), Hand wash (Dettol) , Dish wash (Vim) and Shampoo (Clinic Plus). Surface tension of different solutions was measured by Stalagmometer (Drop weight method). Dish wash shows lower surface tension than the other three. Low surface tension helps to better cleansing property. All (house hold) surface active substances are compared with Sodium Laryl Sulfate.

**Keywords:** Cleansing Action, Surface Tension, Surface Active Agents, Dish Wash, Face Wash, Hand Wash, Shampoo.

## I. INTRODUCTION

In continuation of our earlier study [1] on lowering of surface tension by surface active agents, we report here similar study with some different house hold surface active(washing) substances which show high cleansing property with decrease their surface tension.

In the present report, I describe the solution preparation, density measurement, measuring the surface tension and their cleansing action. It is expected that dish wash (Vim) reduced surface tension in a great extent than the other washing liquids [14-16].

Dish washing liquid is usually a high-foaming mixture of surfactants with low skin irritation, and is primarily used for hand washing of glasses, plates and cooking utensils in a sink.

This is what happens at the interface between water and a hydrophobic surface such as a plastic mixing bowl or a windshield coated with oily material.

The surface tension of water can be reduced to about one-third of its normal value by adding some soap or synthetic detergent. These substances, known

collectively as *surfactants*, are generally hydrocarbon molecules having an ionic group on one end. The ionic group, being highly polar, is strongly attracted to water molecules; we say it is *hydrophilic*.

The hydrocarbon (*hydrophobic*) portion is just the opposite; inserting it into water would break up the local hydrogen-bonding forces and is therefore energetically unfavorable. What happens, then, is that the surfactant molecules migrate to the surface with their hydrophobic ends sticking out, effectively creating a new surface. Because hydrocarbons interact only through very weak dispersion forces, this new surface has a greatly reduced surface tension.

Surfactants are substances that, when present in low concentrations, have the ability to significantly alter the surface properties of the solvent. These compounds are generally composed of lyophobic and/or lyophilic when the lyophobic group lies within the solvent it disrupts the structure of the surface, thus decreasing the free energy of the system, while the lyophilic group prevents the complete expulsion of the surfactant from the solvent.

Of the hundreds of existing surfactants, many have numerous applications. The choice of surfactant for a

specific purpose is difficult and the following information can aid in the selection of the surfactant (Rosen, 1978).

1. Characteristic features of commercially available surfactants.
2. Expected interfacial phenomenon involved and the role of the surfactant.
3. Surface chemical properties of various structural types of surfactants.

All surfactants, however, can be categorized by the charge on the surface active component into : (1) anionic, (2) cationic, (3) nonionic, and (4) zwitterionic (both positive and negative charges) .

Surfactants[11] reduce the amount of work necessary to create unit surface area, i . e . , surface tension of a solution is lowered[1] when surfactants are present. The following are a few generalizations regarding surface tension and surfactants, followed by a discussion of surfactants and dynamic surface tension.

Higher concentrations of surfactants lower the surface tension in comparison to the pure solvent state. The limiting value of surfactant concentration that produces a surface tension decrease is the critical micelle concentration [12] .

Furthermore, the steeper decrease in surface tension is evident only at high surfactant concentrations. Here we use to determine surface tension by Stalagmometer (drop volume method)[2-3, 10].

## II. METHODS AND MATERIAL

### Materials

Household substances i.e, Face wash, dish wash, hand wash and Clinic Plus Shampoo are used to determine the surface tension in aqueous solution at 20<sup>0</sup>C.

### Apparatus

- a) Stalagmometer fitted with rubber tubing and pinch cock b) Burette stand with clamp c) Burette d) Pipette e) Beaker.

### Preparation of solutions

One ml. of each surface active agent was dissolved in 30 ml. of distilled water. Then shake it rigorously and after that solutions were settled for 30 minutes. Then the experiments were performed.

### Surface tension study

Then surface tension of the above solutions was measured by Stalagmometer at 20<sup>0</sup>C.

### pH Study

pH study of the above solutions were measured with a pH meter [ Elico LI 614 pH Analyzer ].

## III. RESULTS AND DISCUSSION

### Surface tensions of common liquids

Substance	Surface tension (dyne/cm)
water (H <sub>2</sub> O)	72.7
diethyl ether (CH <sub>3</sub> -CH <sub>2</sub> ) <sub>2</sub> O	17.0
benzene C <sub>6</sub> H <sub>6</sub>	40.0
glycerin C <sub>3</sub> H <sub>2</sub> (OH) <sub>3</sub>	63.0
mercury (15°C)	487.0
<i>n</i> -octane	21.8
sodium chloride solution (6M in water)	82.5
sucrose solution (85% in water)	76.4
sodium oleate (soap) solution in water	25.0

The table shows the surface tensions of several liquids at room temperature. Note especially that

- hydrocarbons and non-polar liquids such as ether have rather low values
- one of the main functions of soaps and other surfactants is to reduce the surface tension of water
- Mercury has the highest surface tension of any liquid at room temperature. It is so high that mercury does not flow in the ordinary way, but breaks into small droplets that roll independently.

## Reference liquid: Water

Here we use reference liquid as water. At 20°C we can easily know the data of density and surface tension of water from the standard chart. Then we can calculate the value of surface tension of unknown washing liquid and shampoo solutions.

The walls of these bubbles consist of a thin layer of water molecules sandwiched between two layers of surfactant molecules. Their spherical shape is of course the result of water's surface tension. Although the surfactant (soap) initially reduces the surface tension, expansion of the bubble spreads the water into a thinner layer and spreads the surfactant molecules over a wider area decreasing their concentration. This, in turn, allows the water molecules to interact more strongly, increasing its surface tension and stabilizing the bubble as it expands.

The bright colors we see in bubbles arises from interference between light waves that are reflected back from the inner and outer surfaces, indicating that the

thickness of the water layer is comparable the range of visible light (around 400-600 nm).

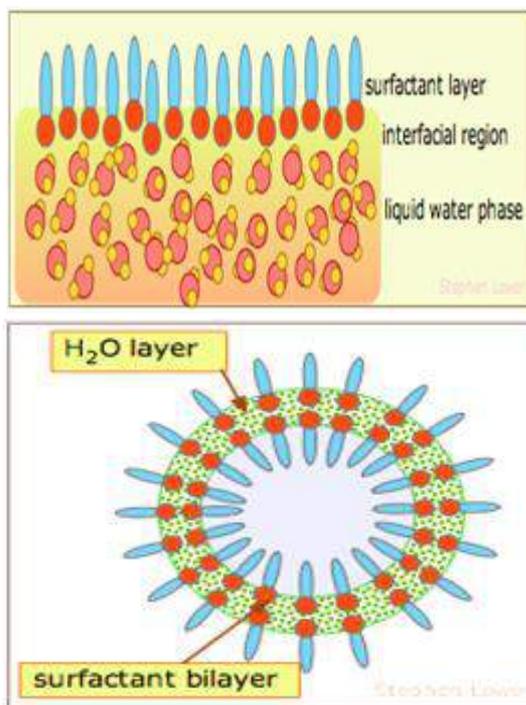


Figure 1: Surfactant reduces surface tension of liquid

Table 1. Temperature, Density and Surface tension of the reference liquid water

W A T E R	Temperature	Density(gm/cc)	Surface tension(Dyne/cm)
	20°C	0.99823	72.75

Table 2. Density of the solution measured by Sp. Gravity Bottle Weight Method

Empty Gravity Bottle( $w_1$ ) gm	Sp. Gravity Bottle + water( $w_2$ ) gm	Sp. Gravity Bottle + solution( $w_3$ ) gm		Relative Density ( $w_3-w_1/w_2-w_1$ )	Density of solution ( $w_3-w_1/w_2-w_1$ ) $\times\rho_{\text{water}}$ gm/cc
11.5090	38.8294	1. Face wash	38.8090	0.9992	0.9974
		2. Hand wash	38.7437	0.9968	0.9950
		3. Dish wash	38.7852	0.9983	0.9965
		4. Clinic plus	38.9304	1.0037	1.0019
		5.SLS(Sodium Laryl Sulphate)	38.8470	1.0006	0.9988

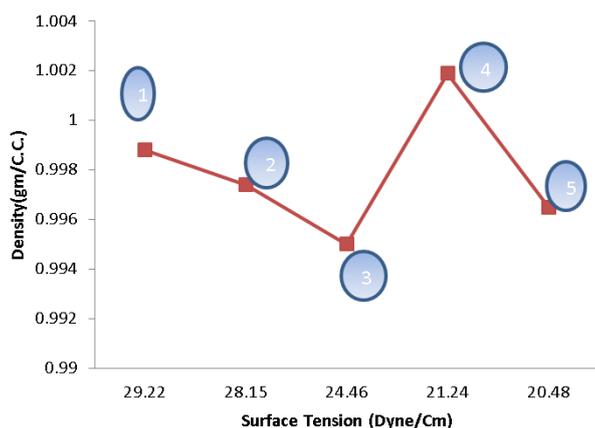
Table 3. Number of drops and measurement of surface tension with the help of reference liquid water of different washing solutions

Substances	Number of drops	Surface tension(Dyne/cm)
1. Water	55	72.75
2. Face wash(Neem)	142	28.15
3. Hand wash(Dettol)	163	24.46
4. Dish wash(Vim)	195	<b>20.48</b>
5. Clinic plus	189	21.24
6.SLS(Sodium Laryl Sulphate)	137	29.22

Table 4. Density Vs. Surface tension of different washing solutions

Substances	Density (gm/cc)	Surface tension(Dyne/cm)
1. Water	0.99823	72.75
2. Face wash(Neem)	0.9974	28.15
3. Hand wash(Dettol)	0.9950	24.46
4. Dish wash(Vim)	0.9965	<b>20.48</b>
5. Clinic plus	1.0019	21.24
6.SLS(Sodium Laryl Sulphate)	0.9988	29.22

Density Vs Surface Tension Plot



Where 1. SLS(Sodium Laryl Sulphate), 2. Face Wash, 3. Hand Wash. 4. Clinic Plus, 5. Dish Wash

Figure 2 : Variation of Surface Tension with Density.

From Table 1, shows the data of reference liquid of water at 200C.

From Table 2, it has been found that different solution shows different density value. Density of the shampoo solution and washing solutions were measured by weight of the Sp. Gravity Bottle weight method with the known density value of water at 200C. Clinic Plus shampoo shows greater density than the other household washing solutions. But surface tension of dish wash is to some extent less than that of Clinic Plus Shampoo.

This experiment was carried out by different washing liquids, liquid shampoo and they were standardized with standard surface active agent i.e, SLS(Sodium Laryl Sulphate). Then we determined the original density of the washing solutions and liquid shampoo with the help of specific gravity bottle method. The trends of the densities are as follows:  $d_{\text{Clinic Plus}} > d_{\text{SLS}} > d_{\text{Face Wash}} > d_{\text{Dish wash}} > d_{\text{Hand Wash}}$ . So, density trend and surface tension are not same, i.e, to some extent different.

This experiment was further carried out by (22ml water+5ml Bengene+0.02ml solution) this solution. Here we observed that the trend of the densities of these solution were same as above.

We test the additivity rule for these solutions to check the densities of the solutions. Here we also found that the density patterns of the solutions are same as above. So, additivity rule helps us to determine the density of these washing solutions.

Table 3 shows the number of drops and measurement of surface tension (with the help of reference liquid water) of washing solutions and different shampoo solutions. With increase the number of drops of the corresponding solutions, decrease the surface tension of those solutions. These are the surfactants which reduce the surface tension i.e, support the basic properties of surface tension of liquid.

Table 4 shows Density and Surface tension of different washing solutions and shampoo solution.

With increase the number of drops of the corresponding solutions, decrease the surface tension of those solutions.

Table 5 shows that pH value of standard surface active substances (SLS) is not so high than that of other washing substances as well as potential decreases. It is a better indication that house hold surface active substances that we choosing here are more or less same pH ranges i.e, nearly neutral pH range substances which helps us low skin irritation.

Fig. 1 shows reduction of surface tension with the help of surface active agents, i.e, the surfactant molecules migrate to the surface with their hydrophobic ends sticking out, effectively creating a new surface. Because hydrocarbons interact only through very weak dispersion

forces, this new surface has a greatly reduced surface tension.

Fig. 2 shows the Surface tension vs. Density plot of different household substances i.e, washing liquids and shampoo solution at 200C. With increase the number of drops of the corresponding solutions, decrease the surface tension of those solutions.

In case of solutions of long chain fatty acid and its salts, higher member alcohols, alkyl sulphonates, long chain quarternary amine and their salts, higher member esters etc. where the hydrophobic part contains a chain containing more than 8 to 10 carbon atoms. Such solutes due to the presence of long hydrophobic chain tendency to remain in the bulk phase. They remain mainly at the surface while the lower member analogues prefer to remain at the surface but have considerable bulk phase concentration also.

Here we found that all the washing liquids and shampoo solution act as surfactants depending on the increase of surface area. These findings support the basic properties of surface tension of liquid.

#### IV. CONCLUSION

This study tried to compare different household surface active substances. All are very essential in our daily life. These surface active agents protect us in every space of our life. So, from our study it has been found that dish wash act as a good surfactant at 200C with increase their surface area than other washing liquids even shampoo solutions. Here also, it was observed that surface tension of water can be reduced to about 1/3.5 of its normal value by adding surfactants i.e, dish wash (Vim). This study will also helpful for consultant who easily differentiate between different house hold surface active substances with this easy method. We already showed our earlier studies that the surface tension of water can be reduced to about 1/3 of its normal value by adding surfactants i.e, Clinic Plus shampoo.

#### V. ACKNOWLEDGEMENTS

The author is thankful to the Department of Chemistry, A.K.P.C. Mahavidyalaya for all types of necessary support.

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# Case Study on Project Performance of Cast in-Situ Bridge

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## ABSTRACT

The present study proposed an improving procedure of the bridge's upper structure, which focuses on the feature of cast in-situ bridge construction management. After applied the improving procedure, the progress days reached in advance was about 23.8%. The costs of redesign construction procedure was only about half of the original design. There is no serious occupational injuries occur during the project period. The quality and customer's satisfaction were all reached relatively high levels. These results showed that the improving procedure which proposed by present study is validity.

**Keywords:** Construction Management, Project Performance, Cast in-situ Bridge.

## I. INTRODUCTION

Bridge is one of the most important elements of transportation system. The selection of bridge construction methods need to consider many criteria simultaneously, such as construction time (schedule), cost, safety, quality, in-situ conditions, structure of bridge, and legal regulations. Determining an appropriate alternative encompasses is a complex trade-off process which requires all the decision criteria to be considered. Today, there are many bridge construction methods have been developed by engineers (Trayner, 2007). Furthermore, the engineers still effort to develop new bridge construction methods for specific field conditions.

Generally, there are two main kinds of bridge construction methods in Taiwan: cast in-situ and pre-cast. Further, there are five cast in-situ construction methods were wildly used in Taiwan: full-span launching (FSL), advancing shoring bridge construction (ASBC), balanced field cast cantilevered (BFCC), incremental launching method (IL), and field cast cantilever construction (FCCC).

FSL used heavy-duty carrier and launching equipment to launch the pre-cast box-girder onto the piers (Fig.1). The

girders with different length are allocated carefully to avoid the conflicts of the pier locations against existing roads and immovable obstacles. Also, the settlement of reinforcement cage prefabrication yards and pre-cast production plants can facilitate better control of quality and schedule easily.

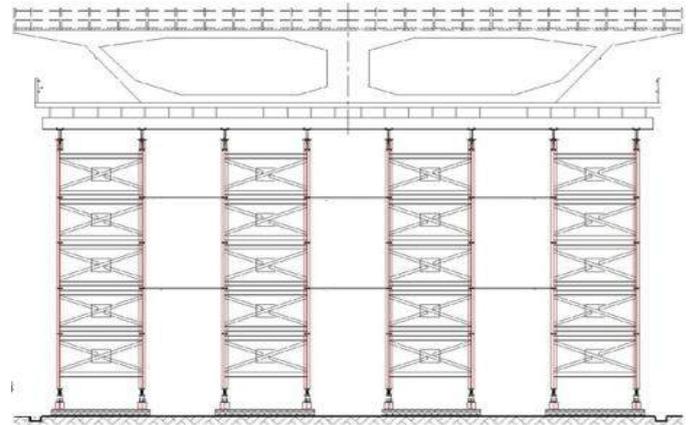


Figure 1: The illustration of FSL

ASBC assembles moldboard on the forward-movable main girder and finishes the process of steel bar binding, pre-stressed steel cable placement, internal moldboard installation, and concrete casting (Fig.2). Pre-stressed pulling will be applied when the concrete reach the

necessary strength to finish the construction of one span. Then the propelling device on the supporting rack will be applied to push the main supporting steel girder and advance the moldboard to the next span.



**Figure 2:** The illustration of ASBC

BFCC is chosen where a bridge has few spans which range from 50 to 250m (Fig.3). Construction begins at each bridge pier. Special formwork is positioned and cast-in-situ pier segment is begun. The complete pier segment is then used as an erection platform and launching base for all subsequent travelling formwork and concrete segment construction. Segment construction is continued until a joining midpoint is reached where a balanced pair is closed. Stability of the end cantilever is maintained by using temporary pier supports as the end span is begun.



**Figure 3:** The illustration of BFCC

By using IL, the bridge superstructure is assembled on one side of the obstacle to be crossed and then pushed longitudinally (launched) into its final position (Fig.4). The launching is typically performed in a series of

increments so that additional sections can be added to the rear of the superstructure unit prior to subsequent launches. The launching method has also been applied to tied-arch or truss spans, although these are fully assembled prior to launching.



**Figure 4:** The illustration of IL

FCCC used cantilever work car to coordinate the suspension template and anchorage system in high-tensile steel bars to fix the pre-cast concrete block section. Employed elevate and floating way to advance the pre-cast upper structure section by section. Therefore, it must first set up the stigma facilities for the base of the cantilever work car.



**Figure 5:** The illustration of FCCC

Unfortunately, the bridge designer might lack of ascertain of the exact field situations or practice experience. The original design might not suitable for the field situations to construct the bridge. Therefore, the present study aimed to enhance the project performance of cast in-situ bridge construction methods. Meanwhile, the occupational injury was also considered.

## II. BRIDGE CONSTRUCTION MANAGEMENT

Generally, the scope of bridge construction management includes: technical, cost, time, safety, and quality management. Furthermore, the customer's satisfaction is one of the most important criteria of the project performance.

### A. Technical Management

Technical management is an effective identification, selection, acquisition, development, development and protection of product-related technologies to maintain or strengthen their market position and operating performance (Ramazani and Jergeas, 2015; Walker, 2015). The technical management usually includes task, content, and management System.

### B. Cost Management

Cost management is to reduce engineering costs and increase profits of the project. It is promoting the successful completion of construction enterprises play an important role in the construction tasks (Budayan, et al., 2015; Walker, 2015).

### C. Time Management

Time (schedule) management is to ensure that the construction contractor to achieve the duration of the guarantee commitment. Therefore, bridge construction project in demand forecast should be prepared to work throughout the project, good progress plan by drawing construction network diagram, rod-shaped diagram and other methods. The construction schedule by week, month, or year to quantify, rational use of project construction the schedule, based on the actual duration of the project and construction of resource supply and other factors (Doloi, 2015).

### D. Safety Management

Because of the particularity and complexity of bridge construction, the losses of the accidents are more serious than other construction tasks. Therefore, safety management plays a decisive role in the bridge project management. There are several ways to prevent accidents: enhance staff in-situ safety and health education and training, to strengthen the management of construction machinery equipment (Shen, et al., 2015), to develop effective preventive measures, and enhance construction safety management.

## E. Quality Management

The quality management of the bridge project includes the early stage and the construction process of the project. The quality management in the early stage usually includes: check the blueprint, in-situ control, organization control of the construction units, and measurement data control. The quality management during the project generally includes: critical paths and special schedule quality control, defects construction, used new materials, employed new technology, and used new equipment. Especially focus on the hidden works. These construction quality problems occur easily, should pay more attention to develop appropriate quality assurance measures.

## III. BRIDGE PROJECT PERFORMANCE

Earned value analysis (EVA) is an approach which integrated three reference categories, scope, time (schedule), cost, etc., in order to measure the overall performance of the project. Simultaneously, EVA used the cost performance index (CPI) and the schedule performance index (SPI) to predict the progress of the project execution costs, early warning, avoid until near the end of the stage the project only to find risk.

## IV. IMPROVING PROCEDURE

Figure 6 shows the improving procedure of the present study.

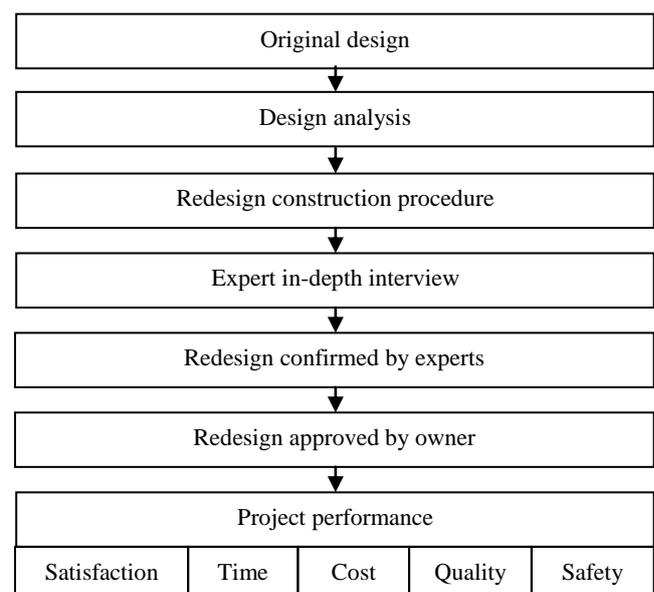


Figure 6: Improving procedure of the present study

## V. IN-DEPTH INTERVIEWED

Table I showed the profile of the in-depth interviewed experts in present study.

Table I

THE PROFILE OF THE IN-DEPTH INTERVIEWED EXPERTS IN PRESENT STUDY

No.	Type	Title	Experience	Main duty
01	owner	owner and CEO	30 years	decision-making and execution
02	manager	associate manager	22 years	technical guidance and safety plan
03	manager	finance manager	20 years	finance
04	manager	engineer manager	26 years	in-situ services and construction management
05	manager	engineer manager	18 years	equipment management and scheduling
06	manager	engineer associate manager	23 years	in-situ services and construction management
07	manager	senior engineer	18 years	in-situ services and construction management
08	manager	senior commissioner	17 years	purchase
09	manager	Senior foreman	25 years	construction management
10	owner	owner	13 years	decision-making
11	manager	manager	22 years	technical guidance and safety plan
12	manager	in-situ director	20 years	in-situ services and construction management
13	owner	owner	13 years	decision-making
14	manager	manager	18 years	construction management
15	scholar	professor	26 years	teaching and research
16	scholar and manager	associate professor and general manager	36 years	teaching, research, and decision-making
17	scholar	assist professor	16 years	teaching and research

## VI. CASE STATEMENT

### A. Project Position

The project located is at green island marina recreation area in Mirs bay park, Pingtung County, Taiwan.

### B. Project Scope

Table II shows the scopes (construction contents) of the case bridge.

Table II

THE CONSTRUCTION CONTENTS OF THE CASE BRIDGE

No.	Items	Contents
1	Flat road	Lanes: 2 car, 1 person, and 1 bicycle, 1 pro access road of car, and lane side; Wide: 25m- 34m.
2	Bridge	Main lane: long: 4K+977-5K+556; wide: 30m.
3	Miscellaneous	Marking, semaphore, flag (frame), and the controller.
4	Water conservation	Lane side ditch, culverts box and pipe.
5	Geotechnical	Earthwork, culverts, and retaining wall.
6	Landscape	Sidewalk, bicycle path, and planting.
7	Illumination	Lamp post, switch box, and the wire pipe.
9	Building	Control room, engine room, power room, and water drainage system.

Figure 7 and 8 shows the outward appearance of the case bridge.



Figure 7: Outward appearance of the case bridge (close gate)



Figure 8: Outward appearance of the case bridge (open gate)

Figure 9(a) to 9(f) shows the main construction stage 1 to stage 6 of the case bridge.

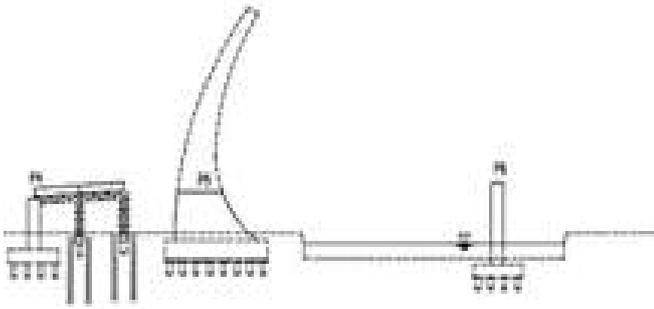


Figure 9(a): Stage 1: construction the bridge pier and tower

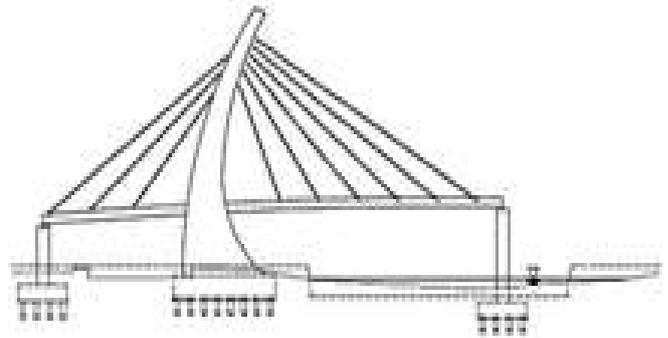


Figure 9(f): Stage 6: removed temporary support

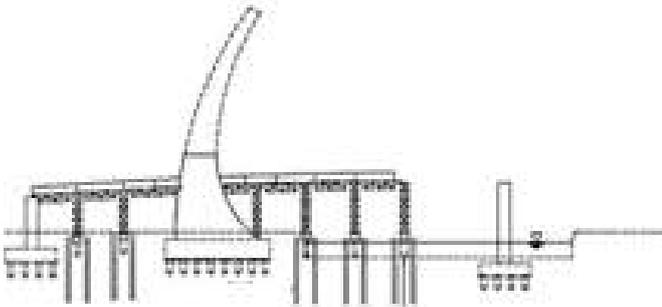


Figure 9(b): Stage 2: construction the main upper structure

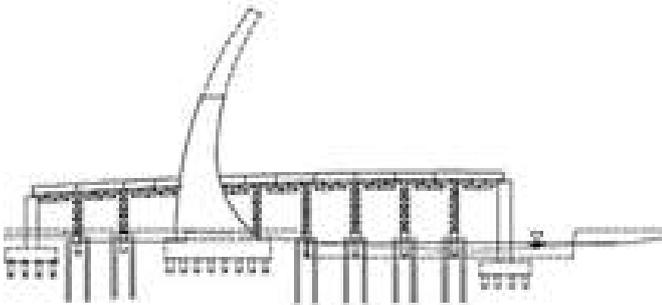


Figure 9(c): Stage 3: construction the box beam of upper structure

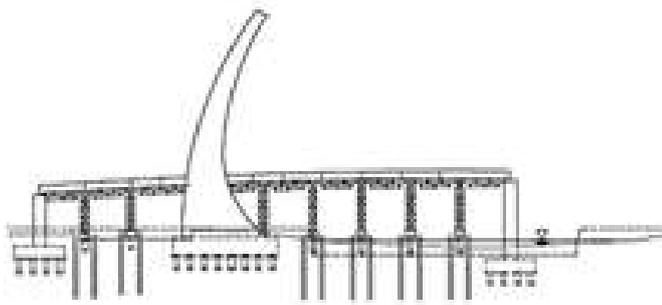


Figure 9(d): Stage 4: finished the bridge tower

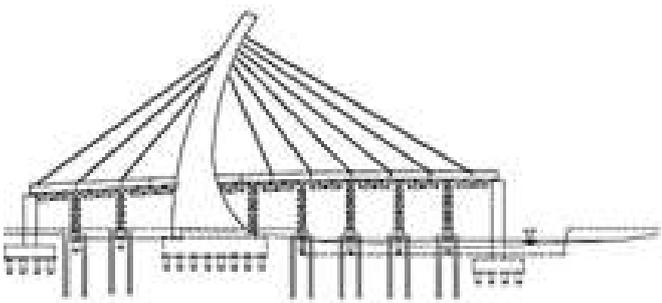


Figure 9(e): Stage 5: finished the steel cable installation

## VII. REVIEW AND REDESIGN THE UPPER STRUCTURE

After design analysis the original design the upper structure of the case bridge. The engineers found many waste design due to the designer might lack of ascertain of the exact field situation. Therefore, the engineering managers redesigned the construction procedure and contents. The redesign was confirmed by the experts and approved the owner. Table III shows the engineering content compared of the original design and redesign of the case bridge.

Table III

COMPARED OF ORIGINAL DESIGN AND REDESIGN

No.	Original design	No.	Redesign
1	setup and remove of 9m steel plate	1	setup and remove of 9m steel plate
2	setup and remove of temporary bridge	2	setup and remove of worker cover board
3	set up and remove of temporary bridge	3	setup and remove of type H 800 * 300 * 14/26 steel plate
4	pile foundation of $\phi$ 150cm * 20m full pipe	4	setup and remove of type H 400 * 400 * 13/21 steel bar
5	remove full pipe pile foundation $\phi$ 150cm * 6m	5	shipment of worker cover board, steel plate, and type H steel bar
6	production and remove of 25m * 3m * 2.5m pile cap	6	earthwork
7	earthwork		

## VIII. RESULTS

The project results were described regard with time, cost, safety, quality, and customer's satisfaction.

## A. Time

The case data is the pre-cast engineering, which is the upper structure of the cast in-situ bridge project. The total schedule days of the pre-cast engineering are 662 calendar days. However, the actual finished are 504 days, the project schedule reached in advance about 23.8%.

## B. Cost

The costs data of the original design and redesign were shown in Table IV and V.

Table IV

THE BUDGET OF THE ORIGINAL DESIGN

No.	Items	unit	Q.	Price	Total
1	setup and remove of 9m steel plate	m	140	5,600	784,000
2	setup and remove of temporary bridge	m <sup>2</sup>	1,350	6,000	8,100,000
3	setup and remove of temporary bridge	st	6	1,250,000	7,500,000
4	pile foundation of $\phi$ 150cm * 20m full pipe	st	36	294,640	10,607,040
5	remove full pipe pile foundation $\phi$ 150cm * 6m	st	36	36,000	1,296,000
6	production and remove of 25m * 3m * 2.5m pile cap	st	6	1,059,088	6,354,528
7	earthwork	m <sup>3</sup>	7,226	262	1,893,212
Total					36,534,780

Table V

THE ACTUAL COST OF THE REDESIGN

No.	Items	unit	Q.	Price	Total
1	setup and remove of 9m steel plate	m	320	5,600	1,792,000
2	setup and remove of worker cover board	m <sup>2</sup>	1,500	900	1,350,000
3	setup and remove of type H 800 * 300 * 14/26 steel plate	m	750	2,226	1,669,500
4	setup and remove of type H 400 * 400 * 13/21 steel bar	m	4,480	2,025	9,072,000
5	shipment of worker cover board, steel plate, and type H steel bar	st	1	1,170,000	1,170,000
6	earthwork	m <sup>3</sup>	12,432	262	3,257,184
Total					18,310,680

The differences of the two design were the setup and remove of steel plate, H steel bar, amount of concrete, setup and remove of temporary bridge, and full pipe pile foundation. The costs of redesign was about half of the original design.

The major difference of the redesign was the correct demand forecasting through technology management tools, good progress plan, reasonable arrangement project construction schedule, for the actual duration of the construction project supply of resources and other factors, arrangements for project construction progress convergence and effective cost control.

## C. Safety

There is no serious occupational injuries occur during the project period. The frequency and the loss of the minor occupational injuries were lower than the average of the industrials.

## D. Quality

The case bridge is still unshakable after the Sudi Le typhoon (the strongest typhoon in 2015) at Aug 8, 2015. Though typhoon resulted in more than 400 millions houses were power out, more than 10 bridges were damaged, 6 people were dead, 4 people were missing, and 379 people were injured.

## E. Customer's Satisfaction

The owner is very satisfied with the project performance.

## IX. CONCLUSION

The total schedule days of the case were 662 calendar days and the actual finished days after redesign were 504, the project schedule reached in advance about 23.8%. The costs of redesign was only about half of the original design. There is no serious occupational injuries occur during the project period. The quality and customer's satisfaction were all reached relatively high levels. These results showed that the improving procedure which proposed by present study is validity.

There results also indicated that familiar with design and related construction sequence, construction method, technical measures, construction progress and the schedule of the in-situ construction requirements, and clear identified difficulties to complete the construction project are the major ways to improve the project performance.

## X. REFERENCES

Furthermore, coordination is a critical technique to perform the construction preparation, to clearly explain the requirements of the project time, quality, and safety to workers, and teaching the construction method to workers. Therefore, coordination technique is the most important task for engineering managers to complete the construction tasks to achieve the project goals.

In addition, correct forecast of workload demand, good plan of schedule and reasonable arrangement schedule, simultaneous operation of facilities, design safety construction procedure, and efficiency used owned heavy machinery are the must have abilities of the engineering managers.

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# Solving Short Term Multi Chain Hydrothermal Scheduling Problem by Artificial Bee Colony Algorithm

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## ABSTRACT

This paper presents an artificial bee colony algorithm for solving optimal short term hydrothermal scheduling problem. To demonstrate the effectiveness of the proposed algorithm, hydrothermal test system consists of three thermal units and four cascaded hydro power plants has been tested. The valve point loading effect is taken into consideration. In order to show the feasibility and robustness of the proposed algorithm, a wide range of thermal and hydraulic constraints are taken into consideration. The numerical results obtained by ABC algorithm are compared with those obtained from other methods such as genetic algorithm (GA), simulated annealing (SA), evolutionary programming (EP) and constriction factor based particle swarm optimization (CFPSO) technique to reveal the validity and verify the feasibility of the proposed method. The experimental results indicate that the proposed algorithm can obtain better schedule results with minimum execution time when compared to other methods.

**Keywords:** Hydrothermal Generation Scheduling, Artificial Bee Colony, Valve Point Loading Effect

## I. INTRODUCTION

The hydrothermal scheduling problem is a non linear programming problem including a non linear objective function and subjected to a mixture of linear and non linear operational constraints. Since the operating cost of hydro electric power plant is very low compared to the operating cost of thermal power plant, the integrated operation of the hydro and thermal plants in the same grid has become the more economical [1]. The primary objective of the short term hydrothermal scheduling problem is to determine the optimal generation schedule of the thermal and hydro units to minimize the total operation cost of the system over the scheduling time horizon subjected to a variety of thermal and hydraulic constraints. The hydrothermal generation scheduling is mainly concerned with both hydro unit scheduling and thermal unit dispatching. Since there is no fuel cost associated with the hydro power generation, the problem of minimizing the total production cost of hydrothermal scheduling problem is achieved by minimizing the fuel cost of thermal power plants under the various constraints of the system [2]. Several mathematical optimization techniques have been used to solve short

term hydrothermal scheduling problems [3]. In the past, hydrothermal scheduling problem is solved using classical mathematical optimization methods such as dynamic programming method [4-5], lagrangian relaxation method [6-7], mixed integer programming [8], interior point method [9], gradient search method and Newton raphson method [2]. In these conventional methods simplified assumptions are made in order to make the optimization problem more tractable. Thus, most of conventional optimization techniques are unable to produce optimal or near optimal solution of this kind of problems. The execution time of these methods increases with the increase of the dimensionality of the problem. The most common optimization techniques based upon artificial intelligence concepts such as evolutionary programming [10-11], simulated annealing [12-13], differential evolution [14], artificial neural network [15-16], genetic algorithm [17 -19], particle swarm optimization [20-23], bacterial foraging algorithm [24] and artificial bee colony algorithm [25-27] have been given attention by many researchers due to their ability to find an almost global or near global optimal solution for short term hydrothermal scheduling problems under various operating constraints of the

system. The ABC algorithm is a population based optimization technique proposed by Devis Karaboga in 2005. It mimics the intelligent behaviour of honey bees. In ABC algorithm, the colony of artificial bee consists of three groups of bees: employed bees associated with specific food sources, onlooker bees watching the dance of employed bees within the hive to choose a food source and scout bees searching for food sources randomly. Both scouts and onlookers are also called unemployed bees. The first half of the colony consists of the employed artificial bees and the second half includes the onlookers. Compared to other evolutionary computation techniques, the ABC algorithm is simple and robust and can solve optimization problems quickly with high quality solution and stable convergence characteristic.

## II. Objective Function and Operational Constraints

The main objective of short term hydro thermal scheduling problem is to minimize the total fuel cost of thermal power plants over the optimization period while satisfying all thermal and hydraulic constraints. The objective function to be minimized can be represented as follows:

$$FT = \sum_{t=1}^T \sum_{i=1}^N nt F_1^t(P_{gi}^t) \quad (1)$$

In general, the fuel cost function of thermal generating unit  $i$  at time interval  $t$  can be expressed as a quadratic function of real power generation as follows:

$$F_1^t(P_{gi}^t) = a_i(P_{gi}^t)^2 + b_i P_{gi}^t + c_i \quad (2)$$

Where  $P_{gi}^t$  is the real output power of thermal generating unit  $i$  at time interval  $t$  in (MW),  $F_1^t(P_{gi}^t)$  is the operating fuel cost of thermal unit  $i$  in (\$/hr),  $FT$  is the total fuel cost of the system in (\$),  $T$  is the total number of time intervals for the scheduling horizon,  $nt$  is the numbers of hours in scheduling time interval  $t$ ,  $N$  is the total number of thermal generating units,  $a_i, b_i$  and  $c_i$  are the fuel cost coefficients of thermal generating unit  $i$ .

By taking the valve point effects of thermal units into consideration, the fuel cost function of thermal power plant can be modified as:

$$F_{i,v}^t(P_{gi}^t) = a_i(P_{gi}^t)^2 + b_i P_{gi}^t + c_i + \left| e_i \times \sin(f_i \times (P_{gi}^{\min} - P_{gi}^t)) \right| \quad (3)$$

Where  $F_{i,v}^t(P_{gi}^t)$  is the fuel cost function of thermal unit  $i$  including the valve point loading effect and  $f_i, e_i$  are the fuel cost coefficients of generating unit  $i$  with valve point loading effect.

The minimization of the objective function of short term hydrothermal scheduling problem is subject to a number of thermal and hydraulic constraints. These constraints include the following:

1) Real Power Balance Constraint: The total active power generation from the hydro and thermal plants must be equal to the total load demand plus transmission line losses at each time interval over the scheduling period.

$$\sum_{i=1}^N P_{gi}^t + \sum_{j=1}^M P_{hj}^t = P_D^t + P_L^t \quad (4)$$

Where,  $P_D^t$  is the total load demand during the time interval  $t$  in (MW),  $P_{hj}^t$  is the power generation of hydro unit  $j$  at time interval  $t$  in (MW),  $P_{gi}^t$  is the power generation of thermal generating unit  $i$  at time interval  $t$  in (MW),  $M$  is the number of hydro units and  $P_L^t$  represents the total transmission line losses during the time interval  $t$  in (MW). For simplicity, the transmission power loss is neglected in this paper.

2) Thermal Generator Limit Constraint: The inequality constraint for each thermal generator can be expressed as:

$$P_{gi}^{\min} \leq P_{gi}^t \leq P_{gi}^{\max} \quad (5)$$

Where  $P_{gi}^{\min}$  and  $P_{gi}^{\max}$  are the minimum and maximum power outputs of thermal generating unit  $i$  in (MW), respectively.

3) Hydro Generator Limit Constraint: The inequality constraint for each hydro unit can be defined as:

$$P_{hj}^{\min} \leq P_{hj}^t \leq P_{hj}^{\max} \quad (6)$$

Where  $P_{hj}^{\min}$  and  $P_{hj}^{\max}$  are the minimum and maximum power generation of hydro unit  $j$  in (MW), respectively.

4) Reservoir Storage Volume Constraint:

$$V_{hj}^{\min} \leq V_{hj}^t \leq V_{hj}^{\max} \quad (7)$$

Where  $V_{hj}^{\min}$  and  $V_{hj}^{\max}$  are the minimum and maximum storage volume of reservoir j, respectively.

5) Water Discharge Rate Limit Constraint:

$$q_{hj}^{\min} \leq q_{hj}^t \leq q_{hj}^{\max} \quad (8)$$

Where  $q_{hj}^{\min}$  and  $q_{hj}^{\max}$  are the minimum and maximum water discharge rate of reservoir j, respectively

6) Initial and Final Reservoir Storage Volume Constraint:

This constraint implies that the desired volume of water to be discharged by each reservoir over the scheduling period should be in limit.

$$V_{hj}^0 = V_{hj}^{\text{begin}} = V_{hj}^{\max} \quad (9)$$

$$V_{hj}^T = V_{hj}^{\text{end}} \quad (10)$$

Where  $V_{hj}^{\text{begin}}$  and  $V_{hj}^{\text{end}}$  are the initial and final storage volumes of reservoir j, respectively.

7) Water Dynamic Balance Constraint: The water continuity equation can be represented as:

$$V_{hj}^t = V_{hj}^{t-1} + I_{hj}^t - q_{hj}^t - S_{hj}^t + \sum_{u=1}^{R_{uj}} (q_u^{t-\tau_{uj}} + S_u^{t-\tau_{uj}}) \quad (11)$$

Where  $I_{hj}^t$  is water inflow rate of reservoir j at time interval t,  $S_{hj}^t$  is the spillage from reservoir j at time interval t,  $\tau_{uj}$  is the water transport delay from reservoir u to reservoir j and  $R_{uj}$  is the number of upstream hydro reservoirs directly above the reservoir j.

8) Hydro Plant Power Generation Characteristic: The hydro power generation can be represented by the following equation:

$$P_{hj}^t = C_{1j}(V_{hj}^t)^2 + C_{2j}(q_{hj}^t)^2 + C_{3j}(V_{hj}^t)(q_{hj}^t) + C_{4j}(V_{hj}^t) + C_{5j}(q_{hj}^t) + C_{6j} \quad (12)$$

Where  $C_{1j}$ ,  $C_{2j}$ ,  $C_{3j}$ ,  $C_{4j}$ ,  $C_{5j}$  and  $C_{6j}$  are the Power generation coefficients of hydro generating unit j

### III. Overview of ABC Algorithm

Artificial bee colony (ABC) is one of the most popular swarm intelligence algorithms for solving constrained and unconstrained optimization problems. It was first developed by Karaboga in 2005, inspired intelligent behaviors of real honey bee colonies [25]. The algorithm simulates the intelligent foraging behaviour of honey bees to achieve global optimum solutions for different optimization problems. The foraging behaviour of bees is to collect nectar from food sources around the hive in nature. In ABC algorithm, the position of food sources represents a possible candidate solution to the optimization problem, and the nectar amount of a food source corresponding to the profitability of associated solution. In ABC algorithm, the number of employed bees is equal to the number of food sources existing around the hive. If an employed bee could not improve the self solution in a certain time, it becomes a scout bee and the main purpose of which is to increase search ability of the ABC algorithm. The scout bees carry out a random search process for discovering new food sources. Compared to the other swarm based algorithms, the ABC algorithm has become very popular and it is widely used, because of its good convergence properties.

### IV. Search Mechanism of ABC Algorithm

The colony of artificial bees consists of two groups of bees called employed bees and unemployed bees. The unemployed bees consist of onlookers and scouts. The main steps of the ABC algorithm are explained as follow:

1) Initialize a randomly food source positions by the following equation:

$$X_{ij} = X_j^{\min} + r \times (X_j^{\max} - X_j^{\min}) \quad (13)$$

$X_i = \{x_{i1}, x_{i2}, \dots, x_{iD}\}$ ,  $i = 1, 2, \dots, N_s$ ,  $j = 1, 2, \dots, D$  Where  $N_s$  is the number of food sources;  $D$  is the number of decision variables.  $r$  is uniformly distributed random value between  $[0, 1]$ ,  $X_j^{\min}$  and  $X_j^{\max}$  are the lower and upper bounds of the decision variable  $j$ , respectively. After initialization, the population of the food sources (solutions) is subjected to repeated cycles of the search process of the employed bees, the onlooker bees and the scout bees. Then the fitness of each food source is calculated.

2) Each employed bee searches the neighborhood of its current food source to determine a new food source using Equation (14):

$$V_{ij} = X_{ij} + \phi_{ij} \times (X_{ij} - X_{kj}) \quad (14)$$

Where:

$k \in \{1, 2, \dots, N_s\}$ ,  $j \in \{1, 2, \dots, D\}$  are randomly chosen indexes. It must be noted that  $k$  has to be different from  $i$ .  $\phi_{ij}$  is a uniformly distributed real random number between  $[-1, 1]$ , it controls the production of neighbor food sources around  $X_{ij}$  and represents the comparison of two food positions visually by the bee.

If the new food source position produced by Equation (14) which exceeds their boundary values, it can be set as follow:

$$\text{If } X_i > X_i^{\max} \text{ then } X_i = X_i^{\max}$$

$$\text{If } X_i < X_i^{\min} \text{ then } X_i = X_i^{\min}$$

3) After generating the new food source, the nectar amount of food sources will be evaluated and a greedy selection will be performed. If the quality of the new food source is better than the current position, the employed bee leaves its current position and moves to the new food source, otherwise, the bee keeps the current position in the memory.

4) The onlooker bee chooses a food source by the nectar information shared by the employed bee, the probability of selecting the food source  $i$  is calculated by the following equation:

$$P_i = \frac{fit_i}{\sum_{j=1}^{NS} fit_j} \quad (15)$$

After selecting a food source, the onlooker generates a new food source by using equation (14). Once the new food source is generated it will be evaluated and a greedy selection will be applied same as the case of employed bees.

5) If the solution represented by a food source position cannot be enhanced for a predetermined number of trials (called limit), the food source is abandoned and the employed bee associated with that food source becomes a scout. The scout generates a new food source randomly using the following equation:

$$V_{ij} = X_j^{\min} + r \times (X_j^{\max} - X_j^{\min}) \quad (16)$$

Where  $j \in \{1, 2, \dots, D\}$

6) If the termination criterion is satisfied (maximum number of cycles), the process is stopped and the best food source is reported; otherwise the algorithm returns to step 2.

The schematic diagram shows the mechanism search of ABC algorithm is illustrated in “Fig. 1”.

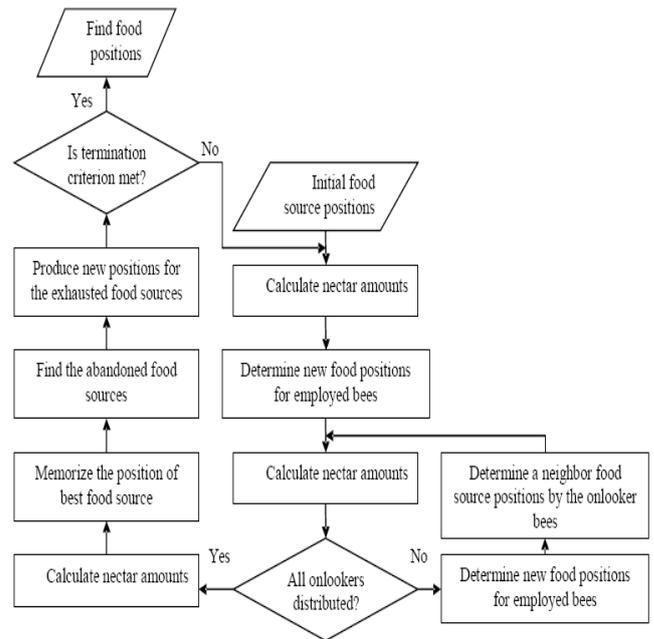


Figure 1: Schematic outline of ABC algorithm

## V. ABC Optimization for Short term Hydrothermal Scheduling Problem

The artificial bee colony algorithm for solving short term hydrothermal scheduling problem is described as follow:

### A. Construction of Solutions

In the initialization process, a set of food source positions are created at random. In this paper, the construction of solution for short term hydro thermal scheduling problem is composed of a set of elements which represent the water discharge rate of each reservoir and the power generation of thermal units over the whole scheduling period. Thus, the structure of solution is defined as follows:

$$X = \begin{bmatrix} q_{h1}^0 & q_{h2}^0 & \dots & q_{hj}^0 & P_{g1}^0 & P_{g2}^0 & \dots & P_{gi}^0 \\ q_{h1}^1 & q_{h2}^1 & \dots & q_{hj}^1 & P_{g1}^1 & P_{g2}^1 & \dots & P_{gi}^1 \\ M & M & O & M & M & M & O & M \\ q_{h1}^T & q_{h2}^T & \dots & q_{hj}^T & P_{g1}^T & P_{g2}^T & \dots & P_{gi}^T \end{bmatrix} \quad \text{--- (17)}$$

In the initialization process, the ABC algorithm generated the initial solutions by using the Equations (18) and (19) defined below.

$$P_{gi}^t = P_{gi}^{\min} + r_g \times (P_{gi}^{\max} - P_{gi}^{\min}) \quad (18)$$

$$q_{hj}^t = q_{hj}^{\min} + r_h \times (q_{hj}^{\max} - q_{hj}^{\min}) \quad (19)$$

Where  $r_g$  and  $r_h$  are uniformly distributed random real numbers in the range  $[0, 1]$ ;  $i = 1, 2, \dots, N$ ,  $j = 1, 2, \dots, M$  and  $t = 1, 2, \dots, T$

The feasible candidate solution of each element must be initialized within the feasible range.

The elements  $p_{gi}^t$  and  $p_{hj}^t$  are the output power generation of thermal unit  $i$  during the time interval  $t$  and the water discharge rate of hydro unit  $j$  at time interval  $t$ , respectively. The range of the elements  $p_{gi}^t$  and  $p_{hj}^t$  should satisfy the generating capacity limits of thermal generators and the water discharge rate constraints.

If any food source position is not satisfy the constraints, then the position of the food source is fixed to its minimum and maximum operating limits as follows:

$$P_{gi}^t = \begin{cases} P_{gi}^t & \text{if } P_{gi}^{\min} \leq P_{gi}^t \leq P_{gi}^{\max} \\ P_{gi}^{\min} & \text{if } P_{gi}^t \leq P_{gi}^{\min} \\ P_{gi}^{\max} & \text{if } P_{gi}^t \geq P_{gi}^{\max} \end{cases} \quad \text{----- (20)}$$

$$q_{hj}^t = \begin{cases} q_{hj}^t & \text{if } q_{hj}^{\min} \leq q_{hj}^t \leq q_{hj}^{\max} \\ q_{hj}^{\min} & \text{if } q_{hj}^t \leq q_{hj}^{\min} \\ q_{hj}^{\max} & \text{if } q_{hj}^t \geq q_{hj}^{\max} \end{cases} \quad \text{----- (21)}$$

$$V_{hj}^t = \begin{cases} V_{hj}^t & \text{if } V_{hj}^{\min} \leq V_{hj}^t \leq V_{hj}^{\max} \\ V_{hj}^{\min} & \text{if } V_{hj}^t \leq V_{hj}^{\min} \\ V_{hj}^{\max} & \text{if } V_{hj}^t \geq V_{hj}^{\max} \end{cases} \quad \text{----- (22)}$$

### B. Evaluation of Fitness of Solutions:

Evaluate the fitness value of each food source position corresponding to the employed bees in the colony using the objective function described in Equation (1).

### C. Modification of Food source positions by Employed Bees:

Each employed bee produces a new food source position by using equation (14). The modified position is then checked for constraints defined in Equations (5) and (8). If the new solutions violate the constraints, they are set

according to Equations (20) and (21). Then compute the fitness value of the new food source positions using Equation (1). The fitness of the modified position is compared with the fitness of the old position. If the new fitness is better than the old fitness, the employed bee memorized the new position and forgets the old one; otherwise, the employed bee keeps the old solution.

### D. Sending the Onlooker Bees for Selected Positions and Evaluate Fitness:

Place the onlooker bees on the food sources with the nectar information shared by employed bees. Each onlooker bee chooses a food source based on the probability described in Equation (15). Onlooker bees search the new food sources in the neighborhood with the same method as employed bees.

### E. Modification of Food Source Positions by Onlooker Bees:

The onlooker bees produce a modification on the position in its memory using Equation (14). Check the inequality constraints of the new positions. If the resulting value violates the constraint, they are set to the extreme limits. Then check the fitness of the candidate food source positions. If the new food source position is equal or better fitness than the old one, it replaces with the old one in the memory; otherwise, the old one is retained in the memory.

### F. Abandon Sources Exploited by the Bees:

If the solution representing a food source is not improved by a certain number of trials, then that food source is abandoned and the employed bee will be changed into a scout. The scout randomly produces a new food solution by using Equations (18) and (19). Then, evaluate the fitness of the new solutions and compares it with the old one. If the new solution is better than the old solution, it is replaced with the old one. Otherwise, the old one is retained in the memory.

### G. Check the Termination Criterion:

The proposed ABC algorithm is stopped if the cycle is equal to the maximum cycle number (MCN).

## VI. Case Study and Simulation Results

The proposed algorithm has been tested on hydrothermal power system comprises of a multi chain cascade of four hydro units and three thermal units to verify the feasibility and effectiveness of the proposed ABC

algorithm. The effect of valve point loading has been taken into consideration to illustrate the robustness of the proposed method. The transport time delay between cascaded reservoirs is also considered in this case study. The scheduling time period is one day with 24 intervals of one hour each. The data of test system are taken from [16]. The configuration of multi chain hydro sub system is shown in “Fig. 2”. The water time transport delays between connected reservoirs are given in Table 1.

The hydro power generation coefficients are given in Table 2. The reservoir storage limits, discharge rate limits, initial and final reservoir storage volume conditions and the generation limits of hydro power plants are shown in Table 3. Table 4 shows the reservoir inflows of hydro power plants. The fuel cost coefficients and power output limits of thermal units are given in Table 5. The load demand over the 24 hours is given in Table 6. The control parameters of ABC algorithm are given in Table 7.

The optimal solution obtained from ABC algorithm is achieved in 50 trial runs. The optimal hourly hydrothermal generation schedule and hourly total fuel cost obtained by the ABC algorithm is shown in Table 8. Table 9 shows the optimal hourly water discharge of hydro power plants obtained from the ABC algorithm while Table 10 presents the optimal hourly storage volumes of hydro reservoirs obtained from the ABC algorithm.

The proposed algorithm has been implemented in MATLAB language and executed on an Intel Core i3, 2.27 GHz personal computer with a 3.0 GB of RAM.

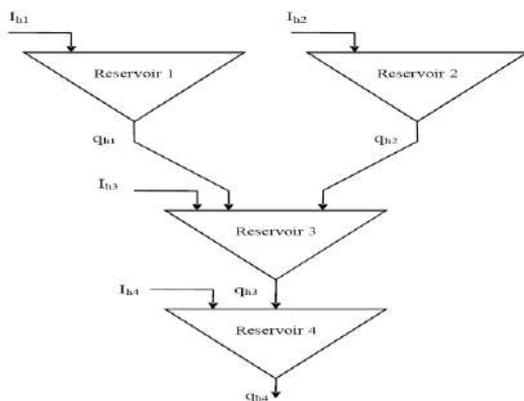


Figure 2: Multi chain hydro sub system networks

TABLE I  
WATER TIME TRANSPORT DELAYS BETWEEN CONNECTED RESERVOIRS

Plant	1	2	3	4
$R_u$	0	0	2	1
$\tau_u$	2	3	4	0

$R_u$  : Number of upstream hydro power plants  
 $\tau_u$  : Time delay to immediate downstream hydro power plant

TABLE II  
HYDRO POWER GENERATION COEFFICIENTS

Plant	$C_1$	$C_2$	$C_3$	$C_4$	$C_5$	$C_6$
1	-0.0042	-0.4200	0.0300	0.9000	10.000	-50.000
2	-0.0040	-0.3000	0.0150	1.1400	9.5000	-70.000
3	-0.0016	-0.3000	0.0140	0.5500	5.5000	-40.000
4	-0.0030	-0.3100	0.0270	1.4400	14.000	-90.000

TABLE III  
RESERVOIR STORAGE CAPACITY LIMITS, PLANT DISCHARGE LIMITS, PLANT GENERATION LIMITS AND RESERVOIR END CONDITIONS ( $\times 10^4 m^3$ )

Plant	$V_h^{\min}$	$V_h^{\max}$	$V_h^{\text{ini}}$	$V_h^{\text{end}}$	$q_h^{\min}$	$q_h^{\max}$	$P_h^{\min}$	$P_h^{\max}$
1	80	150	100	120	5	15	0	500
2	60	120	80	70	6	15	0	500
3	100	240	170	170	10	30	0	500
4	70	160	120	140	13	25	0	500

TABLE IV  
RESERVOIR INFLOWS OF MULTI CHAIN HYDRO PLANTS ( $\times 10^4 m^3$ )

Hour	Reservoir				Hour	Reservoir			
	1	2	3	4		1	2	3	4
1	10	8	8.1	2.8	13	11	8	4	0
2	9	8	8.2	2.4	14	12	9	3	0
3	8	9	4	1.6	15	11	9	3	0
4	7	9	2	0	16	10	8	2	0
5	6	8	3	0	17	9	7	2	0
6	7	7	4	0	18	8	6	2	0
7	8	6	3	0	19	7	7	1	0
8	9	7	2	0	20	6	8	1	0
9	10	8	1	0	21	7	9	2	0
10	11	9	1	0	22	8	9	2	0
11	12	9	1	0	23	9	8	1	0
12	10	8	2	0	24	10	8	0	0

TABLE V  
FUEL COST COEFFICIENTS AND OPERATING LIMITS OF THERMAL UNITS

Unit	$a_i$	$b_i$	$c_i$	$e_i$	$f_i$	$P_{gi}^{\min}$	$P_{gi}^{\max}$
1	0.0012	2.45	100	160	0.038	20	175
2	0.0010	2.32	120	180	0.037	40	300
3	0.0015	2.10	150	200	0.035	50	500

TABLE VIII  
LOAD DEMAND FOR 24 HOUR

Hour	P <sub>D</sub> (MW)						
1	750	7	950	13	1110	19	1070
2	780	8	1010	14	1030	20	1050
3	700	9	1090	15	1010	21	910
4	650	10	1080	16	1060	22	860
5	670	11	1100	17	1050	23	850
6	800	12	1150	18	1120	24	800

TABLE VII  
CONTROL PARAMETERS OF ABC ALGORITHM

ABC algorithm parameters	Value
Colony size (N <sub>p</sub> )	50
Number of food sources (N <sub>s</sub> )	25
Number of employed bees	25
Number of onlookers	25
Maximum cycle number (MCN)	300
Limit value	100

TABLE VIII  
HOURLY OPTIMAL HYDROTHERMAL GENERATION SCHEDULE USING ABC ALGORITHM

Hour	Thermal generation (MW)			Hydro generation (MW)			
	P <sub>g1</sub>	P <sub>g2</sub>	P <sub>g3</sub>	P <sub>h1</sub>	P <sub>h2</sub>	P <sub>h3</sub>	P <sub>h4</sub>
1	102.4411	181.2670	50.0000	73.5131	62.5056	53.8689	226.4043
2	22.1315	126.5600	174.2240	95.0352	55.7258	42.0007	264.3229
3	46.0797	133.2205	140.7144	55.6823	67.3702	00.0000	256.9329
4	20.0000	115.9043	86.1655	68.7149	82.1708	39.6308	237.4138
5	157.3638	40.0000	50.0000	90.1544	75.9778	00.0000	256.5040
6	112.1576	46.8703	226.4150	69.9569	58.6992	46.2835	239.6174
7	65.8606	209.7137	230.4331	52.1114	74.6900	47.6980	269.4933
8	129.3525	211.7074	231.0256	68.6976	46.2139	39.6663	283.3367
9	103.2970	210.0890	352.9299	59.5113	58.6124	51.4951	254.0652
10	103.8887	214.0891	311.2150	64.9068	65.5284	24.2415	296.1305
11	112.9518	212.7889	322.0855	85.1607	43.3682	53.4024	270.2423
12	102.2374	294.5060	324.2013	80.1515	39.6928	53.7267	255.4843
13	174.9917	289.5292	229.0650	89.3898	48.5825	49.0964	229.3454
14	101.1384	293.7887	212.6150	92.7409	43.7653	54.1977	231.7539
15	45.1796	210.6628	320.3588	96.1910	53.4461	53.8540	230.3078
16	127.0743	210.0301	319.3423	55.0720	64.9931	56.2501	227.2381
17	173.9267	208.9086	227.9670	85.5090	49.8735	56.3430	247.4722
18	172.0041	293.4674	229.1950	98.5474	39.9007	59.0776	227.8078
19	100.4064	207.9049	317.0150	81.9545	45.9833	40.7904	275.9455
20	175.0000	208.8229	229.0754	61.5574	46.8905	46.6797	281.9741
21	25.0439	210.1011	230.1837	74.5008	58.9527	54.8876	256.3302
22	76.0859	124.3536	229.2102	68.0906	52.6797	53.5424	256.0375
23	20.0000	147.0406	230.3625	99.3709	47.9393	58.6357	246.6510
24	103.3851	126.2126	140.6553	66.8475	50.1730	57.0228	255.7036

TABLE IX  
HOURLY HYDRO PLANT DISCHARGE USING ABC ALGORITHM

Hour	Hydro plant discharges ( $\times 10^4 \text{ m}^3/\text{hr}$ )			
	Q <sub>h1</sub>	Q <sub>h2</sub>	Q <sub>h3</sub>	Q <sub>h4</sub>
1	7.6477	8.0963	16.3437	14.0836
2	12.8578	6.8962	19.2194	18.9513
3	5.3388	8.8177	25.6760	16.0064
4	6.9983	13.0735	15.8037	13.4801
5	11.5135	12.0182	24.9377	14.4783
6	7.4305	8.3711	11.4678	13.0000
7	5.0000	14.7109	10.5282	17.2684
8	7.0322	7.3416	18.0360	19.7492
9	5.7130	10.0189	14.4034	15.6409
10	6.2676	11.9777	21.8457	23.4767
11	9.1414	6.9553	12.6409	19.3910
12	8.2567	6.1849	13.7150	17.7008
13	9.7922	7.5317	17.0259	13.8451
14	10.3885	6.4754	15.3728	13.9507
15	11.1753	7.9140	16.0444	13.5071
16	5.0000	10.6610	14.7236	13.0000
17	8.8969	7.7224	15.3980	15.2572
18	11.6836	6.1585	13.4430	13.0000
19	8.4627	7.1085	20.9065	18.8872
20	5.7406	7.0701	19.3231	20.1202
21	7.3458	9.0879	16.7212	16.0635
22	6.4966	7.7739	17.5217	15.7994
23	12.0324	6.8722	14.9395	14.6374
24	6.3383	7.1300	15.7040	15.7211

TABLE X  
HOURLY STORAGE VOLUME OF HYDRO RESERVOIRS USING ABC ALGORITHM

Hour	Reservoir storage volume ( $\times 10^4 \text{ m}^3$ )				Total fuel cost (\$/hr)
	V <sub>h1</sub>	V <sub>h2</sub>	V <sub>h3</sub>	V <sub>h4</sub>	
1	100.0000	80.0000	170.0000	120.0000	0
2	102.3523	79.9037	161.7563	125.0601	353.81
3	98.4945	81.0075	150.7369	127.7282	356.62
4	101.1557	81.1898	129.0609	138.9978	332.43
5	101.1574	77.1163	115.2572	141.3214	143.54
6	95.6439	73.0981	109.0635	151.7802	128.25
7	95.2134	71.7270	121.3497	148.7231	146.50
8	98.2132	63.0161	127.9780	141.9829	179.53
9	100.1812	62.6745	132.0138	140.2696	196.18
10	104.4682	60.6556	142.1421	139.0321	228.57
11	109.2006	60.0000	137.0980	137.4011	207.02
12	112.0592	62.0447	145.1680	130.6510	213.21
13	113.8025	63.8598	147.8268	126.6652	227.81
14	115.0103	64.3281	150.5328	129.8460	224.63
15	116.6218	66.8527	156.4053	131.2680	203.31
16	116.4465	67.9387	159.4576	133.8053	198.91
17	121.4464	65.2777	161.1756	135.5289	218.51
18	121.5496	64.5553	165.1015	135.6696	200.51
19	117.8660	64.3968	170.5224	136.1126	224.31
20	116.4033	64.2883	169.7052	138.1319	201.41
21	117.4127	65.8682	167.0431	137.3348	199.42
22	117.8669	66.7803	168.9412	137.9925	156.32
23	119.3703	68.0064	171.2616	139.7149	158.82
24	116.3383	69.1342	172.8933	140.0171	148.92
24	120.0000	70.0000	170.0000	140.0000	289.12

In order to verify and validate the effectiveness of the proposed algorithm, its simulation results will be compared with those obtained from the SA, EP, GA and CFPSO technique. Table 11 shows the comparison of total fuel cost and execution time of the proposed algorithm among other methods. From Table 11, it is clear that the ABC algorithm performs better than SA, EP, GA and CFPSO technique in terms of total fuel cost and execution time. "Fig. 3" shows the hourly thermal plant power generation by using proposed method, the hourly hydro plant power generation by using proposed algorithm is given in "Fig. 4", the hourly hydro plant discharges using ABC algorithm are shown in "Fig. 5" and "Fig. 6" presents the hourly reservoir storage volumes using proposed technique.

TABLE XI

COMPARISON OF TOTAL FUEL COST AND COMPUTATION TIME OF THE PROPOSED TECHNIQUE AMONG SA, EP, GA AND CFPSO METHODS

Method	Total fuel cost (\$)	CPU Time (Sec)
ABC	42909.009	79.16
SA [22]	45466.000	246.19
EP [22]	47306.000	9879.45
CFPSO [23]	44925.620	183.64
GA [23]	45392.009	198.57

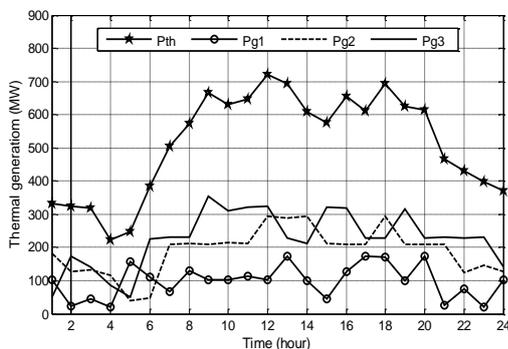


Figure 3: Hourly thermal plant power generation using ABC algorithm

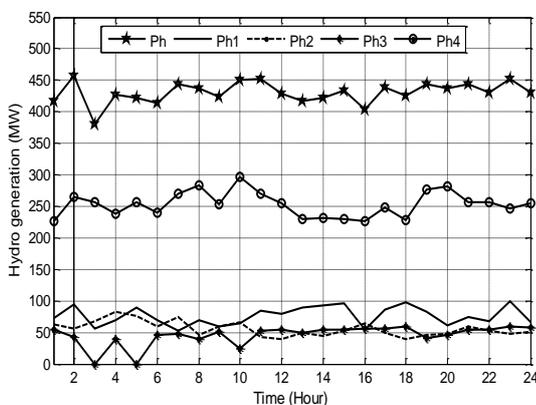


Figure 4: Hourly hydro plant power generation using ABC algorithm

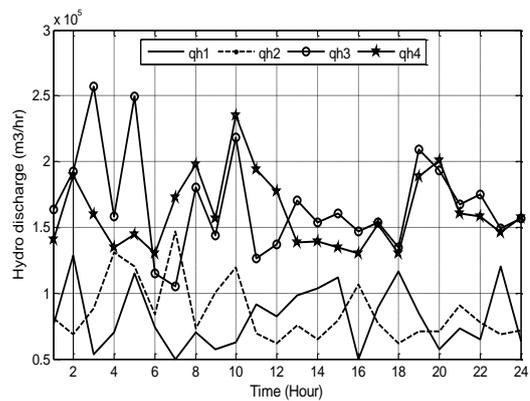


Figure 5: Hourly hydro plant discharges using ABC algorithm

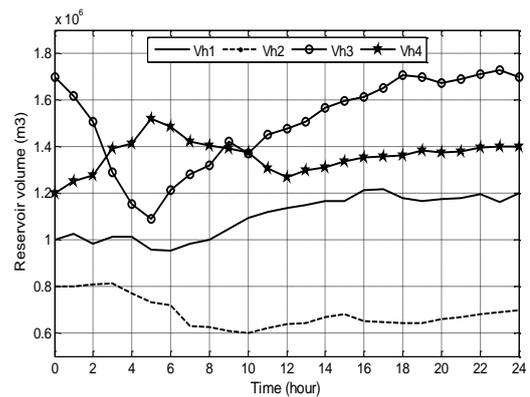


Figure 6: Hourly hydro reservoir storage volumes using ABC algorithm

## VII. CONCLUSION

In this paper, an artificial bee colony (ABC) algorithm has been developed to solve the short term hydrothermal generation scheduling problem. To demonstrate the feasibility and performance efficiency of the proposed algorithm, a multi chain hydrothermal system was tested. The effect of valve point loading is considered in this case study to verify the robustness of the proposed technique. The numerical results indicate that the ABC algorithm can obtain better schedule results with minimum generation cost and lower execution time when compared with other evolutionary algorithms such as SA, EP, GA and CFPSO technique.

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# Viscometric, Volumetric and Thermodynamic Studies L-Alanine in Aqueous and Ethanol Media at 293 – 313 K

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## ABSTRACT

Valuable information regarding solute – solvent, solute – solute and solvent – solvent interactions are obtainable from volumetric, viscometric and other thermodynamic data in a solute solvent system. Consequently we study the density and viscosity of L-Alanine in aqueous and ethanol media as a function of concentration at the temperature ranges between 293 and 313 K. The apparent molar volume ( $\phi_v$ ) of the amino acid is linearly related to its concentration in the two media. Apparent molar volume at infinite dilution ( $\phi_v^o$ ) were estimated from least square fit of  $\phi_v$  vs molarity. Viscosity coefficients A and B were calculated from the viscosity data Jones–Dole equation. The activation parameters for viscous flow  $\Delta\mu_1^\ddagger$ ,  $\Delta\mu_2^\ddagger$ ,  $\Delta S_1^\ddagger$  and  $\Delta H_1^\ddagger$  were also estimated using Eyring equation. The data are explained vis-à-vis structure making or breaking effect of L-Alanine at the temperature under consideration.

**Key words:** L-Alanine; ethanol; apparent molar volume; infinite dilution; structure breaking and structure making

## I. INTRODUCTION

The physicochemical properties of solutes in solutions provide valuable information on solute – solvent, solute – solute and solvent – solvent interactions. When applied to proteins, information that are important in understanding their stability and several other biochemical and physiological processes in a living cell are obtainable (Pal *et al.*, 2009; Zhao, 2006; Zhuo *et al.*, 2005; Banipal *et al.*, 2009; Nain, 2013; Thirumaran and Imban, 2011).

Proteins are large complex molecules with high difficulty in direct study of their interactions with solvents. Therefore, one useful approach is to investigate interactions of the model compounds of proteins, i.e. amino acids, in aqueous or mixed solvent system. The extensive studies of volumetric properties of amino acids in various solvent media can act as effective probes of their confirmation and properties in

solutions (Wadi *et al.*, 1992; Banipal *et al.*, 2004; Yan *et al.*, 2001).

L-Alanine, a nonessential  $\alpha$ -amino acid is manufactured by the human body and does not need to be obtained directly through the diet. It is manufactured in the liver from other amino acids. It is one of the most widely used amino acids in the “fabrication” of proteins, reinforcing their flexibility, fibre-strength and resilience. It is readily converted to glucose when blood sugar levels fall. Life style, work place and dietary are major sources of getting alcohol absorbs into the body system, with effects depending on amount absorbed. When mixed with body fluids can affect the conformation, transportation and the products of their reactions. In this study we desired to study L-Alanine in aqueous and ethanol system under different conditions to see whether it has no net effect on water and ethanol structures either by enhancing or breaking their structure.

## II. METHODS AND MATERIAL

L-alanine (98.5 %) and Ethanol (99.8 %) were from BDH, London. L-Alanine was recrystallized in water + ethanol and dried in desiccator for 24 hours prior to use. Water for the solutions was doubly distilled. The densities were measured by using a  $25 \pm 0.01$  ml density bottle. The viscosities were measured by the Ubbelohde viscometer (Calibrated CUC (9721-K50) Series) from Cannon Instrument with sufficiently long efflux time to avoid kinetic energy correction. The time of flow was recorded by electronic digital clock capable of reading up to 0.01 s. A METTLER PM-200 electronic balance with accuracy of 0.0001 g was used for weighing. The temperature was controlled by a thermostatic water bath fluctuating to  $\pm 0.1$  °C.

## III. RESULTS AND DISCUSSION

The values of densities ( $\rho$ ) and viscosities ( $\eta$ ), obtained for various concentrations of L-Alanine in water and ethanol at  $T = (293.15, 298.15, 303.15, 308.15, \text{ and } 313.15)$  K are listed in Tables 1 and 2. The apparent molar volumes, ( $\varphi_v$ ) were calculated from  $\rho$  data using the following relation:

$$\varphi_v = M/\rho - 1000(\rho_0 - \rho)/\rho_0\rho m \quad (1)$$

where  $\rho$  and  $\rho_0$  are the densities of solution and solvent (water or ethanol), respectively,  $m$  is the concentration of the solute (L-Alanine) in molality and  $M$  is its molar mass of the solute.

The apparent molar volume at infinite dilution ( $\varphi_v^\circ$ ) was obtained using a least-square fit to the linear plots of  $\varphi_v$  vs  $m$  using the Masson equation (Masson, 1929):

$$\varphi_v = \varphi_v^\circ + S_v \sqrt{m} \quad (2)$$

where  $S_v$  is the experimental slope, also known as the volumetric pairwise interaction coefficient (Desnoyers, 1982; Hedwig, 1991). The values of the limiting apparent molar volume,  $\varphi_v^\circ$  and the volumetric pairwise interaction coefficient,  $S_v$  are also presented for L-Alanine in ethanol and water are as shown in Tables 1 and 2. There are slight variations in the values of  $\varphi_v$  and  $\varphi_v^\circ$  as the temperature varies. The positive

values of  $\varphi_v^\circ$  (Tables 1 and 2) suggest the presence of strong solute – solvent interactions in these media throughout the L-Alanine concentrations and temperatures under investigations. The variation of  $\varphi_v^\circ$  with molar concentration of L-Alanine can be explained with co-sphere overlap model (Gurney 1953). This model assumes that hydrophilic – ionic group interaction resulted in positive contribution to  $\varphi_v^\circ$  and that negative contribution is due to hydrophilic-hydrophobic interactions. The zwitterionic forms of amino acids (i.e.  $-\text{NH}_3^+$ ,  $\text{COO}^-$ ) as well as their side chains play significant roles in their interaction with solvents ion hence a positive value of  $\Delta\varphi_v^\circ$  (Banipal *et al.*, 2009, Nain 2009). The trends are also presented in Figures 1 and 2.

**Table 1:** The density ( $\rho$ ), viscosity ( $\eta$ ), partial molar volume ( $\varphi_v$ ) apparent molar volume ( $\varphi_v^\circ$ ) and pairwise coefficient ( $S_v$ ) of L-Alanine in Water at various concentration and temperature.

Temp /K	molality. / (mol kg <sup>-1</sup> )	$\rho$ / (g cm <sup>-3</sup> )	$\eta$ / (Ns m <sup>-1</sup> )	$\varphi_v$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$\varphi_v^\circ$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$S_v$ / (cm <sup>3</sup> dm <sup>3/2</sup> mol <sup>-1/2</sup> )
293	0.000	0.998			61.104	-2.786
	0.050	1.000	1.0148	60.341	60.483	
	0.099	1.001	1.0500	60.226	60.227	
	0.148	1.002	1.0500	60.141	60.031	
	0.197	1.004	1.0407	59.999	59.867	
	0.484	1.012	1.0611	59.068	59.166	
298	0.000	0.997			60.342	-1.155
	0.050	0.999	1.0585	59.772	60.085	
	0.099	1.000	1.0245	60.179	59.978	
	0.148	1.001	1.0094	60.053	59.897	
	0.197	1.003	1.0981	59.919	59.829	
	0.483	1.011	1.1226	59.405	59.539	
303	0.000	0.996			61.000	-2.727
	0.050	0.997	1.1104	60.279	60.392	
	0.099	0.999	1.1083	60.166	60.142	
	0.148	1.000	1.0833	60.028	59.951	
	0.197	1.001	1.0729	59.866	59.790	
	0.483	1.010	1.1646	59.036	59.105	
308	0.000	0.999			61.096	-2.510
	0.050	1.001	1.0224	60.468	60.535	
	0.099	1.002	1.0306	60.305	60.305	
	0.149	1.004	1.0306	60.165	60.128	
	0.197	1.005	1.0469	60.064	59.980	
	0.484	1.013	1.0755	59.299	59.348	
313	0.000	0.992			60.951	-2.652
	0.049	0.994	1.0319	60.260	60.362	
	0.099	0.995	1.0298	60.161	60.119	
	0.147	0.996	1.0277	59.944	59.933	
	0.196	0.998	1.0213	59.877	59.777	
	0.481	1.006	1.0787	59.046	59.113	

**Table 2:** The density ( $\rho$ ), viscosity ( $\eta$ ), partial molar volume ( $\varphi_v$ ) apparent molar volume ( $\varphi_v^\circ$ ), pairwise coefficient ( $S_v$ ) and transfer volume,  $\Delta\varphi_v^\circ$  of L-Alanine in ethanol at various concentrations and temperature.

Temp /K	molality / (mol kg <sup>-1</sup> )	$\rho$ / (g cm <sup>-3</sup> )	$\eta$ / (Ns m <sup>-2</sup> )	$\phi_v$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$\phi_v^o$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$S_v$ / (cm <sup>3</sup> dm <sup>3/2</sup> mol <sup>-1</sup> )
293	0.000	0.785			59.324	-1.700
	0.039	0.787	1.225	58.681	58.988	
	0.078	0.788	1.203	59.167	58.850	
	0.116	0.789	1.165	58.751	58.744	
	0.155	0.790	1.143	58.746	58.656	
	0.377	0.798	1.176	58.173	58.281	
298	0.000	0.781			60.238	-3.882
	0.039	0.782	1.140	59.265	59.472	
	0.077	0.784	1.061	59.285	59.157	
	0.116	0.785	1.073	59.012	58.917	
	0.154	0.786	1.044	58.780	58.716	
	0.375	0.794	1.044	57.762	57.862	
303	0.000	0.777			60.307	-3.849
	0.039	0.778	1.091	59.404	59.550	
	0.077	0.779	1.008	59.331	59.239	
	0.115	0.781	1.063	58.947	59.001	
	0.153	0.782	1.077	58.973	58.803	
	0.372	0.789	1.056	57.856	57.958	
308	0.000	0.790			50.453	-5.062
	0.039	0.791	1.064	49.292	49.449	
	0.078	0.793	1.036	49.168	49.036	
	0.117	0.794	1.035	48.846	48.721	
	0.156	0.796	1.051	48.426	48.457	
	0.380	0.805	1.035	47.296	47.332	
313	0.000	0.772			48.404	-5.432
	0.038	0.774	1.353	47.157	46.900	
	0.077	0.776	1.073	47.024	46.565	
	0.115	0.777	1.066	46.679	46.285	
	0.152	0.779	1.056	46.229	45.092	
	0.372	0.788	1.062	45.015	48.404	

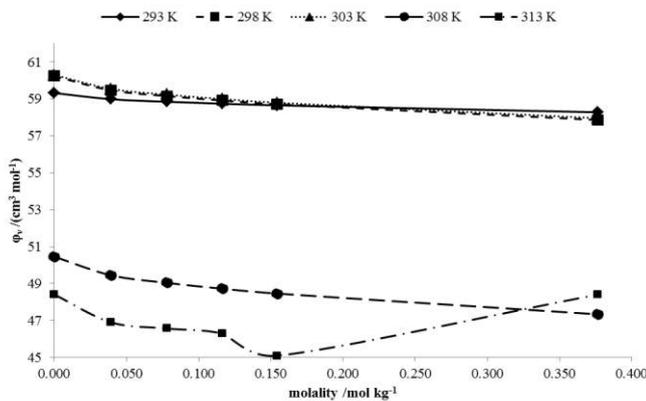


Figure 1: Partial molar volume vs concentration for Ethanol- Alanine system.

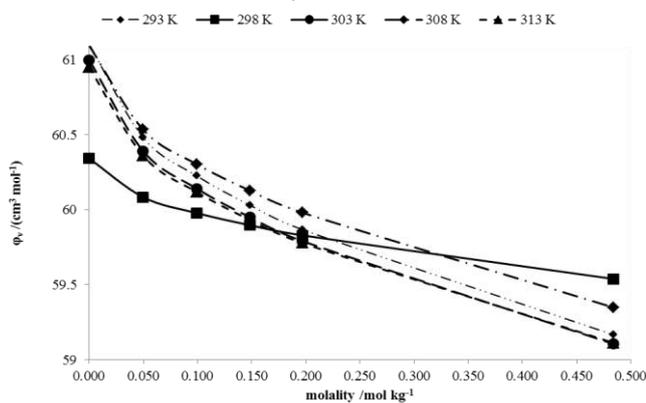


Figure 2: Partial molar volume vs concentration for Water - Alanine system

The negative values of  $S_v$  obtained for L-Alanine in the two solvents suggested weak solute – solute or ion ion interactions.  $\phi_v^o$ , the intercept, which is the limiting apparent molar volume of the solute, provides information concerning solute – solvent interactions. Tables 1 and 2 also revealed increasing density of L-Alanine with increasing concentrations; this is also a

confirmation of attraction between the solute and solvent molecules. The temperature dependence of  $\phi_v^o$  was according to the relation in equation 3 below:

$$\phi_v^o = a_1 + a_2T + a_3T^2 \quad (3)$$

Figures 3 and 4 also depict the variations in the limiting apparent molar volume with temperature.

Table 3: Values of coefficients  $a_i$  for L-Alanine in water and ethanol

Conc (mol dm <sup>-3</sup> )	Coefficient of expansion Alanine – Ethanol system			Coefficient of expansion (Alanine – water system)		
	$a_1$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$a_2$ / (cm <sup>3</sup> mol <sup>-1</sup> K <sup>-1</sup> )	$a_3$ / (cm <sup>3</sup> mol <sup>-1</sup> K <sup>-2</sup> )	$a_1$ / (cm <sup>3</sup> mol <sup>-1</sup> )	$a_2$ / (cm <sup>3</sup> mol <sup>-1</sup> K <sup>-1</sup> )	$a_3$ / (cm <sup>3</sup> mol <sup>-1</sup> K <sup>-2</sup> )
0.05	162.90	-0.69	0.0012	-3858.30	26.50	-0.04
0.10	49.22	0.07	-0.0001	-3599.25	24.82	-0.04
0.15	34.51	0.17	-0.0003	-3641.85	25.10	-0.04
0.20	70.25	-0.07	0.0001	-3716.80	25.61	-0.04
0.50	-83.59	0.95	-0.0016	-3497.54	24.19	-0.04

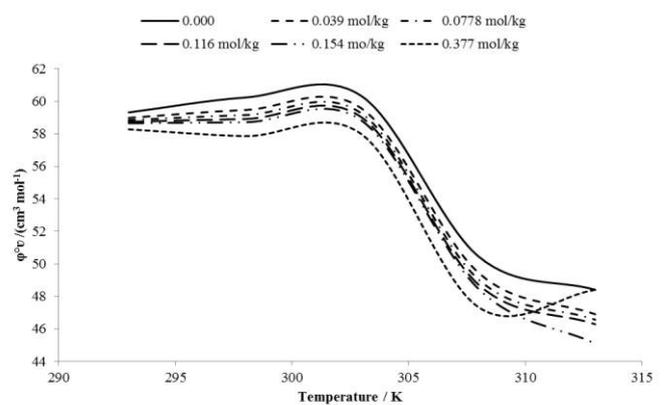


Figure 3: Change in limiting apparent molar volume with temperature in ethanol - L- alanine system.

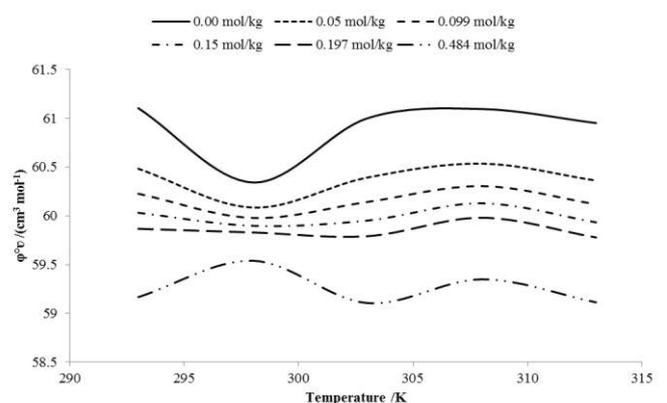


Figure 4: Change in limiting apparent molar volume with temperature in water - L- alanine system.

Values of coefficients  $a_i$  and the limiting apparent molar expansivity  $\frac{\partial \phi_v^o}{\partial T}$  obtained by least square fit are presented in Table 3 and 4. The values limiting apparent molar expansivity decreases with increasing temperature in ethanol which is a clear indication of

common salt behaviour, and that at high temperature bonded solvent molecules are less hence expansivity become weaker (Huiyong et al., 2012). The increase in temperature lead to increase in the molar expansivity at 0.05 and 0.2 mol dm<sup>3</sup> this is attributed to complete solute-solvent co-sphere behaviour of alanine in water at these concentrations. The values thermal

expansivities  $\frac{\partial^2 \phi_v^0}{\partial T^2}$  estimated (Table 4) showed that L-Alanine is a structure maker in both solvents with more effect in water than in ethanol at high concentration (Gupta and Singh, 2005).

**Table 4: Values of coefficients Limiting apparent molar and thermal expansivity for L-Alanine in water and ethanol**

Temp /K	293	298	303	308	313	$\frac{\partial^2 \phi_v^0}{\partial T^2}$	293	298	303	308	313	$\frac{\partial^2 \phi_v^0}{\partial T^2}$
	Limiting apparent molar expansibility in water $\frac{\partial \phi_v^0}{\partial T}$						Limiting apparent molar expansibility (Ethanol) $\frac{\partial \phi_v^0}{\partial T}$					
0.05	-0.0124	-0.0009	0.0107	0.0222	0.0338	0.0023	0.2361	-0.2122	-0.6604	-1.1087	-1.5569	-
0.10	0.0023	0.0011	-0.0001	-0.0013	-0.0025	0.0002	0.1538	-0.2671	-0.6881	-1.1090	-1.5299	-
0.15	0.0003	-0.0027	-0.0056	-0.0086	-0.0116	0.0006	0.1648	-0.2607	-0.6862	-1.1117	-1.5372	-
0.20	-0.0041	-0.0030	-0.0020	-0.0009	0.0001	0.0002	0.1609	-0.2734	-0.7078	-1.1421	-1.5764	-
0.50	0.0283	0.0127	-0.0030	-0.0187	-0.0343	0.0031	0.0869	-0.3244	-0.7356	-1.1469	-1.5582	-

**Table 5: Viscosity coefficients and Activation for viscosity of L-Alanine in Water and Ethanol**

(Temperature /K)	Water + L – Alanine					Ethanol + L – Alanine				
	293	298	303	308	313	293	298	303	308	313
A /(dm <sup>3/2</sup> mol <sup>-1/2</sup> )	0.135	0.669	1.568	2.139	2.496	0.077	0.038	0.348	0.798	1.931
B /(dm <sup>3</sup> mol <sup>-1</sup> )	-0.073	-0.433	-1.359	-1.963	-2.296	-0.121	-0.146	-0.347	-0.830	-2.257
$\Delta\mu_1^\#$ /(kJ mol <sup>-1</sup> )	62.208	62.208	62.208	62.208	62.208	62.490	62.490	62.490	62.490	62.490
$\Delta\mu_2^\#$ /(kJ mol <sup>-1</sup> )	56.92	42.01	3.64	-22.55	-38.38	55.26	54.19	45.64	18.00	-61.25
T $\Delta S_2^\#$ /(kJ mol <sup>-1</sup> )	-1495.18	-1520.69	-1546.21	-1571.724	-1597.24	-1577.51	-1604.43	-1631.35	-1658.272	-1685.19
$\Delta H_2^\#$ /(kJ mol <sup>-1</sup> K <sup>-1</sup> )	-1438.26	-1478.68	-1542.57	-1594.27	-1635.61	-1522.25	-1550.25	-1585.71	-1640.27	-1746.44

The viscosity was determined from the relative viscosity using the relation:

$$\eta/\eta_0 = t\rho/t_0\rho_0 \quad (4)$$

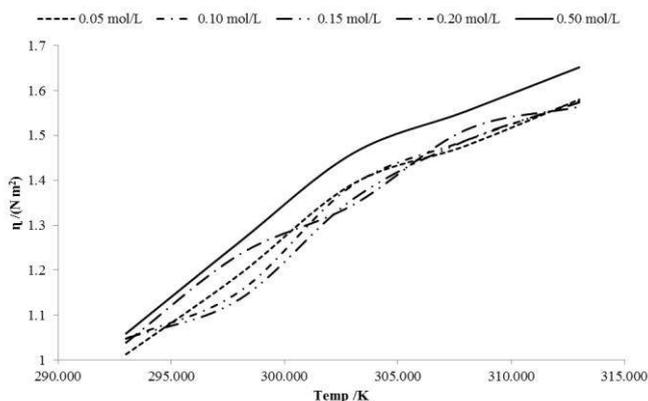
where  $\eta$ ,  $t$  and  $\rho$  are the absolute viscosity, time of flow and density of solution, while  $\eta_0$ ,  $t_0$  and  $\rho_0$  are same quantities for the solvent (water or ethanol).

The viscosity data was analysed by least square fit of relative viscosity vs concentration variation according to Jones- Dole equation below:

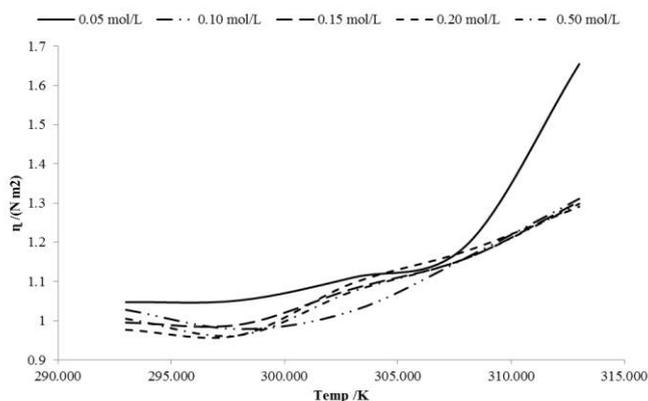
$$\eta/\eta_0 = \eta_r = 1 + A * C^{1/2} + B * C \quad (5)$$

where  $\eta_r$  is relative viscosity,  $C$  is molar concentration the constant  $A$  is the Falkenhagen coefficient and  $B$  is the Jones-Dole coefficient related

to solute-solvent and solute-solute interaction respectively. The value of viscosity co-efficient  $A$  is associated with the size of the soluteues of coefficients  $A$  and  $B$  of the Jones–Dole equation have been determined and the results is presented in Table 5 along with thermodynamic parameters of dissolution of L-Alanine in water and alcohol. Figures 5 and 6 also depict the change in viscosity with increase concentration of L- alanine in water and ethanol respectively. It is observed that the values of  $A$  are positive, while the values of  $B$  are negative in both solvent. This is a clear indication of presence of strong ionic interactions between the L-alanine molecules but weak ion – solvent interactions.



**Figure 5 :** Change in viscosities with temperature in ethanol- L- alanine system.



**Figure 6:** Change in viscosities with temperature in water- L- alanine system.

The thermodynamic properties were estimated using the Nightingale and Benck and Eyring equations (Hossain *et al.*, 2010). The average Gibbs energy of activation,  $\Delta\mu_1^\#$  of a solute for viscous flow in a solvent can be calculated from the viscosity by the relation:

$$\Delta\mu_1^\# = RT \ln \left( \frac{\bar{V}_1^0 \eta_0}{h N_A} \right) \quad (6)$$

#### IV. CONCLUSION

The density and viscosity of L-Alanine in water and ethanol as a function of concentration at the temperature ranges between 293 and 313 K have been studied. The results were used for the estimations of partial molar volume ( $\varphi_v$ ), apparent molar volume at infinite dilution ( $\varphi_v^o$ ), and viscosity coefficients A and B. The activation parameters for viscous flow  $\Delta\mu_1^\#, \Delta\mu_2^\#, \Delta S_1^\#$  and  $\Delta H_1^\#$  were also estimated using Eyring equation. The data were explained vis-à-vis structure making or breaking effect of L-Alanine at the temperature under consideration. This study shows that there greater solute

where  $\eta_0$ ,  $h$  and  $N_A$  are the solvent viscosity, Planck's constant and Avogadro's number, respectively, and  $\bar{V}_1^0$  is the average molar volume which is practically equal to  $\varphi_v^o$ . The activation Gibbs energy,  $\Delta\mu_1^\#$  for viscous flow of the L-Alanine in water and ethanol is related to the coefficient B of viscosity by:

$$B = \left[ \frac{\bar{V}_1^0 - \bar{V}_2^0}{1000} \right] + \bar{V}_2^0 \left[ \frac{\Delta\mu_2^\# - \Delta\mu_1^\#}{1000RT} \right] \quad (7)$$

Upon rearrangement the expression in Eq. (7) above becomes:

$$\Delta\mu_2^\# = \Delta\mu_1^\# + \left( \frac{RT}{\bar{V}_1^0} \right) \left[ 1000B - (\bar{V}_1^0 - \bar{V}_2^0) \right] \quad (8)$$

where  $\bar{V}_2^0$  is the molar volume of the solute, R is gas constant and T is the absolute temperature respectively.

The calculated values of  $\Delta\mu_1^\#$  and  $\Delta\mu_2^\#$  values are positive for all the studied systems as displayed in Table 5. This is interpreted on the basis of solute – solvent interaction leading to the formation of more structured systems. Other activation parameters i.e.

$\Delta H_1^\#$  and  $\Delta S_1^\#$  were obtained from the relations:

$$\Delta\mu_1^\# = \Delta H_1^\# - T\Delta S_1^\# \quad (9)$$

Considering the activation parameters, the activation Gibbs energy,  $\Delta\mu_1^\#$  in the two solvent seems to be closer, which according to Feakin's model (Feakin *et al.*, 1974) that the higher this value is the greater is the structure making ability. Therefore the solute may be said to have similar effect on the solute as regard the structure making with a little variation based on concentration. The values of  $\Delta S_1^\#$  obtained suggest that the attainment of transition state for viscous flow is by bond formation. The values of  $\Delta H_1^\#$  suggested that additional work is required to dissolve L-Alanine in water than ethanol.

- solute interaction between L-alanine molecules than ionic interactions with the solvent molecules.

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# Export Earnings Instability and Economic Growth in Nigeria (1981-2014)

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## ABSTRACT

This paper investigates the impact of export earnings instability on economic growth in Nigeria through the application of regression analysis. The study describes the trend of oil and non-oil export in Nigeria, examines the impact of export earnings instability on economic growth and identifies adequate policy measures and suggestions based on the research findings, towards reducing the undesirable effects of export instability in Nigeria.

Secondary data from various sources were used in the study. Augmented Dickey-Fuller technique was adopted in testing Unit root property of the series. Using the Ordinary Least Squares regression method, the study first examines the impact of export earnings instability on economic growth with the aid of aggregated and disaggregated models. It further uses the Granger causality test to examine the direction of causality between GDP and export earnings (using the same aggregated and disaggregated models).

From the result obtained in the regression of the disaggregated model,  $R^2$  is 0.954. This indicates that oil and non-oil exports actually account for 95.4% of the total variation in economic growth during the years under study. A percentage increase in oil export will cause about 4% economic growth (0.042785), which is statistically significant at all levels. A percentage increase in non-oil export will cause an economic growth of about 10.4%, at 10 per cent level of significance. Also, with a positive and significant value of the intercept, the result indicates that GDP does not only depend on oil and non-oil export, as other variables affect the GDP. The F- Statistics 316.9, which is a measure of the joint significance of the explanatory variables, is found to be statistically significant as indicated by the corresponding probability value 0.0000, and the Durbin-Watson statistics of 1.90, which is in the neighborhood of 2 indicates that there is no autocorrelation. The Granger Causality test of the disaggregated model shows that both Oil and non-oil exports actually granger causes GDP. It also show that non-oil exports granger cause Oil exports. It was found that of all export earnings in Nigeria, oil export has stronger grasp in terms of economic growth compared with the non-oil exports. It was also found that export earnings, from both oil and the non-oil sectors, affects economic growth in Nigeria, and not otherwise. Thus policies geared towards the development of the oil and non-oil sectors will have a positive effect on the economy and thereby resulting to an increase in the gross domestic product.

**Keywords:** Export Earnings, Instability and Economic Growth

## I. INTRODUCTION

Next to the maintenance of national peace, it can reasonably be claimed that raising the standard of living of the Nigerian populace is the most important objective of the country in this era. Less developed countries device different economic measures and social policies to ensure a quick and steady increase in their per capita

income, Price stability, Balance of Payments viability and other desirable economic growth goals and objectives. This is aimed at reducing the development gap between them and developed countries. Foreign trade has been an area of interest to decision makers, policy makers as well as economists; it enables nations to sell their locally produced goods to other countries of the world (Adewuyi, 2005). Foreign trade is the

exchange of capital, goods and services between countries it allows a country or nation to expand her markets for both goods and services that otherwise may not have been available to her citizens. Foreign trade augments per capita income as domestic production, consumption activities, and in conjunction with foreign transaction of goods and services are factored into its calculation.

There is no gainsaying that the pivotal role played by the country's export policy explains its wide macroeconomic importance. The importance of export earnings cannot be overemphasized, as it forms the major source of foreign revenue and exchange to the Nigerian government. These gains are ploughed back into the economy to achieve certain desirable economic conditions such as generation of domestic employment, increase in foreign reserves, aggregate consumption, savings and investments:

The export of most less developed countries, Nigeria inclusive, is characterized by the fact that primary product constitute an overwhelming percentage of total export, the prominent ones in the Nigerian export include crude oil, cocoa, coffee, groundnut, rubber, cotton etc. Although, there is no doubt about the importance of primary commodities, it is frequently claimed that this dependence makes the attainment of economic goals and objectives difficult.

It is generally accepted that both the prices and quantities of export of these primary products vary much more sharply from year to year than that of manufactured goods. Since the prices and quantities of export determine the size of export earnings, variations in any or both of the variables give rise to export earning instabilities. The concern of the less developed countries with the issue is that export instability has damaging effect on both the internal stability and economic development of their countries.

The earnings of primary products are notoriously volatile and the damaging effects of this volatility on the economies of the exporting countries are beyond question. Although, these instabilities may not necessarily be detrimental, it has been observed that the instability exerts notable influences being felt on key indicators of economic growth which may include: the Balance of payments, capital accumulation process,

level of employment of productive resources, Domestic distribution of income, general price level, Terms of Trade (to mention just a few)

Many research works on export instability are aimed at curbing the debilitating effects of Nigerian export problems, however, the desired results of these research works are yet to manifest significantly. Hence, there is a need to analyze the true nature of the Nigerian export earnings fluctuations in order to understand its major underpinnings, impact as well as implications on the economic growth of the country.

### **Export Earnings Instability**

Export earnings instability otherwise known as export earning fluctuations can be defined as short-term fluctuations corrected for trend. Some of trend correction is needed to avoid interpreting a constant year-to-year increase or decrease as indicating instability. Recent investigations have presented convincing evidence supporting the fact that developing countries experience greater export earning instability than the developed countries. Studies by Matheison and Mckinnon (1998), S, Naya (2006), Glezakors (1992) and Lawson (2002) all support the view that while export earnings fluctuate for both group of countries, developing countries in general suffer a greater degree of export earnings instability than the developed countries. Individual commodity data presented by Thomas Morrison and Lorenzo Perez in 1990 also support the existence of considerable export instability in developing countries.

It should be noted however, that the major concern of this study is with export proceeds and not export price. This is because statement about price may not necessarily hold for proceeds. Export price maybe very unstable (due to inflationary pressure, or other factors affecting export price) while the total proceeds received remain very relatively stable. Quantities may have compensated for changes in price more often than not. It is export earnings rather than price that influence the national income and a country's ability to purchase capital goods which is important for a developing country like Nigeria.

Export earning Fluctuations means excessive departure from some normal level (or trend). However, it is

difficult to determine a priori the meanings of 'excessive' and 'normal'. Hence it is a *sin-qua-non* for this study to develop a measure of instability on a common sense basis in the light of available facts.

## II. METHODS AND MATERIAL

### A. Review of Related Literature

Most, if not all, international trade and development theories portray a positive relationship between the volume of trade and economic growth, right from classical Comparative Advantage model of David Ricardo, the Neoclassical model of Heckscher and Ohlin, to the contemporary Endogenous Growth models. Although the various models assume that different factors cause the trade, but the end result portrays improvement in the output and welfare.

#### The Ricardian Model

This model as developed by David Ricardo (1817) is based on some simplified assumptions, the model assumes that each country involved in the trade has a fixed endowment of resources, and all units of each particular resource are identical. Also, the factors of production are completely mobile between alternative uses within a country, thus, the prices of factors are also the same among these alternative uses. However, factors are relatively immobile externally, that is, they do not move between countries. This model further employs labor theory of value, thus, the relative value of a commodity is based solely on its relative labor content. This implies that either other factors are not used in the production process or they are measured in terms of labor hours. It also assumes fixed level of technology for the country and full employment of resources, with constant cost of production, and there is no transportation cost both internally and externally.

Again, the model assumes differences in the production function (Labor Productivity) in different countries that are involved in trade, with each production function depicting constant return to scale. And there is perfect competition in the countries so no government-imposed obstacles to economic activity. The model of Comparative Advantage as it is called asserts that "a country should specialize in the export of the commodities that it can produce at the lowest relative

cost". Germany may be able to produce cameras and cars as well as fruits and vegetables at lower absolute unit costs than Kenya, but because the commodity cost differences between countries are greater for the manufactured goods than for agricultural products, it will be to Germany's advantage to specialize in the production of manufactured goods and exchange them for Kenya's agricultural products, whereas Kenya which has absolute disadvantage in the production of both goods in relation to Germany may still benefit from trade with Germany if it will specialize in the production of agricultural produce which the absolute disadvantage is less than that of manufactured goods (Todaro, 2009). It is this phenomenon of differences in comparative advantage that gives rise to beneficial trade even among the most unequal trading partners. However, there are contradicting views on the relationship between exports and productivity. Some argue that increase in export increases foreign competition, and this may have detrimental effect on growth of GDP, as it may lead to marginalization or even closure of factories. On the other hand, some argue that growth of export brings about higher growth of GDP through educative process. For example, higher contact with foreign competitors as a result of export growth can motivate rapid technological changes and managerial know-how, and enhance efficiency. For instance, Nashimizu and Robinson (1994) accepted the hypothesis that export growth causes productivity growth in Japan, Turkey, Yugoslavia, and South Korea. They concluded that the larger the share of output that goes into exports the higher the productivity growth. These contradicting views are the reasons for conducting the empirical test using Nigeria as a case study.

#### Empirical Literature

Many writers in Nigeria's export have chosen the stance of relating the behavior of the country's exports to change national income as one of the major determinants of the country's imports from Nigeria. One of such works undertaken by Olayide (1980) covered the pricing of Nigeria's export commodities. He observed that Nigeria's approach to empirically obtain the co-efficient of flexibility for prices of numbers of Nigeria.

Many empirical studies have been carried out to determine or evaluate the role of export promotion on economic growth and development. Most of these

studies employed cross sectional analysis of inter – country data on export and Gross Domestic product (GDP) or Gross National Product (GNP). Maizzls (1968) carried out a study on the relationship between exports and economic growth in sixteen countries. In estimating the relationship, he performed time series analysis of exports and GNP. He found out that, there is no strong association between export and the growth of the economy. He however, offered two plausible explanations for this.

First, is the small sample sizes, and second, the relative instance of export in national incomes is not taken into account in each of the countries.

Fajana's (1979) study was meant to test the validity of the widely held view that trade has been a major relate to economic growth in Nigeria. Fajana employed a chancery. Generally, the result indicates a positive and strong relationship between output changes and hence provides empirical support for this thesis that trade has been an important factor in Nigeria's growth.

In 2001, Olayide conducted another study on the demand for Nigeria's export for the period 2000-2001. He employed a linear correlation co-efficient analysis and included that only groundnuts, groundnut oil, palm kernel, and cotton in their investigation. His interest lied mostly in determining the elasticity of demand for the mentioned non-oil export products and the other factors responsible for fluctuations in the demand for those products. He included changes in income of the importing countries in their model. But again, his work was rendered rather detective by the inclusion of a variable for a measure of export control. Another defect of Olayide work is that total Nigeria cocoa export was regressed on the means of real income of only four importers. This formulation wrongly presumes that the demand of the four countries whose real income was used constitutes the total demand for Nigeria's exports. It would have been more logical to estimate the individual function in each country. He forgot to acknowledge the fact that the conditions that influence the demand for Nigeria cocoa for instance, may vary from one country to the other.

Oni (1986) conducted a research in Nigeria's palm oil export using the person and spearman correlation analysis. His main point of deviation from other

researchers' work is that instead of aggregating, he took a separate study of the quantities exported to each of the major trading partners. This new approach used information on the demand conditions that exist in each of the countries importing Nigeria palm oil.

Akinole (2001) in his study adopted the ordinary least square (OLS) regression technique. He investigated the prospects for Nigerian petroleum, groundnut, coca and palm oil in the expanded economic commodity. He discovered that the demand for Nigeria oil by the common market countries is price elastic. But the membership of Nigeria in the Organization of Petroleum Exporting Countries, a collective bargaining organization makes the exploitation of the high price elasticity of demand unlikely. He said that there exist an effective competition between Nigeria's groundnut and soya bean in the following common market countries, France, Netherlands, Belgium, Luxemburg and United Kingdom. He said that Nigeria groundnut oil and cake are not inferior goods in these markets. He observed that this might be due to the fact that the quantities of proportions of total quantities observed. As a result, Nigeria should shift from the export of groundnuts by groundnut oil and cake and this should be boasted by an effective export promotion in market currently exploited. Helleiner (2002) carried out a study using the Keynesian export multiplier approach and two variants of the two – gap frame work, incorporating, and the Harrod Domar model, which shows that only a small part of total agricultural output of the developing countries receive elaborates local processing, since the bulk is usually sent abroad. He points out that the agriculture normally better in the supply of intermediate inputs to other rectors than in the use of other intermediate inputs.

Asanebi (2007) carried out a research using linear correlation coefficient analysis and observed that the performance of exports was below expectation in aggregate terms and so, has not made significant impact on the GNP of the country, cannot sustain the country in terms of economic growth and development. He also came up with the following findings; That primary commodities dominates Nigeria's basket of non-oil export That introduction of the Structural Adjustment Program (SAP) came with export promotion policy that saw some improvement in the proportion of semi – manufactures and manufactures. Though the performance of non-oil exports was below expectation in terms of market diversification, it however, recorded

some success in terms of a gradual growth in the proportion of value added exports.

Furthermore, he identified some major constraints that militated against non – oil export performance especially inefficient credit scheme and his period of research covered 1990 – 2000. Okoro (2009), in his work on the impact of export on the Nigeria economy using econometric growth without the industrial, agricultural and manufacturing sectors improving from their present state. He states that a very strong link exists between these three sectors and other sectors of the economy. His period of study covered 1995 – 2005. Ogbonna (2010) emphasize that the contributions of the non-oil export to the GDP is still marginal and almost insignificant. What this implies is that all the export promotion strategies adopted failed to achieve the desired results, which is to improve the performance of the sector. In her research on “the impact of export promotion policies on Nigeria’s export” using ordinary least square (OLS) regression technique she noted that there is a general need for policy frame work, otherwise, the non – oil sector will continue to make less contribution to the country’s balance of payments, and her research work covered the period from 1981 – 2000.

Ozoudo (2010) also discovered using econometric method, that the dominance of petroleum / crude oil in the export sector’s export. He recorded that the inefficient performance of the non – oil marketing of board deterred progress of the non – oil sector. His research covered the period from 1991 – 2008.

## B. Research Methodology

The method of analysis used is the econometric analysis with focus on the regression analysis. This method was adopted because economic theory is mainly concerned with relationship between economic variables hence; this method of analysis would help to establish the relationship that exists between export earnings instability and economic growth.

### i. Nature and Sources of Data

For the purpose of this study, secondary data was employed and were generated from the CBN Statistical Bulletin. However, there is no doubt envisaged on the reliability of secondary data used, but, the possibilities

of random errors were not neglected. The research work covers a period of 34 years (1981-2014).

### ii. Method of Analysis

The study used simple regression analysis to measure the impact of export earnings instability (given by the Export Earnings Instability Index) on economic growth in Nigeria. This will be achieved by using the Gross Domestic Product (GDP) as the dependent variable and the export earnings as the independent variable.

### iii. Model Specification

The variable of interest for economic growth is Gross Domestic Product which is the dependent variable while percentage growth rate of export is the independent or explanatory variable.

Aggregated Model

$Y_t = F(\text{EXP})$  Implicit function

$Y_t = A_0 + A_1 \text{EXP}_t + U_t$  Explicit function

Where  $Y_t$  = Gross Domestic Product proxy for Economic Growth (Dependent Variable)

EXP = Growth Rate of Total Export (Independent Variable)

$U_t$  = Stochastic Error Term

Disaggregated Model

$Y_t = A_0 + A_1 \text{OIL} + A_2 \text{NOIL} + U_t$

Where  $Y_t$  = Gross Domestic Product proxy for Economic Growth (Dependent Variable)

OIL = Oil Export (Independent Variable)

NOIL = Non oil Export

$U_t$  = Stochastic Error Term

### iv. Apriori Expectation

On a Priori ground, we would expect the coefficient of the equation  $A_1$  to be positive and the constant term  $A_0$  to be positive since export earnings is positively related to the gross domestic product (GDP). For the disaggregated model, we also expect the coefficients of the equation  $A_1$  and  $A_2$  to be positive and constant term  $A_0$  to be positive since both oil and non-oil export earnings is positively related to the GDP.

We would also expect both the oil and non-oil export earnings to cause gross domestic product and not the other way round meaning we expect one way causation flow that is, export earnings should Granger cause GDP.

### III. RESULT AND DISCUSSION

#### A. Trend of Export Earnings

The export earnings of Nigeria which stood at 11.02 billion naira as at 1981 decreased to 8.20 billion naira in the year 1982. It reduced further in 1983 to 7.50 billion naira but started increasing in 1984 and stood at 9.09 billion naira. It increased again in 1985 to 11.72 billion naira but went back again in the year 1986 to 8.92 billion naira. It increased again to 30.36 billion naira in 1987 and it had been increasing ever since then and stood at 205.61 billion naira in the year 1992 and also increased to 218.77 billion naira in 1993 but still dropped to 206.06 billion naira in the year 1994. Ever since, export earnings of Nigeria have been on the same pattern; increasing and decreasing year by year and stood at 1.95 trillion naira as at year 2000. The export earnings began to drop in the year 2001 and dropped to 1.87 trillion naira and dropped again to 1.74 trillion naira in the year 2002 but increased tremendously to 3.09 trillion naira in the year 2003. It kept increasing and stood at 7.33 trillion naira in the year 2006 and rose to 10.16 trillion naira in the year 2008 but reduced drastically to 8.36 trillion naira in 2009. Since then, it had been increasing, rising to 15.13 trillion naira in 2011, but it reduced to 14.69 trillion naira in 2012 and later rose to 14.81 trillion naira in the year 2014.

The trend shows that the export earnings of Nigeria have increased since 1981 to 2014 which is the period that this study covers but the increment in the export earnings have not experienced stability not even for consecutive five years. It has always been increasing and decreasing over the years.

From the findings of this study, the trend of export earnings in Nigeria has been that of continuous fluctuation during the period of this study.

#### B. Table 1: Unit Root Test Results for GDP

The results of the unit root tests in table 1 above revealed that the two variables of the model were found to be stationary at 1 percent, 5 percent, and 10 percent level, which is indicated by ADF results at all levels less than the critical values in the negative direction. The ADF value for GDP is 5.2417 and the critical values are -3.6576, -2.9591 and -2.6181 at 1, 5, and 10 percent

respectively. The Durbin-Watson statistics of 1.88 which is in the neighborhood of 2 means that the data are stationary.

ADF Test Statistic	5.241666	1%	Critical Value*	-3.6576
		5%	Critical Value	-2.9591
		10%	Critical Value	-2.6181

\*MacKinnon critical values for rejection of hypothesis of a unit root.

Augmented Dickey-Fuller Test Equation

Dependent Variable: D(GDP)

Method: Least Squares

Date: 05/08/15 Time: 12:46

Sample(adjusted): 1983 2014

Included observations: 32 after adjusting endpoints

Variable	Coefficient	Std. Error	t-Statistic	Prob.
GDP(-1)	0.125990	0.024036	5.241666	0.0000
D(GDP(-1))	-0.467084	0.185630	-2.516218	0.0179
C	-15733.84	8168.511	-1.926158	0.0643

R-squared	0.817082	Mean dependent var	24207.38
Adjusted R-squared	0.782587	S.D. dependent var	27655.78
S.E. of regression	19893.19	Akaike info criterion	22.72591
Sum squared resid	1.11E+10	Schwarz criterion	22.86468
Log likelihood	-349.2516	F-statistic	14.99041
Durbin-Watson stat	1.882121	Prob(F-statistic)	0.000038

#### C. Table 2: Regression Results

Dependent Variable: GDP

Method: Least Squares

Date: 05/08/15 Time: 12:46

Sample: 1981 2014

Included observations: 34

Variable	Coefficient	Std. Error	t-Statistic	Prob.
EX	0.045582	0.001745	26.12483	0.0000
C	248293.7	10440.22	23.78241	0.0000

R-squared	0.956553	Mean dependent var	409620.8
Adjusted R-squared	0.955151	S.D. dependent var	228347.3
S.E. of regression	48358.39	Akaike info criterion	24.46936
Sum squared resid	7.25E+10	Schwarz criterion	24.56006
Log likelihood	-401.7444	F-statistic	682.5068
Durbin-Watson stat	0.982233	Prob(F-statistic)	0.000000

The result in table 2 above shows that  $R^2$  is 0.956. This implies that about 96 percent of the total variation in economic growth is being explained by export earnings. The coefficient of export earnings is positive; implying that a percentage increase in export earnings will lead to a 4 percent increase in economic growth. The result is significant at all levels. The significance of the result is also corroborated by the t-statistics of 26.125 which is greater than the critical t.

The constant is statistically significant implying that GDP does not only depend on export but other variables may affect the GDP. The F-statistics 682.5, which is a measure of the joint significance of the explanatory variables, is found to be statistically significant as indicated by the corresponding probability value 0.0000.

The Durbin-Watson statistic 0.982 in Table 1 is observed to be higher than  $R^2$  0.956 indicating that the model is non-spurious (meaningful).

#### D. Table 3: Causality Test Result

##### Pairwise Granger Causality Tests

Date: 05/08/15 Time: 22:09

Sample: 1981 2014

Lags: 2

Null Hypothesis:	Obs	F-Statistic	Probability
EX does not Granger Cause GDP	31	1.77684	0.18910
GDP does not Granger Cause EX		8.62742	0.00134

The results of table 3 revealed that export earning Granger causes GDP, the null hypothesis is rejected as indicated by the probability value 0.18910. The null hypothesis is rejected as indicated by the probability value of 0.00134 and this is confirmed by the F-statistics value 1.78. This result therefore indicates a one-way causation flowing from GDP to export earnings. Therefore, the alternative hypothesis is accepted meaning that there is unidirectional relationship between the Gross Domestic Product (GDP) and export earnings, that is, export earnings Granger cause GDP.

#### E. Results of Disaggregated Model

Regression Result

Dependent Variable: GDP

Method: Least Squares

Date: 05/09/15 Time: 13:37

Sample: 1981 2014

Included observations: 34

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	250229.4	11019.92	22.70700	0.0000
OIL	0.042785	0.005845	7.320559	0.0000
NOIL	0.104333	0.155658	0.670273	0.5078
R-squared	0.954816	Mean dependent var	409620	
Adjusted R-squared	0.951804	S.D. dependent var	228347	
S.E. of regression	50130.45	Akaike info criterion	24.569	
Sum squared resid	7.54E+10	Schwarz criterion	24.705	
Log likelihood	-402.3910	F-statistic	316.97	
Durbin-Watson stat	1.903091	Prob(F-statistic)	0.0000	

Level of significance: 1% \*\*\*; 5% \*\*\*; 10% \*\*\*

$$GDP = 250229.3503 + 0.4278546035 * OIL + 0.1043332699 * NOIL + U_t$$

#### Analysis of the Regression Coefficients:

From the result above, when all the independent variables are equal to zero, the intercept for GDP becomes 250,229.3503 million while unit change in oil export revenue increases Gross Domestic Product by 0.4279 units and unit change in non-oil export revenue increases Gross Domestic Product by 0.1043 units.

From the result obtained in the regression,  $R^2$  is 0.954 showing a goodness of fit of 95.4%, on the grounds that the explanatory or independent variables explain 95.4% of the total variation in the dependent variable.

The coefficient of export earnings is positive; this implies that increase in export earnings will lead to increase in economic growth. However, the export earnings are significant at 10 percent.

The constant is statistically significant implying that GDP does not only depend on oil and non oil export but other variables may affect GDP. The F-statistics 316.9, which is a measure of the joint significance of the explanatory variables, is found to be statistically significant as indicated by the corresponding probability value 0.0000.

The Durbin-Watson statistic 1.90 which is the neighborhood of 2 indicating that the model is non-spurious (meaningful), that is, there is no autocorrelation. From the results, oil and non-oil export was revealed to have a positive relationship with the gross domestic product in Nigeria. Thus, policies geared towards the development of the oil and non-oil sectors will have a positive effect on it and thereby resulting to an increase in the Gross Domestic Product.

#### F. 4.6 Results of Granger Causality Test

Pairwise Granger Causality Tests

Date: 09/05/15 Time: 17:24

Sample: 1981 2014

Lags: 2

Null Hypothesis:	Obs	F-Statistic	Probability
OIL does not Granger Cause GDP	31	1.79771	0.18567
GDP does not Granger Cause OIL		9.02267	0.00106
NOIL does not Granger Cause GDP	31	0.28425	0.75488
GDP does not Granger Cause NOIL		1.89785	0.17008
NOIL does not Granger Cause OIL	31	2.90036	0.07294
OIL does not Granger Cause NOIL		7.59527	0.00253

The results of the Granger Causality test show that:

HYPOTHESIS	PROBABILITY	DECISION	DIRECTION
OIL does not Granger Cause GDP	0.18567	Reject	Unidirectional
GDP does not Granger Cause OIL	0.00106	Accept	
NOIL does not Granger Cause GDP	0.75488	Reject	Unidirectional
GDP does not Granger Cause NOIL	0.17008	Accept	
NOIL does not Granger Cause OIL	0.07294	Reject	Unidirectional
OIL does not Granger Cause NOIL	0.00253	Accept	

The results of the Granger Causality revealed that oil export earning Granger causes GDP, the null hypothesis is rejected as indicated by the probability value 0.18567. The null hypothesis is rejected as indicated by the probability value of 0.00106 and this is confirmed by the F-statistics value 1.79.

The results of the Granger Causality revealed that non oil export earning Granger causes GDP, the null hypothesis is rejected as indicated by the probability value 0.75488. The null hypothesis is rejected as indicated by the probability value of 0.17008 and this is confirmed by the F-statistics value 0.28.

The results of the Granger Causality revealed that non oil export earning Granger causes oil export earnings, the null hypothesis is rejected as indicated by the probability value 0.07294. The null hypothesis is rejected as indicated by the probability value of 0.00253 and this is confirmed by the F-statistics value 2.90.

These results therefore indicate a one-way causation flowing from GDP to export earnings.

#### IV. CONCLUSION

This paper investigated the impact of export earnings instability on economic growth in Nigeria through the application of regression analysis. We also adopted the Augmented Dickey-Fuller technique in testing the unit

root property of the series and Granger causality test of causation between GDP and export earnings.

The result showed that there was fluctuating trend in export earnings during the period of the study. The results of unit root suggest that the two variables in the model were stationary at 1%, 5% and 10% critical value with first difference. The results of Causality suggest that there is bi-directional causation between GDP and export earnings. The results concluded that export earnings instability had a negative impact on economic growth of the country.

## V. RECOMMENDATIONS

Sequel to the result of our empirical investigations, the following policy options which if pursued vigorously would help in no small measure to minimize the hardship caused by export earnings instabilities in the Nigerian economy are prescribed. Priority should be given to the establishment and operations of industries with export potentials, (like petro-chemical industries) by providing adequate flow of the needed raw materials and spare parts for the smooth running of their operations. This will help to shift our focus from dependence on oil and primary goods. The government should endeavor to support various export promotion programs and institutions. This could be achieved by encouraging financial institutions, both formal and informal; to make loans available at reduced rates of interest for investors so as to increase the level of investment in this country thereby leading to a more expanded export. There should be a quick diversion from monoculture economy to a multicultural one. This is so since the oil which Nigeria depends on is prone to shocks beyond the control of the nation. As such, crude oil revenue should be put to use so as to make Nigeria's economy self-sustaining.

The Nigerian government should encourage the use of local raw materials as substitutes for the imported ones used in production. This will go a long way in reducing the marginal propensity to import of producers Tariffs on export should be reduced or removed in order to provide incentives for the exporters. Over the years, policies have been made without their full implementation. So to review the economy, proper policies must be squarely implemented as to promote exports. Collection and Banking of Data in modern

world play vital roles in planning. The government should make provisions for a systematic collection of data and their banking by equipping the relevant ministries, departments and agencies with computers and other enabling ICT infrastructures that will improve the collection and processing of these data by researchers. Lastly, intensive research should be embarked upon which will help in discovering new areas of export opportunities to exploit from which we can derive some comparative cost advantage. This should be done in the area of non-oil export such as agricultural products and manufacturing. This will boost the export earnings potentials of the country

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# Law Enforcement and Water Resources of the Malacca River A Case Study of Public Perception

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## ABSTRACT

Malacca state is a historical tourism based economy, and has been recognized by UNESCO as a world heritage site. However, Malacca River pollution can be attributed to human activities such as agriculture, livestock, factories, commercial, and settlements. So, this research study has been conducted to determine the effectiveness of law enforcement of water resources in the Malacca River. The research involves a quantitative approach, which is a questionnaire divided into two parts. Part A is a demographic profile and part B concerns water pollution in the river and the effectiveness of law and regulation towards water resources in Malacca River. The sample size of 400 was decided with a sampling area of Alor Gajah and Melaka Tengah. Analyses involve chi-square ( $\chi^2$ ) analysis and correlation analysis. A majority of respondents agree that the Malacca River is dirty due to industrial and excretory waste turning the water black, smelly, and contaminated. Polluted water cause disease and poisoning aquatic animals to death, and affect plant species through nutrients loss. At worst, polluted river leads to aquatic species becoming extinct and dirty the habitat of animals. Hence, the Water Act 1920, Environmental Quality Act 1974, Sewage and Industrial Effluent 1979, Sewage Service Act 1993, and River Act could stop pollution and protect the water resources of the Malacca River.

**Keywords:** Human Activities, Water Pollution, Law Enforcement, Wastes, Protect.

## I. INTRODUCTION

Water is one of the important resources among the air, soil, solar energy, metals, minerals, and so on, which act to protect the Earth from overheating through the evaporation process, generate food sources, serve as transportation, and help the growth of animals and plants. According to a research entitled 'Water in Crisis: A Guide to the World's Fresh Water Resources', ice caps, glaciers and permanent snow have 68.7% of freshwater, followed by ground freshwater with 30.1%, lake freshwater with 0.26%, swamp freshwater with 0.003%, and river freshwater with 0.006% [5]. However, only 0.26% of freshwater is available as a source of water for drinking, bathing, washing, and others activities, and these water sources are lakes and rivers [5]. The situation becomes worst as 90% of wastewater produced in underdeveloped countries is discharged untreated into water bodies and cause water pollution to happen [16].

For example, 80% of China's major river is polluted and no longer able to support aquatic life while 90% of all groundwater systems under major cities in China are contaminated. 75% of India's rivers and lakes are polluted and cannot be used for drinking or bathing, while 60% of rural Russian drink water from contaminated wells [4]. So, increasing of river water pollution is not only happening worldwide, but these problems also affect a country like Malaysia.

According to Department of Environment Malaysia [2] stated that among 473 rivers monitored, only 278 (59%) are believed to be clean, but 161 (34%) are considered as slightly polluted and 34 (7%) are totally polluted. In other words, among 34 polluted rivers, 19 rivers are classified as Class III, 14 rivers as Class IV, and only one river is Class V [2]. The rivers that categories as polluted are Sungai Rajang, Sungai Selangor, Sungai Sarawak, and etc., which also include Sungai Melaka or

Malacca River in Malacca state [2]. As proved, water contamination in the Malacca River had affected local residents in carrying out daily activities such as fishing, bathing, washing, and so on [13] [9] [6]. Generally, Malacca is a state that based on historical tourism which been recognized by UNESCO as a world heritage site on July 07, 2008 [15] [1]. Tourism is an industry that generates economic value to the country through arrival of tourists from local and international [14]. Therefore, if water pollution is continuously happen in Malacca River, so this will not only affect the environment but also have a negative impact on the tourism industry. So, this issues and problems should be taken seriously in dealing with water pollution in Malacca River.

Malacca River pollution can be attributed to human activities such as agriculture, livestock, factories, commercial activities, and settlements [7]. According to a research done by Hua [8], stated that application of policy in controlling and managing the water resources in Malacca River can be successfully implement. However, the policy will take a long term to implement. So, there are suggestion to use law and regulation in preventing water pollution from continuously happen in Malacca River for this short term. The law can be categorized into several sections, namely Water Act 1920, Environmental Quality Act 1974, Sewage and Industrial Effluent 1979, Sewage Service Act 1993, and River Act [3]. Generally, the main purposed of Water Act 1920 is that any individual is prohibited from releasing or removed any material into the river that can change the original quality of the water; continued by Environmental Quality Act 1974, which states any individual release, remove, settling of waste in an area, segment, or any feature of environment without following the prescribed conditions; Sewage and Industrial Effluent 1979 states that individual is prohibited from removing or causing or permit the discharge of any material involve with liquid or solid form in upstream area which can cause river water quality to decrease. The Sewage Service Act 1993 states that any individual shall not discharge or allow any public sewer or public treatment of any industrial effluent except formal permission from Director General of Sewage Services into river water; and the last one is the River Act which imposes restrictions and sanctions for inland water pollution (only for those who have licensed and followed the conditions can release or discard wastes into inland water or inland waters) [3].

Therefore, research studies have been conducted to determine the effectiveness of law enforcement for the water resources in the Malacca River.

## II. METHODS AND MATERIAL

The method used in this research study is a quantitative approach, which involves questionnaires for collecting the data. In other words, the data as collected and gathered will only involve primary data. In this questionnaire, will be divided into two main parts. Part A is respondent's demographic profile and part B is respondent's perception on water resource in Malacca River. There are another two parts in part B, while part B (I) contains respondent perception on water pollution in Malacca River and part B (II) contains respondent perception on the effectiveness of law and regulation on the environment, especially towards water resources in Malacca River. The questionnaire in part B (I) and part B (II) only has 5 questions for each part and is based on a Likert Scale (5 points: 1-Strongly Disagree, 2-Disagree, 3-Normal, 4-Agree, 5-Strongly Agree), which is referred to as a 'close-ended' approach. However, the part A is based on Thurstone scale of 'close-open ended', where respondent are given an answer to fill in the empty space (where applicable).

Since this research study is based on the Malacca River, the sampling area will be in Malacca state, especially respondents settled near the Malacca River. In general, the Malacca River spans two districts in Malacca State, namely Alor Gajah and Melaka Tengah (or Malacca Central) [11]. So, the questionnaire is concentrated on and distributed to respondents within Alor Gajah and Melaka Tengah, especially along and near the Malacca River. Once the sampling area is specified, the researcher determines the sample size needed. Since total population that lives in Malacca state is 830,900 [12], so the appropriate amount to collect data is 400 respondents [10]. The questionnaire was collected and gathered after the respondents successfully answered all parts. The analysis used in this research study is chi-square ( $\chi^2$ ) analysis and correlation analysis. So, the value of reliability test (or Cronbach's Alpha) is 0.813; means items are satisfactory, suitable, and eligible for use in this analysis study.

### III. RESULT AND DISCUSSION

The analysis for this research study can be seen in Table 1 for respondent's demographic profile, Table 2 for respondent's perception on water pollution in Malacca River, and Table 3 for respondent's perception on the effectiveness of law and regulation on the environment, especially regarding water resources in the Malacca River.

**Table 1:** Respondent's demographic profile

Category	Frequency	Percentage (%)	Total
<b>Gender</b>			
Man	200	50	400 (100%)
Women	200	50	
<b>Age</b>			
<20	1	0.25	400 (100%)
21-30	78	19.5	
31-40	183	45.75	
41-50	130	32.5	
51>	8	2	
<b>Education Level</b>			
Primary School	52	13	400 (100%)
Secondary School	166	41.5	
College	108	27	
University	74	18.5	
<b>Occupation</b>			
Government	30	7.5	400 (100%)
Private	86	21.5	
Self-Employment	131	32.75	
Retirees	78	19.5	
Student	75	18.75	
<b>Number of Years Live in Malacca (years)</b>			
1-10	40	10	400 (100%)
11-20	70	17.5	
21-30	130	32.5	
31-40	119	29.75	
41-50	40	10	
>51	1	0.25	

According to the Table 1, the total respondents involved in this research study were 200 males and 200 females with a majority at age 31-40 (45.75%) and working as a business owner or self-employment (131 respondents or 32.75%). Basically, minority respondents that run business will have higher education, but the respondents will only be studied until the secondary level (166 respondents or 41.5%) or even primary level (52 respondents or 13%) because they must continue the family business to prevent from closing, and most of respondents are in the age of 41-50 with 130 respondents (32.5%). There are also respondents that working in the private sectors with 86 respondents (21.5%) and government sector with 30 respondents (7.5%). In other words, a majority of respondents have a higher education level, with 74 respondents (18.5%) are

graduate university and 108 respondents (27%) are graduate college. Only a small number of respondents at 8 respondents (2%) have an age of more than 51 and have chosen to retire and stay at home (78 respondents or 19.5%; with certain respondents are age 41-50). It is important to know the duration of respondents living in Malacca state especially near to the river (with a majority of respondents at 130 or 32.5% stay at Malacca for 21-30 years, 1 respondent or 0.25% resided more than 51 years in Malacca) because the exposure and experience of respondents with water resources in Malacca River will provide accurate and precise answers to the questions in the questionnaire provided.

Analysis of respondent's perception on water pollution in Malacca River may be seen in Table 2. This analysis involves a chi-square ( $\chi^2$ ) test between education level and perception on water pollution in Malacca River.

**Table 2:** Respondent's perception on water pollution in Malacca River

Variable A		Malacca River is dirty.					Total
Education Level		SD	D	N	A	SA	
University	EC	-	6.5	19.8	26.1	21.6	74
	SR	-	-2.2	-1.5	0.2	2.4	
College	EC	-	31.6	9.5	28.9	38.1	108
	SR	-	-1.4	0.5	0.8	0.3	
Secondary school	EC	-	14.5	44.4	58.5	48.6	166
	SR	-	-0.1	-1.1	0.1	1.1	
Primary school	EC	-	15.2	18.3	4.6	13.9	52
	SR	-	-2.9	-0.8	2.1	2.7	
<b>Total</b>							400

\*EC means Expected Count; SR means Standard Residual; SD means Strongly Disagree; D means Disagree; N means Normal; A means Agree; SA means Strongly Agree.

Chi-Square Test ( $\chi^2$ )			
	Value	df	Asymp. Sig (2-sided)
Pearson Chi-Square	38.649 <sup>a</sup>	9	0.000
Likelihood Ratio	42.094	9	0.000
Linear-by-Linear Association	17.298	1	0.000
N of Valid Cases	400		

a. 1 cells (6.3%) have expected count less than 5. The minimum expected count is 4.55.

Variable B		Industrial waste and excretion waste will cause the water in the river to become black color, smelly, and contaminated.					Total
Education Level		SD	D	N	A	SA	
University	EC	-	5.9	25.7	30.3	12	74
	SR	-	-0.8	-1.5	-0.1	2.9	
College	EC	-	44.3	8.6	37.5	17.6	108
	SR	-	-0.6	1.5	0.9	-1.3	
Secondary school	EC	-	13.3	57.7	68.1	27.0	166
	SR	-	-0.9	0.3	0.2	-0.2	
Primary school	EC	-	8.5	4.2	21.3	18.1	52
	SR	-	-1.2	0.4	0.6	0.0	
<b>Total</b>							400

\*EC means Expected Count; SR means Standard Residual; SD means Strongly Disagree; D means Disagree; N means Normal; A means Agree; SA means Strongly Agree.

Chi-Square Test ( $\chi^2$ )			
	Value	df	Asymp. Sig (2-sided)
Pearson Chi-Square	19.291 <sup>a</sup>	9	0.023
Likelihood Ratio	18.197	9	0.033
Linear-by-Linear Association	3.211	1	0.073
N of Valid Cases	400		

a. 1 cells (6.3%) have expected count less than 5. The minimum expected count is 4.16.

Variable C		Polluted water river can cause disease, cause aquatic animals to death and become habitat to dirty animals.					Total
Education Level		SD	D	N	A	SA	
University	EC	-	0.4	8.1	32.2	33.3	74
	SR	-	-0.6	-2.5	0.3	1.0	
College	EC	-	0.5	11.9	47.0	48.6	108
	SR	-	-0.7	0.0	-0.3	0.3	
Secondary school	EC	-	0.8	18.3	72.2	74.7	166
	SR	-	-0.9	0.4	-0.6	0.5	
Primary school	EC	-	23.4	0.3	22.6	5.7	52
	SR	-	-2.6	3.4	1.1	2.2	
<b>Total</b>						<b>400</b>	

\*EC means Expected Count; SR means Standard Residual; SD means Strongly Disagree; D means Disagree; N means Normal; A means Agree; SA means Strongly Agree.

Chi-Square Test ( $\chi^2$ )

	Value	df	Asymp. Sig (2-sided)
Pearson Chi-Square	34.443 <sup>a</sup>	9	0.000
Likelihood Ratio	33.472	9	0.000
Linear-by-Linear Association	16.068	1	0.000
N of Valid Cases	400		

a. 4 cells (25%) have expected count less than 5. The minimum expected count is 26.

Variable D		Contaminated water can cause aquatic species to become extinct.					Total
Education Level		SD	D	N	A	SA	
University	EC	-	0.4	10.9	27.4	35.3	74
	SR	-	-0.6	-1.2	-0.8	1.5	
College	EC	-	40.0	0.5	15.9	51.6	108
	SR	-	-0.5	0.6	1.0	-0.2	
Secondary school	EC	-	0.8	24.5	61.4	79.3	166
	SR	-	-0.9	-0.7	-0.3	0.8	
Primary school	EC	-	24.8	0.3	19.2	7.7	52
	SR	-	-2.8	1.5	2.2	1.2	
<b>Total</b>						<b>400</b>	

\*EC means Expected Count; SR means Standard Residual; SD means Strongly Disagree; D means Disagree; N means Normal; A means Agree; SA means Strongly Agree.

Chi-Square Test ( $\chi^2$ )

	Value	df	Asymp. Sig (2-sided)
Pearson Chi-Square	24.498 <sup>a</sup>	9	0.004
Likelihood Ratio	25.956	9	0.002
Linear-by-Linear Association	7.831	1	0.005
N of Valid Cases	400		

a. 4 cells (25%) have expected count less than 5. The minimum expected count is 26.

Variable E		Water pollution can cause the loss nutrients and will effect the plants species.					Total
Education Level		SD	D	N	A	SA	
University	EC	-	8.5	23.7	30.0	11.8	74
	SR	-	-1.9	-1.2	1.1	1.5	
College	EC	-	43.7	34.6	16	17.3	108
	SR	-	-0.3	0.4	12.4	-1.0	
Secondary school	EC	-	19.1	53.1	67.2	26.6	166
	SR	-	-0.2	-0.6	0.0	1.1	
Primary school	EC	-	21.1	16.6	6.0	8.3	52
	SR	-	-0.9	1.8	1.2	-2.2	
<b>Total</b>						<b>400</b>	

\*EC means Expected Count; SR means Standard Residual; SD means Strongly Disagree; D means Disagree; N means Normal; A means Agree; SA means Strongly Agree.

Chi-Square Test ( $\chi^2$ )

	Value	df	Asymp. Sig (2-sided)
Pearson Chi-Square	22.586 <sup>a</sup>	9	0.007
Likelihood Ratio	25.105	9	0.003
Linear-by-Linear Association	8.835	1	0.003
N of Valid Cases	400		

a. 0 cells (0%) have expected count less than 5. The minimum expected count is 5.98.

According to the Table 2, the analysis of respondent's perception on water pollution in Malacca River can be describe through  $\chi^2$  test between education level with five variables, namely variable A, Malacca River is dirty; variable B, industrial waste and excretion waste will cause the water in the river to become black color, smelly and contaminated; and variable C, that polluted water river can cause disease, cause aquatic animals to die and become a dirty habitat to animals; variable D, contaminated water can cause aquatic species to become extinct; and variable E, water pollution can cause the loss of nutrients and affect plants. The results show that

there is a significant correlation ( $\chi^2 = 38.649$ ,  $df = 9$ ,  $P < 0.05$ ) in variable A between education level (university level: 26 agree, 21 strongly agree; college level: 9 normal, 28 agree, 38 strongly agree; secondary level: 58 agree, 48 strongly agree; primary level: 4 agree, 13 strongly agree) with Malacca River is dirty. Secondly, variable B showed significant correlation ( $\chi^2 = 19.291$ ,  $df = 9$ ,  $P < 0.05$ ) between education level (university level: 12 strongly agree; college level: 8 normal, 37 agree, 17 strongly agree; secondary level: 57 normal, 68 agree; primary level: 4 normal, 21 agree, 18 strongly agree) with agreement that industrial waste and excretion waste will cause the water in the river to become black,, smelly, and contaminated.

Continuously, variable C indicates a significant correlation ( $\chi^2 = 34.443$ ,  $df = 9$ ,  $P < 0.05$ ) between education level (university level: 32 agree, 33 strongly agree; college level: 11 normal, 48 strongly agree; secondary level: 18 normal, 74 strongly agree; primary level: 1 normal, 22 agree, 5 strongly agree) with polluted water river can cause disease, cause aquatic animals to death and become habitat to dirty animals. Fourthly, variable D provided the result of a significant correlation ( $\chi^2 = 24.498$ ,  $df = 9$ ,  $P < 0.05$ ) exist between education level (university level: 35 strongly agree; college level: 1 normal, 15 agree, 51 strongly agree; secondary level: 79 strongly agree; primary level: 1 normal, 19 agree, 7 strongly agree) with contaminated water can cause aquatic species to become extinct. Lastly, variable E showed a result with a significant correlation ( $\chi^2 = 22.586$ ,  $df = 9$ ,  $P < 0.05$ ) between education level (university level: 30 agree, 11 strongly agree; college level: 34 normal, 16 agree, 17 strongly agree; secondary level: 67 agree, 26 strongly agree; primary level 16 normal, 6 agree, 8 strongly agree) and the claim that water pollution can cause the loss nutrient and will affect the plants species.

Based on the results, education level has a significant correlation with all the variables. In other words, education is very important to all aspect because it helps people to think critically and advanced towards a particular issue or problem that may harm or cause dangerous to the society. For example, the Malacca River will never be polluted, except when human activities are carried out such as industries or factories which leading to the releasing of wastes and excretion either directly or indirectly into the river. This is the

main reason for the river to change and become black, smelly, and contaminated. A majority of respondents from university level to the primary level are agreed that when there is water pollution in the river, it will cause disease and poisoning the aquatic animals until death. If contamination in the river is elevated, this will cause aquatic species to become extinct. At the same time, the polluted water can become habitat to the dirty animals. Due to the length of time they have stayed near the Malacca River, some respondents strongly agree that water pollution not only can harm the ecosystem, but also affect plants through the loss of soil nutrients. This issue is affecting the respondents that carry out agricultural activities surrounding the house which cause 'death' to the plant. Therefore, most of respondents are agree that water pollution in Malacca River will cause death poisoning and extinction to aquatic animals, affect the plants through loss of soil nutrient, increase dirty animals through habitat, and destruct the environment if the water pollution in river is continuously happening.

After considering respondent perception on water pollution in Malacca River, these issues should be taken seriously and find a solution for the short term before successfully implementing water resource policies in Malacca River. Among the most suitable suggestions is to introduce and applies the law and regulation towards local residents to protect water resources in Malacca River. Hence, the analysis in this section is to determine the effectiveness of law against river pollution in Malacca through the perceptions of local residents, which can be seen in Table 3.

**Table 3:** Respondent's perception on the effectiveness of law and regulation towards water resources in Malacca River

	Variable A	Variable B	Variable C	Variable D	Variable E
Water Act 1920	r = 0.471** P < 0.01 N = 400 Significant	r = 0.484** P < 0.01 N = 400 Significant	r = 0.420** P < 0.01 N = 400 Significant	r = 0.333** P < 0.01 N = 400 Significant	r = 0.631** P < 0.01 N = 400 Significant
Environmental Quality Act 1974	r = 0.254** P < 0.01 N = 400 Significant	r = 0.258** P < 0.01 N = 400 Significant	r = 0.186** P < 0.01 N = 400 Significant	r = 0.137** P < 0.01 N = 400 Significant	r = 0.296** P < 0.01 N = 400 Significant
Sewage and Industrial Effluent 1979	r = 0.910** P < 0.01 N = 400 Significant	r = 0.726** P < 0.01 N = 400 Significant	r = 0.850** P < 0.01 N = 400 Significant	r = 0.791** P < 0.01 N = 400 Significant	r = 0.748** P < 0.01 N = 400 Significant
Sewage Service Act 1993	r = 0.764** P < 0.01 N = 400 Significant	r = 0.822** P < 0.01 N = 400 Significant	r = 0.839** P < 0.01 N = 400 Significant	r = 0.759** P < 0.01 N = 400 Significant	r = 0.822** P < 0.01 N = 400 Significant
River Act	r = 0.740** P < 0.01 N = 400 Significant	r = 0.749** P < 0.01 N = 400 Significant	r = 0.770** P < 0.01 N = 400 Significant	r = 0.799** P < 0.01 N = 400 Significant	r = 0.740** P < 0.01 N = 400 Significant

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* r means Pearson Correlation, P means Significant (2-tailed), N means Total Number.

Referring to the Table 3, the law and regulation will be versus with water pollution in Malacca River to evaluate the significant between two variables through correlation analysis. Water Act 1920 showed weak-positive significant correlation with variable A (r = 0.471), variable B (r = 0.484), variable C (r = 0.420), and variable D (r = 0.333), but a normal-positive significant correlation with variable E (r = 0.631) at level P < 0.01. The Environmental Quality Act 1974 proved a very weak-positive significant correlation with variable A (r = 0.254), variable B (r = 0.258), variable C (r = 0.186), variable D (r = 0.137), and variable E (r = 0.296) at level P < 0.01. Next, the Sewage and Industrial Effluent 1979 indicates that it has strong-positive significant correlation with variable A (r = 0.910), variable B (r = 0.726), variable C (r = 0.850), variable D (r = 0.791), and variable E (r = 0.748) at level P < 0.01. Fourthly, Sewage Service Act 1993 also showed a strong-positive significant correlation with variable A (r = 0.764), variable B (r = 0.822), variable C (r = 0.839), variable D (r = 0.759), and variable E (r = 0.822) at level of P < 0.01. Lastly, the River Act has a strong-positive significant correlation with variable A (r = 0.740), variable B (r = 0.749), variable C (r = 0.770), variable D (r = 0.799), and variable E (r = 0.740) at level P < 0.01.

Overall, all of the variables show significant with all the law and regulation. In other words, majority of respondents are agreed to apply the law in their daily life with the aims to protect the water resources in Malacca River. Any individuals throwing garbage and wastes, chemicals and toxin, and any object that exist in solid or liquid form which can directly cause the changes to the quality of water resources in the river will be subjected to be punishment (either in the form of money, imprisonment, lashes, and so on, where applicable) according to the law of Water Act 1920, Environmental Quality Act 1974, and River Act. Secondly, if any individuals place tar material or other insoluble liquid, flammable solvents or garbage, sawdust, human or animal waste or solid materials, and any public sewer or public treatment or even any industrial effluent directly into the water and cause river to polluted will be punished (either in the form of money, imprisonment, lashes, and so on, where applicable) according to the Sewage and Industrial Effluent 1979, and Sewage Service Act 1993. However, if there are any individuals that remove, release, or place waste material which may fall, flow down, blown off or even wash away to cause

river water pollution will be punished according to the Environmental Quality Act 1974 and River Act. In the opinion of respondents, they agreed to choose law as a guide for the individual that making mistake which affect the environment, aquatic animals, and community or society, the sentence should be carried out to those offenders. At the same time, punishment in the form of money or jail may not dramatically impact them, so minority of respondents suggested that an offender should be punished through whipping and cleaning the river so that they aware on their actions that can bring negative impact. Therefore, education level (which is not only based on emotional but through critical thinking when giving the answer to questionnaire) is important to evaluate the condition of water resources in the river and helps to determine the laws and regulations that should apply towards the water resources in Malacca River.

#### IV. CONCLUSION

This research study has proven that law enforcement can help to protect the environment especially towards water resources in Malacca River. A majority of respondents agreed that human activities without control can cause Malacca River to become black, smelly, and polluted. Once the river is polluted, the water can bring disease and poison aquatic animals to death and affect the plant species through the loss of nutrients. At the same time, if the water quality conditions become worse, this will not only cause aquatic species to become extinct, but also become a dirty habitat for animals. So, respondents prefer to apply laws and regulations such as the Water Act 1920, Environmental Quality Act 1974, Sewage and Industrial Effluent 1979, Sewage Service Act 1993, and River Act [3] to the Malacca River. The main aim and reason to use laws and regulations is not to blame activities carried out daily (for example factory operators), but to create awareness that every action and attitude of individuals must be controlled to have a responsible attitude towards the environment. Hence, law enforcement should be used and applied in Malacca state especially for the river to protect water resources from continued pollution.

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# Role of Microarray Technology in Diagnosis and Classification of Malignant Tumours

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## ABSTRACT

Typical cancer research including diagnosis and classification of tumours performed via clinical, cytological and histopathological methods. However, some cases show diagnostic disturbances due to inadequate clinical information and aberrant histopathological characters which together demonstrated the disadvantages of typical cancer research in the mentioned branches. Recently, development of microarray technology and gene expression profiles emerged for diagnosis and classification of tumours. This technology could overcome some disadvantages of conventional methods of diagnosis and classification and covered some aspects of unresolved tumour's problems. In this review, we focused on recent discoveries in tumour's diagnosis and classification via microarray technology and importance of this technology in cancer research area. Some important types of cancers recently analysed via microarray technology and reviewed in this paper are such as oral cancers, ovarian cancers, colorectal carcinomas, melanomas and prostate carcinoma and breast cancer. Also classification of some tumours using microarray technology discussed here for emphasis the prominent role of microarray technology in cancer research.

**Keywords:** Microarray technology, Gene expression profile, Cancer research, Diagnosis, classification of tumours.

## I. INTRODUCTION

Cancer is the second cause of death which basically is diagnosed using histopathological and cytological techniques [1]. However, sometimes application of histopathology and cytology show diagnostic disturbances due to inadequate clinical information and aberrant histopathological characters which together demonstrated their disadvantages [2]. Additionally in tumour study and their classification, several molecular diagnosis and observations aided scientists which usually are such as use of diagnosis of tumour biomarkers, genetic mutations and chromosomal variations, looseness of the long arm of chromosome 14 and activation of *c-kit* mutation occurred in gastrointestinal stromal tumours, in acute myelogenous leukemia occurrence of gene rearrangement of *bcr/abl* and its use as biomarkers and so on. [3-6]. Although these diagnostic observations are available but due to inconsiderable number of target tumour markers, molecular diagnosis is not typically accepted for study

and classification of tumours. Moreover, most of genetic variations are not adequately specific to precisely diagnose the tumours and encyclopedic mutation databases for the tumours have not been searched. By development of gene expression profiling via DNA micro-array technology and its application in tumour diagnosis and classification, scientists could advance in diagnosis and classification of tumors. DNA-microarray technology has been facilitated several specific diagnostic marks and observations in different type of tumours such as identification of gene subsets by their characteristic expression, in oral cancers, ovarian cancers colorectal carcinomas, prostate carcinomas and melanomas. Moreover, it provides scientists and researchers with molecular classification of soft tissue sarcomas and classification of tumours to determine primary sites in metastatic cancers. DNA microarray also helps in identification of intertumoral variations within the same histologic tumour types. These variations are used for proper molecular classifications and genetic changes evaluations. Gene expression

profiling via DNA microarray also helps successfully in differentiation of Leukemia types and its oncogenetic route for T-cell type. Additionally this technology helps in identification of a subset genes involved in different biological and genetic changes in solid tumours. [2]. In this paper we reviewed recent discoveries in cancer research including diagnosis and tumour classification in different types of cancers for the purpose of illustration of prominent role of microarray technology and encourage scientists for application of this technology in their cancer research programs for precise diagnosis and tumour classification.

## II. METHODS AND MATERIAL

### A. Recent discoveries in cancer research in association with microarray technology

*Application of cDNA microarray in ovarian cancer research:* Ovarian cancer is one of the most common cause of deaths among the woman. The prognostic factor for this type of cancer is stage of tumour or disease extension at diagnosis. One of the way for treatment of this cancer is its diagnosis at early stage. cDNA microarray technology has facilitated understanding of early stages and exploration of progression steps in ovarian carcinogenesis. Scientists have applied combined genetic methods for analysis of 21 early tumour stage and 17 late stage in ovarian cancer. These applied techniques included expression profiling with cDNA microarrays consisted of approximately 18,000 expressed sequences and comparative genomic hybridization for finding chromosomal loci of copy number for both gain and loose conditions. The result has demonstrated the exhibition of profound alternations of early stage ovarian carcinogenesis in gene expression which showed similarity to those detected in late-stage tumours. However, observation of differences at genomic level proposed differences between the two stages and accommodate a base for a promotion model for development of ovarian cancer [7].

*Oral cavity cancer analysis via microarray contribution:* Scientists carried out a large scale expression profiling using high-density oligonucleotide microarrays for analysis tumor oral epithelial cells. In this analysis research approximately 600 genes were identified in association with oral cancer. These genes include tumor

suppressors, oncogenes, transcription factors, metastatic proteins, differentiation markers, xenobiotic enzymes, and non –implicated genes in oral cancer. This created database serves as a valid comprehensive profile of gene expression for analysis of oral carcinogenesis reporting the significant role of recognized genes as well as and non –implicated genes in oral cancer. In this study, scientists demonstrated the LCM application for harvesting of tumor and normal cells from a solid tumor site of oral cavity cancer type. Linear amplification of mRNAs carry out by three rounds of T7 RNA polymerase reaction, then biotinylation is done and subsequently hybridization to HuGeneFL-microarrays performed.[8].

*Contribution of microarray to identification of genes associated with human melanoma:* The need for identification of new molecular markers for diagnosis and analysis of progression stage of melanoma come from the fact that the typical treatment and diagnosis methods are not effective and not completely responsive. Recently, scientists has been developed a new technique for screening of expression of thousands of genes at the same times via high density oligonucleotide arrays. They have used this technique for comparison of the patterns of mRNA of two human melanoma cell lines which show differentiation in their metastatic behavior. Validation of oligonucleotide array's results carried out by methods of Northern blotting and reverse transcription-polymerase chain reaction (RT-PCR) using eight differentially expressed genes named as transforming growth factor-beta superfamily, tyrosinase-related protein 1, , alpha2-macroglobulin, human cell division cycle 10 and serine/threonine protein kinase (DYRK1A), apolipoprotein CII, subtilisin-like protein, elongation factor 1 alpha2. Finally their data and results demonstrated the reliability and accuracy of the high density oligonucleotide arrays for screening for differentially expressed genes in melanoma. This study may be considered as a fundamental step in the identification of malignant melanoma associated genes .[9].

*Revealing of alterations of gene expression in colorectal carcinogenesis by cDNA microarrays:* Recently scientists identified a set of genes implicated in the progression of colorectal carcinogenesis. They used a DNA microarray including 9216 human genes for comparison of expression profiles of colorectal

cancerous cells from 8 tumors with analogous non-cancerous colorectal cells. Laser-capture microdissection has been applied to this cell community for rendering homogenous. The researchers observed expression change for 235 genes including 191 down-regulated and 44 up-regulated genes in more than half of the tumors. The genes which differentially expressed showed association with metabolizing enzymes, signal transduction, production of reactive oxygen species, mitosis, cell cycle and apoptosis. Their analysis examination provided a reliable and valuable database for colorectal carcinogenesis and provide a strong source of novel target for cancer treatment.[10-11]

*Aid of microarray technology in identification of potential markers and pharmacological targets in prostate cancer:* Recently, researchers monitored expression levels of more than 8900 genes in normal and cancerous prostate cells in order to characterize primary prostate cancer. By analysis gene expression patterns, they revealed a precise difference between normal and tumor cases and also found a remarkable group of about 400 genes that showed overexpression in tumor tissues. Their research and analysis provided identification of prostate implicated genes and their act association with a various biochemical pathways and encoding secreted molecules with diagnostic possibility, such as, MIC-1 and the secreted macrophage inhibitory cytokine, and detection of some genes like fatty acid synthase encoding enzymes which serves as drug targets in other contexts which all together provided new therapeutic approaches. [12-13]

*Breast cancer research via microarray technology:* Due to scant numbers of precise predictive and prognostic factors which are clinically and histopathologically identified and the need of high throughput technology in breast cancer researches for overcome such disadvantages, DNA microarray has taken a place in breast cancer research for assessment of the expression of thousands of genes simultaneously as well as rapidly. Gene expression profiling provided identification of prognostic gene sets that facilitate prediction of a short intervals to remote metastases.[14]. Using microarray technology study of epigenetic mechanism of tumorigenesis is provided which is helpful in management of cancer. Recently scientists identified changes in genome-wide DNA methylation in a breast cancer metastasis using a cell-line model. They

analyzed complicated epigenetic changes and karyotype which led to establishment of hypothesis that multiple genomic changes such as translocations, deletions and ploidy in breast cancer cells are overlapped to over promoter-specific methylation conditions that are involved in gene-specific expression alternations occurred in breast cancer metastasis. Scientists carried out high resolution, whole-genome analyses of MDA-MB-468GFP and MDA-MB-468GFP-LN human breast cancer cell lines simultaneously using combination copy number variant/single nucleotide polymorphism microarrays. Their approach facilitated more precise profiling of functionally related breast cancer associated epigenetic signatures.[15]

## **B. Role of microarray in molecular classification of cancers:**

Although classification of cancers has been started over 30 years and its improvement has been seen but still there is no common approach for identification and prediction of new cancer classes for assessment of tumors to already identified classes. Researchers developed a general approach for classification of cancers based on gene expression profiling via DNA microarray. They developed this approach and applied it to human acute leukemias as a test sample. The discovery achieved using this approach was the identification of difference between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) independent of previous knowledge about their biology. Their approach suggests a generic strategy for discovery and prediction of cancer classes of other types of cancers without knowing previous knowledge of their biology. In another approach, researchers established a project for organ-specific molecular classification of primary lung, ovarian and colon carcinomas. [16]. Scientists has been reported gene expression profiles of 154 primary adenocarcinomas of the lung, ovary and colon. They generated general gene expression profiles of 57 lung, 51 colon, and 46 ovary adenocarcinomas using high-density oligonucleotide arrays consisting 7129 gene probe sets, and then using nearest neighbor classification, their principle component analysis and cross validated prediction analyses carried out. These statistical analyses showed the classification of 152 of 154 of the adenocarcinomas in an organ-specific behavior and determined genes expressed in a putative tissue-specific behavior for each

tumor type. Additionally identification of two tumors, one in the ovarian group and another in the colon group carried out. These two tumors did not integrate to their corresponding organ-specific groups. Their studies suggest the application of gene expression profiles for classification of tumors and determination of organ-specific gene expression profiles and precise molecular diagnosis. [17]. Analysis of patterns of gene expression of soft tissues tumors including fibroblasts, muscle cells, or adipocytes was carried out using cDNA microarrays. In this approach, 41 soft tissue tumors subjected to cDNA microarrays. After analysis of expression patterns of 5520 genes, they carried out separation of tumors into different groups by hierarchical clustering and singular value decomposition. Their results demonstrated that, Gastrointestinal stromal tumours Synovial sarcomas, neural tumours, and a subset of the leiomyosarcomas, exhibited different gene-expression patterns. Liposarcoma, malignant fibrous histiocytoma, , and the other leiomyosarcomas showed molecular profiles that were not previously identified by prediction methods or immunohistochemistry methods . Their studies and results provided a new approach for classification of soft tissue tumors which is not previously carried out via histopathological methods. This approach helps in improvement of histological finding as complementary method for distinction between the tumors. Thus these discussed findings as examples of application and contribution of microarray technology showed its significant role in identification of new cancer associated genes and classification of tumors in distinct groups for better diagnosis and treatment in cancer control programs . [18]

### III. CONCLUSION

The molecular background of cancers has been reevaluated over the past decade. General cancer research mainly bases on clinical, cytological and histopathologically methods but in some cases , due to diagnostic disturbances emerged from inadequate clinical information and aberrant histopathological characters, the need for advanced methods demands. Microarray technology emerged complementary method with providing a enormous source of data on gene expression changes in cancers. Microarray technology has been established a strong tools in unrevealing problems in oncology .The fundamental studies carried

out via microarray technology has been promising enough for providing an individualized dimension to cancer therapy .Microarray technology provides identification of cancer involved genes, cancer associated epigenetic signatures, biomarkers, novel targets genetic changes evaluations and classification of tumors in distinct groups for better study and treatment of cancer. Although microarray analysis is a promising diagnostic method for cancer diagnosis and classification, but it is not separated from limitations. The limitations related to this modality are firstly detailed and exact diagnoses of individual tumors by gene expression profiling lonely are not always feasible. This may be due to unavailability of reference database for encyclopedic gene expression for cancer and lack of development of specific biomarker groups for diagnosis of specific-corresponding tumors .Secondly, the gene expression reports exhibit significant variation within the same tumor due to distinct gene expression profiles in the tumor and the different stromal reaction or peritumoral lymphoid condition. Thirdly, early tumor diagnosis is not possible via microarray technology (gene expression profiling). At the present, cancer diagnosis via DNA microarray is feasible only when large amount and large slice of tumor cells are provided. In spite of these drawbacks, microarray is effectively used for diagnosis of tumors and their molecular classification based on and biological and genetic changes.

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# Role of G-Protein Coupled Receptors in Cancer Research and Drug Discovery

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## ABSTRACT

G-Protein Coupled Receptors as the largest class of cell surface signaling proteins are widely considered in drug discovery programs as therapeutic drug targets; their contribution in drug discovery process is because of their critical role in many physiological functions and their link with emerging different types of diseases including cancer development and cancer metastasis. Their association to hereditary diseases, made them important for examination as novel targets too. As they constitute the target of 50% of the available therapeutic agents in the market, they are still most attractive potential targets for developing new drugs especially anti-cancer drugs. Their structure –function relationship have been made them a powerful basis for screening a large number of pharmaceutical products. In this paper we purpose to review significant role of the GPCRs in cancer development and drug discovery along with the information regarding their structure, classification and recent studies and findings about their position as drug targets. This review provides scientists for development of favorable opportunities for drug discovery in cancer research including prevention and diagnosis and treatment.

**Keywords:** Corporation of G-Protein Coupled Receptors, Drug Target, Drug Discovery, Associated Disesaas, Cancer

## I. INTRODUCTION

G-Protein Coupled Receptors are the largest class of cell surface signaling proteins which form the biggest class of drug targets. Their physiological functions and their link with emerging different types of diseases especially development of cancers and metastasis, have been made them significant for drug discovery programs and hiring them as therapeutic drug targets. In this paper we first focus on their structure, classification, mechanism of peptide and non-peptide interactions then discuss their role in drug discovery and recent finding related to their position as novel drug targets in drug discovery. [1,2].The purpose of this review is to redefine the structure-function relationship of the CPCR as a valuable source for development of novel drugs and provide favorable and tremendous opportunities in prevention and treatment of cancers. Mechanism of their

activation is carried out through ligand binding to a G-protein, which in turn activates or inactivates an enzyme that produces a specific second messenger or induces modulation of an ion channel, results in an alternation in membrane potential. Examples for GPCRs are the receptors for calcitonin, glucagon, serotonin, epinephrine, etc. The CPCR consisting of seven hydrophobic regions that constitute membrane stretching alpha helices, joined by intracellular and extracellular loops. The intracellular loops constitute the heterotrimeric G-protein binding domains and include three subunits: the alpha subunit (35 kDa) family, the beta subunit (35-36 kDa) family and the gamma subunit(6-10kDa) family. After ligand –receptor binding, the heterotrimeric G-proteins detach into two activated subunits:  $G\alpha$  GTP and  $G\beta\gamma$ .The ligand binding stimulus conformational change of the receptor, which leads to alternation of the  $G\alpha$ , decreases the affinity for GDP,

which is replaced by GTP. The Galpha CTP conformation has a decreased affinity for G beta gamma, results in dissociation of subunits from each other and the receptor. Activation of effectors is carried out by Ga GTP and free Gβγ. The hydrolysis of GTP is carried out by its intrinsic GTPase activity. Moreover, acceleration the intrinsic GTPase activity of Ga is aided by regulatory proteins (RGS proteins). This signaling route is deactivated during hydrolysis of GTP to GDP by Ga which subsequently binds free Gβγ to form an inactive heterotrimer which may be bound to a GPCR. This mechanism of signaling serves as a switch that can turn on and off signaling routs. GPCRs are classified into three distinct groups : Class A: Rhodsonian-like-receptors which form the biggest class of GPCRs including receptors for Rhodsonian and adrenaline and most other 7 transmembrane receptor types such as olfactory subclass. Class B: This class consists of approximately 25 membranes, including receptors for corticotropic releasing hormones, calcitonin hormones, parathyroid hormone, growth hormone releasing hormone and gastrointestinal peptide hormone family like secretin, VIP, glucagon. The activation of the receptors of this class is carried out by large peptide including high amino acid identity. Class C: This group is the smallest group of GPCRs and contains calcium sensing receptors and metabotropic glutamate. The prominent characterization of this class is a large N terminal domain with specific motifs containing the proper neurotransmitter or hormone binding sites. Mechanism of G-protein binding is done through the following stages: first the activation of the receptor is carried out by the ligand (agonist), secondly, Binding G-protein to the activated receptor is occurred, during G-protein coupling progression, ligand binding to the receptor become stronger. Then the release of GDP occurred-protein picks GTP up, GTP triggers the dissociation of G-protein from the receptor. Mechanism of peptide interaction is as follow: First binding of C-terminal of the peptide and N-domain of the receptors occurred, subsequently, binding of the N-terminal of the ligand to the J-domain occurred which leads in activation the receptors results in stimulation of intracellular signaling. Non-peptide interaction with class B is as follow: first of all, binding of the non –peptide antagonist to the J-domain occurred leads to formation of antagonist-receptor complex while the peptide ligand is free. In this interaction, a change in the J-domain occurred, inhibiting peptide binding to the J-domain,

subsequently, the interaction between the peptide-J-domain is blocked so that the receptor signaling is blocked by the non-peptide antagonist, this is because of prevention of G-protein activation. One conclusion is obtained from non-peptide interaction which is allosteric modulation of the GPCRs. This deduction suggests the significant role of the GPCRS in drug discovery as drug target using their modulation. [1-5].

## II. METHODS AND MATERIAL

### Role of the GPCRs in cancer research and drug discovery:

In the previous section, we mentioned the role and importance of the GPCRs in drug discovery as therapeutic drug target. Among classes of GPCRs, class B are largely participated in major pathophysiological and biological functions, hence they have verified to be a big opportunity for designation of novel drugs. Their functions made them stimulus for drug discovery programs and also supported the studies performed using GPCR knockout animals. In this section, for better understanding the role and participation of GPCRs in drug discovery we discussed some recent discoveries about their selection as drug target in drug discovery programs. Recently, scientist worked on discovery of novel therapeutic targets and prognostic factors in head and neck squamous cell carcinoma (HNSCC). They succeeded with identification of several G protein-coupled receptors (GPCRs) as potential candidates. They have observed the occurrence of important epigenetic silencing of GPCR expression as compared to healthy tissue which showed significant correlation with clinical behavior. Additionally, they have observed the suppression tumour cell growth by GPCR activity which together indicates potential utilization of the GPCR expression as a prognostic factor. In the previous studies some receptors identified in association with HNSCC such as *galanin receptor type 1 (GALR1)* which function in inhibition of HNSCC cells though ERK1/2-mediated effects on proteins controlling cell cycles such as p27, p57, and cyclin D1, *type 2 (GALR2)*, its role is indicated by its inhibition of activity for cell proliferation and induction of apoptosis in HNSCC cell, *tachykinin receptor type 1 (TACR1)* and *somatostatin receptor type 1 (SST1)* which both are in association with significantly decreased diseases –free survival and a higher frequency rate. Their studies demonstrated the

potential utility of the GPCRs in HNSCC research as its use as therapeutic drug target.[6].In another approach, scientists understood the role of G-protein estrogen receptor (GPER) in breast cancerous cells and cancer associated fibroblasts(CAFs).In this study, they have demonstrated binding of nicotinic acid and nicotinamide to the GPER-mediated signalling pathway results in its activation and played important role in breast cells and CAFs. Both of nicotinic acid and nicotinamide were able to promote the up-regulation of GPER target genes via the EGFR/ERK transduction pathway. Their studies support the supplementary role of the two molecules (nicotinic acid and nicotinamide) in induction of proliferation and migration of cancerous cells in breast and CAFs via GPCR-dependent route. Their study supported significant role of GPCR as therapeutic drug target [7].Another interesting research on GPCRs has shown their significant role in cancer development via GPCR-CARMA3-NF- $\kappa$ B signaling axis which is appearing as a new therapeutic drug target for cancer research. In this signaling route, activation of NF- $\kappa$ B is carried out by GPCR and abnormal regulation of GPCR-NF- $\kappa$ B signalling axis leads to cancer development. However, it has been shown that a new scaffold protein named CARMA3 is crucial in activation of GPCR-NF- $\kappa$ B signalling axis, so GPCR-CARMA3-NF- $\kappa$ B signalling axis forms a opportunity for drug discovery for treatment and control of diseases. [8].The role of GPCRs in development of colon carcinogenesis has been evaluated. In one if related study, scientists investigated the increased gene expression of G protein-coupled receptor 48 (GPR48) in the p27<sup>+/-</sup> cells which form cyclin-dependent kinase inhibitor involved in increased tumor malignancy and weak prognosis in HCT116 human colon carcinogenesis. They have also showed the participation of GPR48 in lung carcinoma and lymph node metastasis. So this potential prognostic factor can be utilized in cancer research and drug discovery as a therapeutic target. [9].Scientists have examined the expression of 929 GPCR transcripts in tissue samples of squamous cell cancer (10 patients) and adenocarcinoma (7 patients) for identification of novel targets for treatment of non-small cell lung carcinoma (NSCLC).As a result, they have identified 5 significantly increased expressed GPCRs associated in squamous cell carcinoma.These five GPCRs are arranged in descending order of expression as follow: GPR87 > CMKOR1 > FZD10 > LGR4 > P2RY11. Out of which, LGR4 and CMKOR1 are orphan receptors and

GPR87 has shown potential utilization in drug discovery as a target validation because of its prominent overexpression and link with squamous carcinoma. [10].In another studies, the role of orphan G-protein-coupled receptor, *Gpr49* in human hepatocellular carcinomas and GPR56 in tumor adhesion evaluated which demonstrated the prominent role of GPCRs family in cancer development and their utilization as valid candidate for target validation [11,12].Recent studies demonstrated that over-expression of some genes encoding GPCR-PCa, PSGR2, CaSR, GPR30, and GPR39 [13]. were linked with carcinogenesis and metastasis in various type of cancers.GPCR-PCa and PSGR2 associated in human prostate cancer [14,15] CaSR associated with breast cancer cells evolved from bone metastases [16,17].GPR30 is in association with breast cancer cells and induces proliferation and migration of them via connective tissue growth factor[18,19]. GPR39 is involved in lymph node metastasis and advanced TNM stage [20]. Additionally, many GPCR ligands such as phingosine-1-phosphate [21]. LPA [22-24], thrombin [25]. platelet-activating factor [26,27], interleukin-8 [28], monocyte chemoattractant protein 1 [29],growth regulated oncogene  $\alpha$ - $\gamma$  [13],and stromal cell-derived factor have also been observed and examined for their participation in vasculogenesis, tumor-induced angiogenesis, tumour growth and metastasis [30]. These findings have been helped in providing new strategies for cancer control including prevention, diagnosis and treatment.

### III. CONCLUSION

The GPCR family as the largest cell-surface proteins in mammalian genomes are physiologically and biologically very important and any disturbance in their function and genes are associated with development of various types of diseases. Among the associated diseases, development of various types of cancers such as breast, prostate, head and neck squamous cell carcinoma (HNSCC), colon cancer, squamous cell carcinoma (SCC) of the lung, hepatocellular carcinoma, basal cell carcinoma (BCC) and lymph node metastasis are investigated well. The GPCRs family have shown potential utility as therapeutic target in cancer research studies and control. Although GPCRs introduced as a significant target group for various type of cancer in for pharmaceutical therapeutics, some limitations are visible

in their role as potential drug targets which is mainly due to problems in the identification of their natural ligands. Although high-throughput screening technologies effectively has been solved these difficulties but due to multiple roles and associations of GPCRs in cancer prevention, diagnosis and treatment, have been demanded more precise and integrative system biology approaches for unrevealing biological mechanism of cancers and to decrease limitations are present in diagnosis and drug development for cancerous diseases. Recently for rapid and precise cancer control, multidisciplinary approaches such as Bioinformatics, Biotechnology, engineering and medicine and biology disciplines related to physics have been utilized together for understanding the dynamic networks of GPCRs interactions within a cell. The need for development of new technologies and new systems biology approaches for identification, study and analysis of GPCRs in the cancers are urgent. The newly developed sciences such as bioinformatics with cooperation mathematics helped in revealing signaling networks linked with GPCRs. The new technologies help in revealing abnormal regulation of GPCRs associated signaling axes. We hope in near future effective and complementary technologies will be developed for cancer control programs depend on GPCRs associations. At the end We have to add this point, although there are various cell surface drug targets such as ion channels, [31], Cyclin independent kinases (CDKs)[32], Aquaporins [33] and so on which have been shown successful results in cancer diagnosis, prevention and treatment, but GPCRs as they have formed the largest drug targets marketed so far, they deserve the most important attention in drug discovery programs.

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# Role of Biomarkers in Cancer Research and Drug Development

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## ABSTRACT

Biomarkers as characterizing agents of patient colony and quantifying factors to a specific degree to which developing drugs reach the intended targets, induce alternation of proposed pathophysiological mechanism and obtain desirable clinical results. These specific molecular signatures have been emerged rapidly in cancer research and cancer related drug development programs and have been helped vigorously in validation of novel drug targets and prediction of drug response which together lead to precise and strong treatment of cancer diseases. In this paper we have discussed emergence of biomarkers in drug development linked to cancer treatment and highlighted their significant role in the mentioned area. The purpose of this review is to redefine the biomarker evolution in drug development related to different types of cancers and a brief background about their classification, their assessment and technologies used for their identification in order to encourage scientists for strong focus on biomarkers in their research programs related to cancer treatment. Several types of biomarkers are discussed in this paper are such as biomarkers for breast, prostate, colon, ovarian, lung, cervical cancers, hepatocellular carcinoma, nasopharyngeal carcinoma and myeloid leukaemia.

**Keywords:** Biomarkers, Cancer Research, Drug Development, Target Validation, Biomarker Classification

## I. INTRODUCTION

Cancer as the second cause of death among high risk human diseases developed due to alternation of genome, hence tumour growth dynamics and diagnosis at molecular level are essential elements for supporting therapeutics. Although classification of human tumours is based on the site of origin and their morphological and anatomical assessment relied on tumor node metastasis (TNM) with the help of histopathological methods, monitoring progression of diseases is supplemented by application of prognostic biomarkers which are able to predict the clinical results of a therapy. So the emergence of biomarkers in drug development is prominent from this point that they play an important role in characterization of cancers and quantifying of new drugs to an specific extent in order to reach the proposed targets, change intended pathophysiological

systems of the body and obtain desirable clinical outcomes. Biomarkers are diagnostic which are considerably measured and assessed as an indicator of normal biological and pathophysiological conditions or pharmacological responds to a therapeutic mediation. [1,2]. They also help in determination of surrogate endpoint and facilitate rational basis for lead compound selection. Generally biomarkers based on their clinical application classified in the following groups: Type 0: These group of biomarkers supposedly measure innate history of a disease and correspond over time with known clinical indicators. Type I: These types of biomarkers indicate the mediation effect of, for example a remedial drug and Type II: These biomarkers are seen to be both surrogate endpoint markers and clinical endpoints. Another classification of biomarkers relied on quantitative indicators of common biological or pathogenic conditions, or drug responses. These

biomarkers are useful for prediction and treatment of diseases and furnished with one or more of the following assets : 1: Biomarkers that are particularly linked with disorders in a population 2: those are inherited 3: Independence and presence state , regardless of the presence of clinical phenotype of the disorder .4: Co-dissociation with illness within families and, 5: presence in family's relatives with illness at a higher rate than in the common population .Classification of above discussed biomarkers which are in association with diseases are as follow: 1: Protein biomarkers: Proteomic assessment provides a direct measurement of the alternation in levels of protein in biological samples 2: NA-base molecular biomarkers : Utilization of mRNA – expression profiling gives highly proper and reproducible estimation of mRNA in patients 3:small molecule as biomarker : Metabolomics can provide understanding intracellular concentrations of small molecules such as proteins , amino acid, organic acids , sugar and other small molecules which are associated with diseases phenotypes 5: Single nucleotide polymorphism (SNP): As single base pair loci in genome that show variation among individuals in one or numerous populations , these biomarkers are used to determine common diseases such as cancer , heart diseases and diabetes which are susceptible in contribution to the traits that make them unique individuals. In clinical attempts biomarkers are classified in the following way: Preventive biomarkers: These types of biomarkers diagnose individuals at increased risk for pathology development. Diagnostic biomarkers: Their role is identification of the disease presence at the earliest stage before clinical symptoms. Prognostic biomarker: Their role is stratification of risk of diseases progression in patients experiencing actual therapy .Predictive biomarkers: Their role is identification of patients who are mostly expected to respond to particular therapy. Therapeutic biomarkers: Their role is measurement of response in patients experiencing therapy .Special biomarkers: Their role is identification of patients at the risk of development of adverse reactions to specific drug and therapy . We

already discussed different classification of biomarkers, the current biomarkers are widely used are mRNA, proteins,SNP, expression profiling and small molecules . The most important and basic issues supported their emergence in biomarker discovery filed are cost, time and appropriate and suitable technologies .Identification of biomarkers involves combination of several technologies which together help in investigation of disease stages at all levels. The stages in biomarker identification are as follow: analysis of samples, target discovery, target validation, preclinical development, clinical development and approval. Assessment of biomarkers are carried out by following assays: single nucleotide polymorphism, bioimaging, immunoassays , mass spectrometry and quantitative polymerase chain reaction. For biomarker discovery some genomic technologies are applicable which are such as SNP genotyping by DNA microarray which is used for identification of disease gene, microsatellite instability through linkage analysis and positional cloning which are used for mapping disease positions, expression arrays which is used for identification of deregulated genes and signalling routs , comparative genomic hybridization arrays and exon arrays which are used for loss of heterozygosity and amplification of gene . Moreover proteomic and metabolic technologies are used in biomarker discovery for example ,proteomic platforms are such as HPLC, ICAT-MS,LC-MS, MALDI-TOF, MALDI-QTOF and Liquid chromatography which participate in identification of hydrophilic and hydrophobic proteins using blood, urine, saliva , CSF and affected tissues as their samples . Gel electrophoresis, DI GE-MS, 2DE and tissue microarrays participate in detection differences between proteins of two different samples, analysis of protein biomarkers, identification of targets and biomarker validation. All these techniques use affected tissue as their analysing samples. Metabolic platforms are used in biomarker discovery are NMR for identification of small molecules such as amino acids, organic acids, sugars and so on and MS used in identification and characterization of small molecules . The metabolic technologies used saliva,

urine, blood, CSF, serum and cells as their analysing samples. [2-4] Although these techniques are helpful in identification of biomarkers but new technologies have emerged in last decay which are very helpful in identification of biomarkers and saving the time and cost for example , bioinformatics tools for SNP detection [5] and emergence of new animal model of zebra fish and it's biomarkers for screening the developing drugs .[6].

## II. METHODS AND MATERIAL

### **Role of biomarkers in cancer research and drug development:**

Biomarkers play significant role in drug development and especially in cancer research. In this section we focus on previous and recent discoveries related to cancer specific biomarkers to demonstrate their broad area of use in cancer research .Cancer specific biomarkers are discussed here are such as biomarkers are associated in detection of prostate cancer, hepatocellular carcinoma, colon cancer, breast cancer, ovarian cancer,myeloid leukaemia ,nasopharyngeal carcinoma, lung cancer and cervical cancer.[2].

*Role of alpha-methylacyl CoA racemase in prostate cancer:* Alpha-methylacyl CoA racemase (AMACR) is a cancer specific biomarker which is show overexpression in prostate cancer as compared to benign prostatic tissue. Researchers explored the use of alpha-methylacyl CoA racemase as a biomarker for prostate cancer. They have used immunohistochemistry for determination of AMACR protein expression. In their research, they have used an image analysis system on two localized prostate cancers groups including radical prostatectomy treated group (204 men ) and 188 men followed waiting . In this research, the end points for the groups were the time to prostate-specific antigen (PSA) failure and time to prostate cancer death in the attentive expectant group. For best differentiation prostate cancer outcome in each of the groups, a regression tree method used to determine optimal AMACR protein expression cut-points separately. For examination of the effect of the AMACR cut-point on prostate cancer outcome, and adjusting for clinical variables, Cox proportional hazard models were used. Their results shown that lower expression of AMACR tissue was in association with worse prostate cancer results. Their research was the

first one which showed that significant association of AMACR expression with prostate cancer and defined it as biomarker of aggressive prostate cancer.[7]

*Role of alfa-fetoprotein (AFP) biomarker in detection of Hepatocellular carcinoma (HCC):* HCC as a large cause of cancer death is usually diagnosed after developing clinical retrogression at which measurement of time survival is carried out in months. Control of high risk patients for HCC is usually carried out using the serum marker alfa-fetoprotein (AFP) along with ultrasonography. Initial elevation of AFP levels is seen in the early stages of HCC and then decline or even normalizes before increasing again as disease progression follows. [8, 9].

*Role of adenomatous polyposis coli (APC) in colon cancer :* Mutation in the adenomatous polyposis coli (APC) sometimes result in sporadic colorectal cancers.Colorectal cancers are raised through a regular course of histological alternations named as 'adenoma-carcinoma' sequence , each associated by a genetic change in a particular oncogene or tumor suppressor gene . Failure in APC function activates this chain of molecular and histological alternations. Generally, an intestinal cell requires to come around with two essential conditions to develop into a cancer. These two requirements are initial colon cancer expansion, and genetic instability which are fulfilled by inactivation of APC.[10].

*Role of biomarkers in breast cancer research:* Recently several biomarkers associated with breast cancer are identified, in this part we explained some of them which are both established and emerging .*Estrogen receptor (ER)* : It is the most significant biomarker in breast cancer, because it provides the indication for endocrine therapy sensitivity . ER-positive tumors which contain approximately 80% of breast cancer use the steroid hormone estradiol as their critical growth cause, therefore ER is the direct target of endocrine treatments. The studies have been confirmed that ER-negative breast cancer patients have no profit from five year adjuvant therapy with tamoxifen, but some profit may be obtained in the exceptional group of ER-negative and progesterone receptor (PgR)-expressing breast tumors. Such therapy decreases the annual breast cancer death by 31% in ER-positive cases.[11].Another biomarkers associated with breast cancer is *progesterone receptor (PgR)* .Progesterone receptor expression highly

dependent on the ER presence. Tumors exhibit PgR expression but not the Er are rare and exhibit <1% of all breast cancer patients. [12]. Because of this reason, tumors expressing PgR without ER expression should be retested for their ER status for elimination of false ER negativity. In some uncommon cases when PgR – expression without ER expression present, Sometimes tamoxifen is described , but endocrine treatment is still highly recommended.[13].HER2 is another breast cancer related biomarker. HER2 is overexpressed in about 15% of all primary breast cancer .The best treatment for such cases is significant benefit from anti-HER2 therapies. Assessment of HER2 status in every detected case of breast cancer should be done. [14,15].Some other biomarkers related to breast cancer are Breast cancer anti –estrogene 1 resistance (BCAR 1) , Glutathion S-transferase \_1 (GSTP\_1), Urokinase type plasminogene activator (uPA) and Tyrosine kinase receptor (HER-2). Some emerging biomarkers in cancer research have been developed such as *ki67* which is first investigated by scientists [16]. In this investigation , scientist used a mouse monoclonal antibody in contrast to a nuclear antigen which is derived from a Hodgkin's lymphoma cell line.*ki67* is a proliferation biomarker and its prominent characteristic is universal expression among proliferating cells and its absence in quiescent cells [17].*Cyclin D1* is another emerging biomarker for breast cancer .Its characteristic is its overexpression at the mRNA and protein levels in about 50% of breast cancer patients containing 15% in which occurrence of a gene amplification exist, [18-20].*Cyclin E* is another emerging biomarker for breast cancer .Its act is similar to cyclin D1 and its gene amplification has been identified in several breast cancer cell lines[21]. It is strongly evidenced that Cyclin E play an important role in tumorigenesis [22,23]. Another emerging biomarker related to breast cancer is *ERβ*. [24,25]

*Role of biomarkers in ovarian cancer: Cancer antigen 125 (CA 125) and cancer antigen 15.3 (CA 15.3) are ovarian cancer biomarkers. Cancer antigen 125 is a protein which is present on the surface of many ovarian cancer cells .This protein can be present in other type of cancer as well as healthy tissue in small amounts. Due to this presence Ca-125 is used as a biomarker for identification of ovarian cancer. In a research study, determination of CA 125 and CA 15.3 antigens is carried out by enzyme immunoassay in 78 cases with ovarian cancer for a total of 540 determinations*

.Investigation of the antigens in sera from 100 women with other gynaecological diseases is done. Evaluation of CA 15.3 reference values in 91 normal healthy women is carried out . The results showed that the sensitivity of CA15.3 at diagnosis and its relapse detection were lower than that of CA 125. Aspecific mesothelial cell reaction does not increase Ca 15.3 which showed that more specificity of CA 15.3 than CA 125. Combination use of both biomarkers are helpful in early detection of relapse which demonstrated their significant role as biomarkers for ovarian cancer.[26,27].

*Role biomarker in Myeloid leukaemia ;: A prognostic marker known as of BCR-ABL play an important role in Chronic myeloid leukemia (CML). A chimeric oncogene Bcr-Abl is formed due to fusion between the Abelson (Abl) tyrosine kinase gene at chromosome 9 and the break point cluster (Bcr) gene at chromosome 22, which after activation result in development of Chronic myeloid leukemia . this activated chimeric oncogene Bcr-Abl is employed as biomarker in detection of CML.[28].*

*Nasopharyngeal carcinoma(NPC) is detected at early stage using combination of ENO1 and CYPA by quantitative proteomic analysis. In this study , scientists combined 2D-DIGE with MALDI-TOF-MS analysis for identification novel biomarkers for early detection of NPC. In this approach they performed the experiment for identification of expressed proteins in the cancer development and progression of NPC via LCM-purified normal nasopharyngeal epithelial tissues and various stages of NPC biopsies. They identified 26 differentially expressed proteins , of two proteins show direct expression change in the carcinogenesis process they named as ENO1 and CYPA,and their validation carried out by western blot analysis. They identifies as biomarkers for detection of early stage of nasopharyngeal carcinoma (NPC). [29]*

Other cancer specific biomarkers are epidermal growth factor receptor (EGFR) which is biomarker for lung cancer identification when mutated. [30].Human papilloma virus DNA (HPV) for invasive cervical cancer, in this case induction of deregulation of miRNA expression is carried out by HPV and this deregulation is through E6 and E7 proteins targeting miRNA transcription factors including p53. [31].Detection of primary tumours of bladders, found in urine sample is

done using microsatellite alterations, Mutl.homologue 1(MLH 1). MLH-2 and MLH-6 are biomarkers of hereditary nonpolyposis cancer and Epstein –Bar virus DNA (EBV) serves as biomarker for detection of nasopharyngeal carcinoma. [2].Although number of established and emerging biomarkers specific to cancer diseases are not limited to above mentioned biomarkers but these are most important and well-studied examples to support the significant role of biomarkers in cancer research and drug development. Additionally biomarkers are helpful in assessment of harmful effects of new drugs; they also are used for determination of potential toxicity of drugs. This broad area of use of biomarkers in cancer research and drug development can be integrated with new technologies and bioinformatics and biostatistics methodologies which result in efficient contribution to better cancer control via biomarker and drug discoveries.[2,3]

### III. CONCLUSION

The fact that the cancer is a flexible disease and consists of various types with different biological behaviour, molecular and risk profiles. Flexibility nature of the cancer requires different factors such as prognostic and predictive factors for individualized therapy of cancers. Biomarkers are the most important prognostic and predictive factors in cancer research and drug development in order to achieve individualization of cancer therapy. Biomarker discovery furnish a broad area of use in cancer research and drug development such as early diagnosis of cancers, progression monitoring of cancer, anti-cancer drug responses validation, providing low risk profiles from extra side effects of over-treatment and establishing endpoints. Although biomarker discovery have been currently improved but due to sensitivity of this research area , special attention requires to be paid for designing and conduction of related clinical trials which support validation of emerging biomarkers for cancer diseases . Careful and appropriate assessment for designing, validation methods and collection of appropriate samples such as blood, quality tissues are actual requirement to address the clinical question for which the biomarker have been emerged and selected. Despite, advancement in biomarker discovery and suitable strategy support identification of biomarkers at all levels such as RNA, DNA, proteins and small molecules but compiling of the brad data resulting from this attempt

will require comprehensive bioinformatics and biostatistics methods. Integration of all these requirements will be helpful in providing important contribution to biomarker discovery. For individualized cancer therapy all aspects and essential elements should be employed not only biomarker identification will be result in individualization of therapy but also all types of drug targets such as CDKs,[32] ion channels,[33] GPCRs [34] and aquaporins [35] should be assessed and their assessment and validation should be correlated in all biological aspects to cover all points of treatment in order to reach a comprehensive specific individualized therapy.

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# Employment and Unemployment Scenario of Jammu and Kashmir

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## ABSTRACT

The unemployment problem in the State of Jammu and Kashmir has reached alarming dimensions which is ever increasing every passing year with thousands of educated and uneducated youth getting added to the list of unemployment largely due to economic distress and mismatch. In a latest survey released by Labour Bureau under Union Ministry of Labour and Employment, Jammu and Kashmir State has emerged as having highest unemployment rate in Northern India. So on the bases of secondary sources the present paper tries to analyse the situation of employment and unemployment in Jammu and Kashmir. We found unlike structural transformation theory, occupational pattern of the J&K State have shifted from Primary sector to Services Sector, without undergoing the development of the Secondary sector. Also our results show that the problem of Unemployment is more prevalent amongst females in the State of J&K.

**Key words:** Unemployment, Employment, Jammu and Kashmir, Educated Youth

## I. INTRODUCTION

The State of Jammu & Kashmir presents peculiar geographical and socio-economic settings. It occupies an important position in the Indian subcontinent by virtue of its rugged topography, high altitude and rough terrain. The State is highly dependent on outside supplies of not only material but also men, the dependency in material is easily understandable but the paradox of unemployment and inward migratory labour force is a grave matter of concern. Whereas, on one hand it employs more than 4.5 lakh skilled and unskilled labours who migrate inwards from different parts of the country; on the other hand unemployment is being claimed as one of the sensitive areas of the state economy. The unemployment problem in the State has reached alarming dimensions which is ever increasing every passing year with thousands of educated and uneducated youth getting added to the list of unemployment largely due to economic distress and mismatch. According to the population census of India the population of J&K State has increased from 101.44 lakhs in 2001 to 125.49 lakhs in 2011. The number of workers also registered an increase of 15.15% during the

same period. Work participation rate for J&K was estimated at 34.5% as against the All India figures of 39.8% as per census 2011. The number of total workers in J&K as per 2011 census stood at 43.23 lakhs of which the main workers constitute 26.44 lakhs (61.77%) and the number of marginal workers constitutes 16.79 lakhs (38.83%). Female workers constituted 26.09% of the total work force. The share of female in the Main workers is only 12.80% as against 47.02% in marginal workers category in J&K. Work opportunities, however, have not kept pace with the increasing population. The problem of unemployment gains more importance because of higher incidence of unemployment among the educated section of youth in the State. Almost 70% of the population is directly or indirectly dependent on agriculture and allied activities which continue to be a major subsistence sector. However, it may be mentioned that near about 70% of the said marginal workers are associated with the Agricultural and allied sector contributing very marginally to the total production thus giving rise to the disguised unemployment in Agriculture. To avoid this situation, out of the said 16.79 lakh of marginal workers, half of this working force suffers from disguised unemployment who can contribute positively on being shifted to the other sectors

of economy without affecting the total agricultural production and yield productivity in the State. In the absence of desirable industrial growth and limited scope for absorption in the private sector, many have been rendered unemployed and have joined the ranks of job seekers.

## II. OBJECTIVES OF THE STUDY

The study is based on following objectives

1. To understand the situation of employment and unemployment in the J&K state.
2. To analyse the sector wise occupational distribution of the state.
3. To examine the sex and area-wise unemployment trends in the state.
4. To compare unemployment situation of northern states of India.
5. To know the approach of J&K state government towards Employment Generation.

## III. METHODOLOGY

In present study data has been collected from all the possible secondary sources like National sample survey organisation, Economic census Government of India, Digest of statistics; Directorate of Economics and Statistics; Government of J&K and Economic Survey of J&K. In addition to this, data have also been collected from other reliable sources like articles, journals and newspapers.

## IV. ANALYSIS AND DISCUSSION

### A. Registration of Unemployed Youth

Due to limited job opportunities available for unemployed youth in the State, the number of unemployed youth has been increasing with every passing year. The number of unemployed youth registered in various District Employment & Counseling Centres of the J&K State had increased from 106130 in 2008 to 307827 in 2013 (ending December, 2013) thereby registering an increase of 190%. The Statistics of employment exchanges does not provide accurate picture of unemployment in the State. The data suffers mainly from two defects; one, all the unemployed persons do not register themselves with these Employment Exchanges and second, some of the

registered persons may not be actually unemployed but only in search of better jobs. However, in the past, the process of placements which was made through employment exchanges has vanished altogether as a result of which there has been decrease in the registration level at these employment exchanges. In anticipation to the announcement of Employment Policy in the State and the invitation of the Government to the unemployed persons to register themselves in the Employment Exchanges, the whole scenario changed. The registration level increased tremendously. The registration of illiterate persons has decreased but that of educated unemployed persons has increased.

### B. Sectoral distribution of employment

Broadly an economy is classified into primary sector, secondary sector and tertiary sector. Agriculture and allied activities like forestry, fishing and dairying and mining and quarrying are the economic activities of the primary sector. Manufacturing, electricity, gas, water supply, and construction constitute the secondary sector. The tertiary sector also known as the services sector includes trade, transport, storage, communications, financial services, community social and personal services.

In table 1.1 we have provided the estimates of employment by sectors. These estimates are based on the census data from 1981 to 2011.

**Table 1.1 Sectoral Occupation Structure (Percentage)**

Occupation	1981	2001	2011
Primary sector	63.88	50.1	41.48
Secondary sector	14.25	6.23	3.99
Tertiary sector	21.87	43.67	54.53

**Source:** Compiled from Census of India, various issues

It is observed from the table 1.1 that the percentage of labor force employed in primary sector has decreased continuously in the state of J&K from 63.88% in 1981 to 41.48% in 2011. This means that since last three decades there has been a decline of 22.4 percentage points in labour force employed in primary sector. Considering the overall performance of secondary sector in terms of

employment of labour force it looks that persistent decline has happened in this sector also. In 2011, 3.99% of labor force was employed in this sector as against 14.25% in 1981 i.e. labour force employed in service sector has decreased by 10.26 percentage points over the same period. There is a terrific and continuous rise in the percentage of labor force employed in service sector since 1981 up to 2011. In 1981 the total labor force employed in tertiary sector was 21.87% which rises to 54.53% in 2011. This means there has been an increase of 32.66 percent rise in labor force employed in tertiary sector.

Thus the analysis of the above table clearly depicts that occupational pattern of the J&K State have shifted from Primary sector to Services Sector, without undergoing the development of the Secondary sector – which provides the opportunities for creation of jobs on a larger scale. This shows that there exists lopsided path of economic development on one hand and agricultural backwardness on the other. This agricultural backwardness has created an alarming situation of unemployment in the State, as the tertiary sector does not have the capacity to absorb the ever increasing unemployed labor force of the State. This is due to the fact that there are limited technological advancements in this sector in the State, thus leading to limited employment opportunities for the unemployed youths of the State. Thus for the sustainable development of the State it is necessary that the Agricultural Sector (Primary) of the economy should be promoted and developed, thereby providing suitable support to the manufacturing units (Secondary sector). Such an accelerated approach would help generate more employment opportunities for the unemployed youth of the State.

### ***C. Concepts of Unemployment***

Keeping in view the recommendations of the Committee of Experts on Unemployment, the National Sample Survey Organisation (NSSO) has developed and standardised concepts and definitions of labour force, employment and unemployment suitable to Indian conditions. These concepts have not only been adopted by the NSSO for conducting surveys on employment since 1972-73, but have also been accepted by the Planning Commission for analysing the dimension of the unemployment problem. The three concepts of unemployment developed by the NSSO are: (i) Usual

Status Unemployment, (ii) Current Weekly Status Unemployment and (iii) Current Daily Status Unemployment.

(i) The Usual Status concept is meant to determine the Usual Activity Status - employed, or unemployed or outside the labour force - of those covered by the survey. The activity status is determined with reference to a longer period; say a year proceeding to the time of survey. The persons covered by the survey may be classified into those working and/or available for work in their principal activity sector, and those working and/or available for work in a subsidiary sector, that is, a sector other than their principal activity sector. Hence, within the Usual Status concept, the estimates are now derived on the Usual Principal Status (UPS) as well as Usual Principal and Subsidiary Status basis. The Usual Status unemployment rate is a person rate and indicates chronic unemployment because all those who are found usually unemployed in the reference year are counted as unemployed.

(ii) The Current Weekly Status (CWS) concept determines the activity status of a person with reference to a period of preceding seven days. If in this period a person seeking employment fails to get work for even one hour on any day, he/she is deemed to be unemployed. A person having worked for an hour or more on any one or more days during the reference period gets the employed status. The Current Weekly Status unemployment rate, like the Usual Status unemployment rate, is also a person rate.

(iii) The Current Daily Status (CDS) concept considers the activity status of a person for each day of the preceding seven days. A person who works for one hour but less than four hours is considered having worked for half a day. If he works for four hours or more during a day, he/she is considered as employed for the whole day. The Current Daily Status unemployment rate is a time rate.

Out of these concepts of unemployment, the Current Daily Status concept provides the most appropriate measure of unemployment. Raj Krishna states, "The daily status flow rate is evidently the most inclusive, covering open as well as partial unemployment. It is therefore, the rate which is most relevant for policy-making." In India, the problem of chronic

unemployment is far less serious as compared with the enormous problem of the discontinuous underemployment of a section of the labour force whose composition keeps on changing over time. This factor has important policy implication and has thus to be kept in mind while employment programmes are chalked out.

#### **D. Comparison of Unemployment Rate in Jammu and Kashmir at National Level**

The latest NSS Survey- 68th round conducted during July, 2011 to June, 2012 throughout the country constitutes an important source of information on unemployment. The unemployment rates revealed by 68th round of NSS for J&K State in comparison to all India figures is given in the following table:-

**Table 1.2 Unemployment Rate\* for J&K State vis-a-vis All India, 68th Round of NSSO Survey (July 2011–June 2012)**

Area	J&K (%)			All India (%)		
	Male	Female	Persons	Male	Female	Persons
<b>Rural</b>						
UPS	2.7	16.6	3.9	2.1	2.9	2.3
CWS	3	6.3	3.8	3.3	3.5	3.4
CDS	5	11.8	6.1	5.5	6.2	5.7
<b>Urban</b>						
UPS	4.7	25.6	7.8	3.2	6.6	3.8
CWS	4.5	21.8	7.6	3.8	6.7	4.4
CDS	5.3	24.2	8.4	4.9	8	5.5
<b>Combined (Rural + Urban)</b>						
UPS	3.2	20.2	4.9	2.4	3.7	2.7
CWS	3.4	8.8	4.7	3.5	4.2	3.7
CDS	5	14.7	6.7	5.3	6.6	5.6

US: Usual Principal Status; CWS: Current Weekly Status; CDS: Current Daily Status

\* : Unemployment rate per 100 persons

The analysis of table 1.2 reveals that the combined Unemployment Rate (Rural+Urban) of J&K state as per 68<sup>th</sup> Round of NSSO, under UPS was 4.9% and under CWS was 4.7%, while as the indicator for CDS was 6.7%. As against this the All India level indicator was lower than that of State level under all the three approaches as indicated in the above table. The table 1.2 further shows that at State level, the unemployment rates for females as per all the three approaches viz UPS, CWS and CDS were on the higher side when compared to corresponding figures for males. Further there is a

huge gap of female unemployment rate in comparison to national level as per all the three approaches. For example as per UPS approach unemployment rate among female in J&K state is 20.2% while at national level it is just 3.7 % means thereby that in J&K state as per the UPS unemployment rate among female is more than five times than at national level. Similarly as per CWS, and CDS unemployment rate in the state among female is 8.8% and 14.7 % while as at national level it is 4.2 % and 6.6 % respectively. Thus the results show that the problem of Unemployment is more prevalent amongst females in the State of J&K.

#### **E. Estimates of Unemployment**

The statistics of unemployment in J&K do not indicate any clear trend over the last 13 years as is shown in table 1.3. However it is quite clear that unemployment rate in the state is highest in urban areas than in rural areas and within urban areas it the female unemployment rate that has shown an increasing trend through all the three approaches. Also in rural areas female unemployment rate has increased from 4.4 % in 1999 to 16.6% as per Usual status which means that rural female unemployment has increased around four times in the last 13 years. Similar trend is evident from current weekly status as female unemployment has doubled from 3.3 % to 6.3 % from 1999 to 2012. The table 1.3 further shows that unemployment rate among male in urban areas has decreased in the last decade as per all the three approaches. Thus the analysis of the table makes it clear that in the state of Jammu and Kashmir unemployment is mostly an urban phenomenon and that too in female population which needs a serious concern by government and policy makers.

**Table 1.3 Unemployment rate in J&K as per Sex, Residence and Status (percent)**

NSSO Rounds/Year	Male			Female		
	US	CWS	CDS	US	CWS	CDS
<b>Rural</b>						
55 <sup>th</sup> (1900-00)	2.3	3.6	4.8	4.4	3.3	11.8
60 <sup>th</sup> (2003-04)	2.03	3.1	5.4	18.18	6.1	10.6
61 <sup>th</sup> (2004-05)	3.8	8.6	10.7	81.8	14.7	36.7
62 <sup>th</sup> (2005-06)	5.1	6.5	7.9	0.6	0.3	0.5
66 <sup>th</sup> (2009-10)	3.1	3.0	3.8	19.3	5.7	10.3
68 <sup>th</sup> (2011-12)	2.7	3.0	5.0	16.6	6.3	11.8
<b>Urban</b>						
55 <sup>th</sup> (1900-00)	6.2	6.8	7.0	5.2	15.1	4.5

60 <sup>th</sup> (2003-04)	5.36	5.6	5.2	19.6	25.96	27
61 <sup>th</sup> (2004-05)	7.3	8.8	13.18	5.8	12.2	15.2
62 <sup>th</sup> (2005-06)	6.7	7.0	7.6	11.7	8.1	8.7
66 <sup>th</sup> (2009-10)	5.0	5.2	5.5	14.5	13.1	15.0
68 <sup>th</sup> (2011-12)	4.7	4.5	5.3	25.6	21.8	24.2

**Source:** Compiled from various Rounds of NSSO

### F. Unemployment in Northern States

As per the results of 68<sup>th</sup> Round of NSS, the Unemployment situation of J&K in comparison to Northern States viz H.P, Punjab, Haryana, Delhi and at All India level is given in the table 1.4 below:-

**Table 1.4 Unemployment Rate as per Usual Principal Status in J&K State in comparison with the neighbouring States/All India as per 68<sup>th</sup> Round of NSS (2011-12)**

State	Rural			Urban			Rural + Urban		
	Male	Female	Persons	Male	Female	Persons	Male	Female	Persons
J&K	2.7	16.6	3.9	4.7	25.6	7.8	3.2	20.2	4.9
H.P	1.8	1.8	1.8	2.1	11.0	4.2	1.8	2.2	2.0
Punjab	2.3	6.1	2.6	2.8	5.1	3.1	2.5	5.6	2.8
Haryana	2.6	4.2	2.8	4.0	5.6	4.2	3.1	4.8	3.2
Delhi	9.4	0.0	7.8	3.4	4.8	3.6	3.9	4.3	4.7
All India	2.1	2.9	2.3	3.2	6.6	3.8	2.4	3.7	2.7

**Source:** NSSO 68<sup>th</sup> Round

The analysis of table 1.4 reveals that based on Usual Principal Status (UPS), J&K has the highest Unemployment rate of 4.9% in comparison to its neighbouring States viz. Punjab (2.8%), H.P (2.0%), Delhi (4.7%), Haryana (3.2%). All India figures for Unemployment rate stood at 2.7% only. The above table also shows that Unemployment rate for Males in J&K was 3.2% only whereas that of females was 20.2% which is far too high when compared to the unemployment of females in neighbouring States viz Punjab (5.6%), Haryana (4.8%), Delhi (4.3%), H.P (2.2%). The analysis of the above table clearly shows that based on Usual Principal Status there is a huge gap of unemployment amongst females in J&K (20.2%) compared to the national aggregate (3.7%). Further the unemployment is more prevalent in urban than in rural areas; unemployment rate in urban area of J&K based on 68<sup>th</sup> Round of NSS stood at 7.8% as against 3.9% in rural area. The Unemployment amongst urban male (4.7%) is higher than that of rural males (2.7%) in J&K. Rural female unemployment in J&K stood at 16.6% which is far too high as compared to the neighbouring States viz Punjab (6.1%), H.P (1.8%), Haryana (4.2%) and far too higher than the national average of 2.9% only. So it is quite clear that based on the Findings of

68<sup>th</sup> Round of NSSO, the rate of unemployment based on UPS indicator is more pronounced and visible in J&K compared to the national average and further the gender differential is prominently visible and highlighted in the unemployment statistics of the State, irrespective of the approaches of measurement used. Furthermore, a regional differential in unemployment is also pronounced with the highest incidence of unemployment found in urban areas than in rural areas of the state, using any of the three approaches viz, UPS, CWS and CDS.

Further increasing rates of literacy seem to exhibit a positive relationship with unemployment which is a serious area of concern for policy makers and development practitioners. The disproportionate growth of the educated persons and employment opportunities have created a chaotic scenario where opportunities for employment are not substantial vis-à-vis educated workforce added. As per Census 2001, the literacy rate of the State stood at 55.52 %, which has increased further to 67.16 percent as per Census 2011. The population of State as per census 2011 is 12,541,302 persons out of which 7,067,233 persons are literate. The literacy is growing at an annual average growth rate of 1.02 percent, which results in addition to the educated youth year after year. This situation requires creation of ample opportunities in terms of employment avenues in the State or otherwise increase in literacy rate and number of literates will culminate into higher unemployment ratios.

### V. Need for Employment Policy

The State of J&K has certain inherent strengths that can be utilized to improve the income of its people and to provide gainful employment opportunities on sustainable basis, which are:

- i. Strong base of traditional skills not found elsewhere;
- ii. Untapped natural resource;
- iii. A natural environment which has been very profitably utilized by other countries for high income- environment friendly tourism industry.

In order to sustain growth and employment in its economy, the State Government should articulate an Employment Policy focusing on:

- i. Improving the productivity of, and thereby income of those engaged in industries based on traditional skills,
- ii. Shifting the agricultural work force to high value-added/high-income agriculture/horticulture;
- iii. Transforming the service industry in the State, driven by tourism, from informal and low income to modern by setting up a world class tourism infrastructure, largely on the basis of private investment and entrepreneurship; and creating a vibrant self-employed-professional workforce, which does not depend on government jobs, or for off-take of its services by government alone.

The policy would need to incorporate the following objectives:

- i. To exploit the full growth potential of the sectors and sub-sectors which are important to the state's Economy.
- ii. To create necessary infrastructure and extension services for diversification of agriculture and setting up new enterprises in manufacturing or services sector.
- iii. To rehabilitate people who have suffered loss in employment due to the slowdown in the economy.
- iv. To assist the self-employed workers to upgrade themselves through provision of credit, marketing, technological and training facilities.
- v. To utilise the government consumption expenditure to promote off take of commodities produced in J&K.
- vi. To improve prospects of long term growth by creating physical infrastructure such as transport and communication services.
- vii. To improve the efficiency of utilization of resources already invested in electricity, irrigation and transport sectors.
- viii. To carry out reforms in delivery of education, health, civic and other community services.

Government of J&K would also need regularly to update the Employment Policy statement. Manpower Planning would be an important element of this Employment Policy of the state, the Planning Department of the state government if required could seek assistance of the Planning Commission of India in this task.

## VI. Approach towards Employment Generation

The economy of Jammu & Kashmir has suffered from disturbed conditions prevailing in the State for almost two decades. It would, therefore, be necessary to put the economy back on the rails to enable the average person to get employment opportunities. This would require giving fillip to the economic activities that have traditionally been the mainstay of the State's economy and continue to hold significant potential for growth and employment. Such activities include Agriculture (including Horticulture), Food Processing, Handicrafts and Handlooms, Tourism etc. It would be equally necessary to ensure diversification of the State economy, especially expanding the industrial base by promoting private capital inflows into the State through various incentives and concessions in the initial stage. The special industrial package announced for Jammu and Kashmir has been a step in the right direction.

The government is taking all possible steps and making all possible efforts in providing gainful employment to the unemployed youth of the state, but it may not be possible for the government to provide government jobs to the educated unemployed the graph of which is increasing at a very faster pace. Under these circumstances possibilities have to be explored for absorbing the youth by way of creating work opportunities in the private sector as well. In this direction the following 8 sectors of economy have been identified for generation of gainful employment opportunities in the state on sustainable basis:-

- 1) Agriculture (including Horticulture, Floriculture , Food Processing and Animal Husbandry),
- 2) Handlooms and Handicrafts,
- 3) Industries (including Small Scale industries and Rural industries)
- 4) Tourism & travels,
- 5) Education & health,
- 6) Large infrastructure projects (Roads & Railways),
- 7) Information Technology & Telecommunication,
- 8) Construction Sector.

While as tourism - both domestic as well as international may provide possibilities for employment generation in the hotels, catering, entertainment and travel sectors as well as a market for handlooms and handicrafts, there

are equal chances of gainful self employment in the other above defined sectors as well.

## V. CONCLUSION

By way of conclusion we can say that occupational pattern of the J&K State have shifted from Primary sector to Services Sector, without undergoing the development of the Secondary sector – which provides the opportunities for creation of jobs on a larger scale. This shows that there exists lopsided path of economic development on one hand and agricultural backwardness on the other. Further based on our analysis and discussion it is quite clear that in the state of Jammu and Kashmir, unemployment is mostly an urban phenomenon and that too in female population which needs a serious concern by government and policy makers.

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# Attitude of Pre- Service Educators toward Including Children with Special Needs in General Classes

## Case study of Education Faculty – University of Jazan. K.S.A

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### ABSTRACT

The study conducted in Jazan university- faculty of education during academic year 2014-2015. The aims of study are to explore pre-service educator's attitudes about the inclusion disabilities with their typical peers in general classrooms. We used descriptive statistic methods. Researchers used The Teacher Attitudes to Inclusion Scale to measure pre-service educator's attitudes toward include students with disabilities in general classes. Study group include (420) pre-service educators from Education School. The research sample consisted of 100 pre-service educators from group sample. The data was analyzed by using SPSS program. The most important results are: The attitude of Pre-Service teacher toward including pupils with disabilities on general education class is positive. The number of pupils in class influenced on attitudes of Pre-Service education students toward inclusion. There are differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family. There are no differences on attitude of pre-service education students toward inclusion according to desired school levels.

**Keywords:** Attitude, Pre-service, Inclusion, Special Need and Education.

### I. INTRODUCTION

Inclusion is an educational practice based on a notion of social justice that advocates access to equal educational opportunities for all students regardless of the presence of a disability. Inclusion involves students with disabilities learning with their peers in regular schools that adapt and change the way they work in order to meet the needs of all students. Preparing teachers for regular class teaching has undergone a major pedagogical shift in recent years. Training institutions are now required to ensure that pre-service teachers are competent to cater for the needs of an increasing range of diverse learners. This move has been furthered by international recommendations (now more than 12 years old) from UNESCO to include content on inclusion as part of teacher training programs. In preparing teachers for inclusive classrooms their attitudes, beliefs, expectations and acceptance of people with diverse needs may well be challenged (Sharma, & et al 2006).

Although this is such an important area, international studies have been carried out to understand pre-service Educators attitudes toward inclusion. There is a study conducted by Thaver, & et al (2014), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Singapore. Researchers indicated that pre-service educators held negative attitudes to educate students with disabilities in general classes. Researchers revealed that interaction with individuals with special needs and previous training in disabilities significantly influence attitudes. Rather Researchers revealed that, elementary and primary specializations and program of the pre- service educators' responses did not significantly influence pre-service educators' attitudes towards inclusion. A study conducted by Sharma, & et al (2006), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Australia, Canada, Hong Kong, and Singapore. Researchers indicated that Canadian trainee educators positively revealed their attitudes more than others did. While trainee educators from Hong Kong and

Singapore revealed the least positive attitudes to include children with disabilities in general classes. A study conducted by Sharma, & Desai (2012), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Australia and Singapore. Researchers told that participants from Australia greatly revealed higher attitudes towards inclusive education than participants from Singapore. There is a study conducted by Toole, & Burke (2013), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Ireland. Researchers told that pre-service educators positively showed their attitudes to educate children with disabilities in general classes. There is a study conducted by Cameron, & Cook (2007), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Ireland. Generally, researchers told that pre-service educators revealed positive attitudes. There is a study conducted by Malak (2013), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Bangladesh. Researcher told that special education trainee educators largely showed positive attitudes to educate children with disabilities in general classes. . Participants highly held positive attitudes to educate children with physical impairments, hearing impairments and vision impairments in general classes. There is a study conducted by Mdikana, Ntshangase, & Mayekiso (2007), and it aimed to explore pre-service teachers' attitudes toward inclusive education in Johannesburg. Researchers indicated that student teachers revealed positive attitudes to educate students with special needs in general classes. Contact, disabled family member, and specialization can influence pre-service educators' attitudes. In addition, researchers found that 60% of college students recorded positive scores to educate pupils with disabilities in general classes while 27% of the students showed negative attitudes and 16% were undecided. . In addition, researchers indicated that 72% of college students revealed their needs to have special skills and abilities to educate pupils with disabilities in general classes. Additionally, researchers indicated that 72% of college students revealed their needs to have special resources to implement successfully: they indicated that 77% of college students revealed positive attitudes towards learners with Special Needs and they revealed their needs to special resources. There is a research study conducted by Costello & Boyle (2013). It was to explore trainee college students' dispositions and

attitudes about the inclusion. Research showed that students' attitudes were more positive to inclusion in the first year of university than in following years. There is a study conducted by Mangope, Mannathoko, & Kuyini (2013) that was to explore the attitudes of college students, specialized in physical education, of the University of Botswana toward including individuals with different disabilities in regular classes. Researchers found that college students had moderately positive dispositions and attitudes to include individuals with disabilities in regular classes. There is a research study conducted by Kuyini & Mangope (2011) that aimed to discover pre-services educators' dispositions and worries about inclusion in Ghana and Botswana. Researchers indicated that pre-service educators presented higher attitudes towards including students with disabilities on the social interaction components, and pre-service educators from Botswana show lower attitudes towards including children with disabilities in regular classrooms than Ghanaian pre-service educators do. There is a study conducted by Ahsan, Sharma & Deppeler (2012) and it aimed to explore trainee college students' teaching-efficacy, concerns, attitudes, and readiness to include individuals with special needs in general classes. Researchers found that trainee students revealed greater teaching-efficacy did not present concerns. Rather they demonstrated higher positive attitudes to include individuals with disabilities in general classes. There is a study conducted by Sharma Moorea, & Sonawaneb (2009), and it intended to explore trainee educators' attitudes to include children with disabilities in general classes. The study revealed that positive attitudes to educate pupils with behavioral issues. In addition, researchers indicated that trainee educators held higher levels of confidence about inclusion showed less concerns and worries among their counterparts. There a study implemented by Mongwaketse & Mukhopadhyay (2013), and it aimed to explore student educators' tenets, attitude and readiness to include students with disabilities in general classes. Researchers found that most of college students revealed and sustained positive attitudes to include students with disabilities in general classes. Researcher indicates that it was important to provide practical courses for college students to have more experience and knowledge about the actual profession.

## II. METHODS AND MATERIAL

### Method Research Approach:

In this study, the descriptive analytic research technique was used.

### Study group:

It formed from pre-service education students in faculty of education – University of Jazan about (420) in three specialization (Special Education- Art Education- Physical Education).

### Study sample:

In these research random sampling methods was used. The individuals who participate in random sampling were chosen randomly. The study was conducted with (100) students. (33) From pre-service art education students, (33) from pre-service physical education students, and (34) from pre-service special education students.

### Tool:

The researchers used The Teacher Attitudes to Inclusion Scale to measure student teachers' attitudes to include students with disabilities in general classes designed by the researchers. In order to ensure the validity and reliability of the scale form, it distributed to four instructors who had completed their doctorates and this form developed in according the opinions of the instructors, then a pilot study were conducted and the value of reliability was found. It was about (0.86) and after that, the scale forms became ready for application.

### Practical Procedures:

The principle of pre-condition of participating in scale, an explanation was prepared. The aims of the study and how the study would be carried out were clearly stated in it. The scale application took place between 1-21 days.

## III. RESULTS AND DISCUSSION

### 3.1 Results

After analysing the data, the results are as follows:

#### 1. What is attitude of Pre-Service teacher toward including pupils with disabilities on general education class?

To answer this question the researchers used one sample T-test and table (1) shows the attitude of education students toward inclusion.

Table (1) shows the attitude of Pre-Service education students toward inclusion

variable	Mean	SD	T	SIG	significant
Attitude	109.30	12	16	0.00	Positive significant

S.D = Standard deviation; T= T value; SIG = sigma value

When we compare the average premise (87) with the mean (109.30), we found that mean greater than average premise as phrases as in table, and this difference is significant at the level (0.05), because the level of significant greater than SIG. this means that the attitude of Pre-Service teacher toward including pupils with disabilities on general education class is positive.

#### 2. What is an influence of number of pupils in class on attitude of Pre-Service students toward inclusion?

For answer, this question the researchers used regression method, and table (2) shows the influences of number of pupils in class on attitude of Pre-Service students toward inclusion.

Table (2) shows the influence of number of pupils in class on attitudes of Pre-Service education student.

PV	Dependent	F	R	SIG	Pen	Result
N of pupils	Attitude	5	0.3	0.04	0.4	Positive

S.D = Standard deviation; T= T value; SIG = sigma value.

After analysis the data, we found that the standardized coefficients value is (0.39) (see table 3), the value of correlation coefficients is (0.23). These values are significant at level (0.05), because the level of significant is greater than SIG. This means that the number of pupils in class influence on attitudes of Pre-Service education students toward inclusion.

### 3. Are there significant differences on attitude of pre-service education students toward inclusion according to desired school levels?

For answer, a question the researchers used ANOVA analyses of variance, table (3) shows the differences on attitude of pre-service education students toward inclusion according to desired school levels.

Table (3) shows the differences on attitude of pre-service education students toward inclusion according to desired school levels.

Compare	Sum of Squares	d.f	F	Sig.	Result
Between	30.900	38	1.5	.32	negative
Within	30.381	43			
Total	61.280	81			

F= F value Sig= sigma value.

As you see in table (3), you found the sig value (0.326) is greater than significant level (0.05), which means there are no differences on attitude of pre-service education students toward inclusion according to desired school levels.

### 4. Are there significant differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family?

For answer, a question the researchers used independent samples test.

Table (4) shows the differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family.

disabilities in friend of family	Mean	Std. D	T	Sig.	Result
Yes= 50	113	12.4	2.9	0.03	Positive
No=50	109	12.5	2.9		

T= T value Sig= sigma value.

As you see in table (4) when we compare the mean of whom have disabilities in friend and families (133) with the mean of have not disabilities in friend and families (109), we found that mean of first group

greater than means of second group as phrases as in table, and this difference is significant at the level (0.05), because the level of significant greater than SIG, this means that there are differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family.

### 3.2 Discussions

When analyzed the results revealed that:

The attitude of Pre-Service teacher toward including pupils with disabilities on general education class is positive. This result is in line with many studies. Study of Sharma, & et al (2006), Researchers indicated that Canadian trainee educators have positive attitudes to include children with disabilities in general classes. A study conducted by Sharma, & Desai (2012), researchers told that participants from Australia greatly revealed higher attitudes towards inclusive education. A study conducted by Toole, & Burke (2013), told that pre-service educators positively showed their attitudes to educate children with disabilities in general classes. A study conducted by Cameron, & Cook (2007), told that pre- service educators revealed positive attitudes. There is a study conducted by Malak (2013), told that special education trainee educators largely showed positive attitudes to educate children with disabilities in general classes. Participants highly held positive attitudes to educate children with physical impairments, hearing impairments and vision impairments in general classes. A study conducted by Mdikana, Ntshangase, & Mayekiso (2007), indicated that student teachers revealed positive attitudes to educate students with special needs in general classes. Contact, disabled family member, and specialization can influence pre-service educators' attitudes. In addition, researchers found that 60% of college students recorded positive scores to educate pupils with disabilities in general classes while 27% of the students showed negative attitudes and 16% were undecided. . In addition, researchers indicated that 72% of college students revealed their needs to have special skills and abilities to educate pupils with disabilities in general classes. Additionally, researchers indicated that 72% of college students revealed their needs to have special resources to implement successfully: they indicated that 77% of college students revealed positive attitudes towards learners with Special Needs and they revealed their needs to special resources. Study conducted by

Costello & Boyle (2013) showed that students' attitudes were more positive to inclusion in the first year of university than in following years. A study conducted by Mangope, Mannathoko, & Kuyini (2013) found that college students had moderately positive dispositions and attitudes to include individuals with disabilities in regular classes. A study of Kuyini & Mangope (2011) indicated that pre-service educators presented higher attitudes towards including students with disabilities on the social interaction components, and pre-service educators from Botswana show lower attitudes towards including children with disabilities in regular classrooms than Ghanaian pre-service educators do. A study conducted by Ahsan, Sharma & Deppeler (2012) found that there are higher positive attitudes to include individuals with disabilities in general classes. A study conducted by Sharma Moorea, & Sonawaneb (2009), revealed that positive attitudes to educate pupils with behavioral issues. In addition, researchers indicated that trainee educators held higher levels of confidence about inclusion showed less concerns and worries among their counterparts. A study implemented by Mongwaketse & Mukhopadhyay (2013), found that most of college students revealed and sustained positive attitudes to include students with disabilities in general classes. Researcher indicate that it was important to provide practical courses for college students to have more experience and knowledge about the actual profession. The researchers pointed that the importance of having positive attitudes toward inclusive education amongst in-service educators has been long recognized. If educators hold positive attitudes towards inclusive education, it may allow and encourage practices that will guarantee, to a large extent, successful inclusion of all students. Positive attitudes can be and need to be fostered through both training and positive experiences with students with disabilities.

In addition, the study indicated that the number of pupils in class influenced on attitudes of Pre-Service education students toward inclusion. So that when the numbers of pupils with disabilities in class large the attitude of pre-service is negative because this situation make the pre-service educator experienced distress. Finally, the study revealed that there are differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family. Moreover, this means that exist of disabilities in friend

of family influenced on attitude of pre-service educators. So that pre-service educators whom live with disabilities have, positive attitude toward including pupils with special needs in general classroom.

#### IV. CONCLUSION

Our study conducted to find out the attitude of pre-service educators toward including pupils with disabilities on general class school, to verify this aims the researcher used pre-service educator's attitude toward inclusion scale. The study indicated that the attitude of Pre-Service teacher toward including pupils with disabilities on general education class is positive. The number of pupils in class influenced on attitudes of Pre-Service education students toward inclusion. There are differences on attitude of pre-service education students toward inclusion according to disabilities in friend of family. There are no differences on attitude of pre-service education students toward inclusion according to desired school levels.

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# The Role of Population Genetics for Ethiopian Farm Animal Genetic Resources Conservation

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## ABSTRACT

This review work is focused on the theory of genes and chromosomes as they occur and vary in members of populations. Conservation of local breeds of farm animal genetic resources should be a part of animal management and the communities should be informed by pertinent parties for the distribution, structures, and trends, productive and adaptive performances of populations of the existing breeds. Conservation farm animal genetic resources still not practiced and managed well in the whole or all over the country except for few breeds such as three cattle breeds and one sheep at research centers. The responsible constraints are lack and gap (is avail) of information of about farm animal genetics resource, climate change the another driving force to change animal production system, increase the exposure of breed populations to unfamiliar epidemic disease and lack of awareness of AnGR among decision makers and lack of consultation with livestock keepers and other relevant stake holder. But there are also opportunities in the country such as practice in little research centers. Farm animal genetic resources conservation must be practice by well-designed management, support by national breeding policy strategy and economic wise to attain the food security of many societies finally to the GDP of the country as the whole. Farm animal genetic resource information should be accessible available for every interested specially for animal keepers and other stakeholders as well. Perceptions and thoughts of the community should include during design and implementation of animal conservation in the country. Animal conservation must have support by government and non-government organization to facilitate, fund and other related aspects.

**Keywords:** Population Genetics, Conservation, Ethiopia

## I. INTRODUCTION

Population genetics is dealing the theories of genes and chromosomes number of every member of a population. Genes, chromosomes and environmental influence are controlled the development of organisms. We observe that different species are different because they develop differently. Population genetics could supply answers these differences having with essential step in animal, human and plant species through demographic cultural developments (Diamond 2002). This subject has effective management dream of farm animal genetic resources (AnGR) that requires comprehensive knowledge of the breeds 'characteristics, population size and structure, geographical distribution, the environment and genetic diversity. Strategic priority areas and Global

Plan Action for Animal Genetic Resources is adopted by 109 countries at the first International Technical Conference held in Switzerland and endorsed by the FAO Conference (FAO, 2007a).

As the genetic diversity, low-production breeds are contributed to current or future traits of interest for maintaining future breeding options (Natter, 1999; Bruford *et al.* 2003; Toro *et al.* 2009). According to the FAO reported that from 7600 breeds about (20%) worldwide, belonging to 18 mammalian species and 16 avian species are at risk and 62 breeds became extinct within the first 6 years of this century (FAO, 2007b). A breed that is common in other countries is likely to be a lower priority for national conservation. A basic requirement is to know whether a given national breed is genetically distinct or whether it is part of a larger

population spread across several countries. In a recently developed classification (FAO, 2007b), breeds present in only one country are termed local breeds and those present in more than one country are termed trans-boundary breeds, the latter being further differentiated into regional and international trans boundary breeds. Depending on the extent of their distribution, in 2008, about 7040 local breeds, 500 regional trans-boundary breeds and 551 international trans-boundary breeds were recorded in FAO Domestic Animal Diversity Information System (DAD-IS; <http://www.fao.org/dad-is/>) (FAO, 2009). Therefore, this review paper was initiated to review the role of population genetics for farm animal genetic resources conservation for present and future uses.

## **The Role of Population Genetics for Ethiopian Farm Animal Genetic Resources**

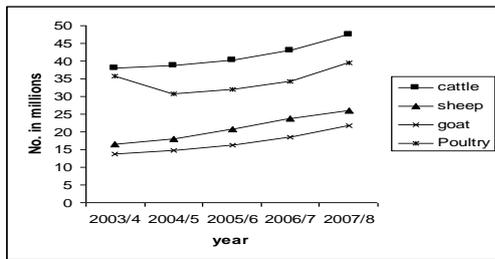
### **Conservation**

According to IBC (2013) cited by CSA, 2009, Ethiopia has served as a gateway of domestic animals migration from Asia to Africa. In terms of livestock population, Ethiopia stands first in Africa and 10<sup>th</sup> in the world. The domestic animal population of the country is estimated to be 47.5 million cattle, 26.1 million sheep, 21.7 million goat, 1 million camel, 39.6 million chickens, 1.8 million horses, 0.4 million mules and 5.6 million donkeys (CSA, 2009). Though, this increased population size doesn't necessarily show the state of the animal diversity; given more than 99% of the livestock population (excepting poultry) being indigenous breeds. However, the trend and the state of individual breeds are needed to be scrutinized, since some of the breeds are threatened. At present there are about 30 cattle, 14 sheep, 14 goat, 4 camel, 4 donkey, 2 horse, 2 mule, 5 chicken and 5 honey bee breeds/strains/populations are identified. However, the status and trend of most of the breeds is not known. At the moment population genetics stated that Sheko cattle is the only Taurus breed in East Africa appears to be highly threatened as a result of interbreeding with the local zebu and change in the system of production. Whereas, Fogera, Begayit, Irob, Ogaden, Afar and Borena cattle breeds, Sinner donkey breed, and afar sheep breed are also facing various degrees of endangered species.

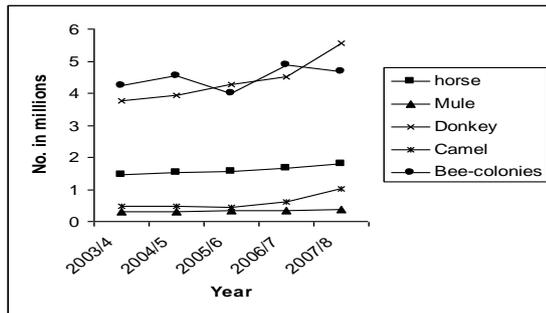
The major threats of livestock genetic resource are feed shortage as a result of degradation of rangelands/grazing areas, overgrazing and overstocking; sporadic invasion of rangelands by weeds and shrubs, expansion of crop cultivation, illegal trafficking, inbreeding and interbreeding and Trypanosomiasis. The major threats for indigenous chicken are the random distribution of exotic breeds, diseases and predations. While the major threats to honeybees are agro-chemicals (pesticides/herbicides) diseases, pests and predators. Some indigenous domestic animals (e.g. Sheko and Ogaden cattle) are interbreeding with other breeds and are losing their unique features. In addition, drought, desertification and abandoning of irrigated areas due to salinity are affecting the pastoralists and possibly the animal genetic resource. Crossbreeding of Menz sheep with Awassi exotic breed is likely to dilute the indigenous Menz sheep breed. Boran cattle breed is also a subject of dilution as a result of crossbreeding and replacement by other breeds during restocking after drought. Introduction of new breeds to a new area is also resulting in appearance of diseases which were not recorded before. Gumboro is a disease of poultry which is being seen in Ethiopia only since recent times.

As a result of increased market demand, the number of cattle, goat, sheep and camel being exported legally and illegally seems to threaten the resource since the size of export is not equal to the off-take rate. This can be verified by the large proportion of young and breeding animals supplied to the market. Conservation of the domestic animal diversity is not getting the attention it deserves. Fogera cattle breed is interbreeding with other cattle breeds and consequently the pure line is declining. Ranches were established in Chagni, Metekel and Andasa for this breed with major objectives of breed Improvement and retaining the pure lines. A ranch was also established for Washera sheep in north western Ethiopia (Amhara region).

The national artificial insemination coverage for cattle is 1%. Exotic or crossbreed bulls, chicken and sheep are also distributed to some parts of the country. Excepting poultry the level of current introduction is low and for cattle is mainly restricted to urban areas. However with the aggressive extension program to increase the artificial insemination service and due to more focus on certain breeds and areas there is serious threat of dilution to some local population.



**Figure 1:** Change in Population Size of the cattle, sheep, goat and poultry in the period



**Figure 2:** Change in Population size of horse, mule, donkey, camel and bee-colonies

Currently as a result of changes in production system and interbreeding with Zebu animals, the breed (Shako cattle) is under severe threat of extinction. The recent estimate of the population is around 4,000 (Takele, 2005) against 31,000 and 18,307 reported by FAO in 1999 and 2001, respectively.

### Diversity of Farm Animal Genetic Resource

According to Moritz (1994), degrees of genetic differentiation are relevant to endangered species conservation. A measure of distinct species is strong application idea to know patterns of distinctness and identify entire systems. This whole idea of prioritization is very controversial. However, genetic distinctness and ecological importances are not necessarily correlated and more important unanswerable issue. Genetic distinctness should be viewed as only one of many criteria in assessing conservation merits.

Genetic diversity provides a way for populations to adapt to changing environments and more variation is more likely to possess variations of alleles determining traits that are suited for the environments. In applying the principles of population genetics to recommend endangered species conservation and biologists are interested to see genetic variation, distribution and

maintenance. However, opposite situation are faced on endangered species, with a small number of populations which are isolated from one another having a small number of individuals. Applying population genetic theory to such situations may point the way to management strategies which will maximize the maintenance of existing variation.

Connectedness is generally measured by examining the frequencies of different alleles, or forms of a specific gene, at several different genes. If the frequencies differ significantly between two areas, it is likely that there is some restriction in gene flow between them. If it appears that there is no difference in frequencies from one area to another, it may be some genetic connection preventing differentiation (other interpretations are, of course, possible). Strong inter population connectedness (presumably through frequent migration) will be good for promoting the maintenance of overall genetic diversity; rare alleles are less likely to disappear in a larger population. However, a disadvantage to strong inter population connectedness is that deleterious alleles and diseases may more easily spread through a species whose populations are in frequent contact with each other. It is important to understand the threats to a species in order to properly interpret information on inter population structure.

In addition to this Avise (1994) said that Meta populations are frequently mentioned in conservation of endangered species. On a landscape scale, this situation arises when appropriate habitats are small and dispersed. Numerous subpopulations must be protected and allowed to maintain and survive in order for the Meta populations. Habitat fragments are often preserved in isolation. While all such efforts should be applauded, it also must be recognized the necessary steps in the protection of some diversified species.



**Figure 3:** Show differences possible in livestock breeds and species over time and space

### **How population genetics play role in conserving farm animals**

Conservation of animal genetic resources for food and agriculture (AnGR) may be undertaken for a number of reasons (FAO, 2012). In developed countries, traditions and cultural values are important driving forces in the conservation of risk breeds and the emergence of niche Markets for livestock products. However, in developing countries the immediate concerns are food security and economic development. Allendorf et al., 2006 and Avise, 2004 stated that conservation genetics is an interdisciplinary science that aims to apply genetic methods to conservation and restoration of biodiversity. Interdisciplinary of conservation genetics is based on the interaction of several field including population genetics, molecular ecology, biology, evolutionary biology, and systematic, Genetic diversity is one of the three fundamental levels of biodiversity with direct impact on conservation of species and ecosystem biodiversity. According Kefyalew, 2013, conservation of animal resources should ideally be undertaken at global level, because of the existence of non- and trans-boundary breeds. However, national conservation programs better serve specific local Interests, such as conservation with the objectives of improving indigenous breeds. There are obviously many reasons why genetic conservation should be considered, e.g. cultural, historic, and scientific interests and on the one hand for more practical and economic considerations.

Conservation is management of animal genetic resources by communities and pertinent parties. Although in Ethiopian much information is lacking for the conservation of farm Animal Genetic Resources (AnGR) from the rational utilization and protection of existing genotypes from genetic erosion (IBC, 2004) cited in Kefyalew, 2013. Therefore, insitu (in which animals are maintained within the environments or production systems in which they were developed) and ex situ conservation strategies are recommended. The latter can be further divided into ex situ– in vivo conservation and cryo conservations (FAO, 2012).

**In situ Conservation:** In the context of domestic animal diversity, in situ conservation primarily involves the active breeding of animal populations for food and

agricultural production in such a way that diversity is optimally utilized in the short term and maintained for the longer term. Activities pertaining to in situ conservation include performance recording schemes, development of breeding programmes and management of genetic diversity within populations. In situ conservation also includes steps taken to ensure the sustainable management of ecosystems used for agriculture and food production.

**Ex-situ Conservation:** In the context of domestic animal diversity, ex situ conservation means conservation away from the habitat and production systems where the resource developed. This category includes both the maintenance of live animals and cryo conservation.

**Ex situ– in vivo conservation:** Ex situ – in vivo conservation is ex situ conservation in which germ plasma is maintained in the form of live animals. As in the case of in situ conservation, it is accepted that improve-ment and natural selection may alter gene frequencies in the conserved population. A key question with respect to this strategy is whether or not long-term finances and commit-ment are available to maintain generations of animals to the standards required for success-full conservation.

### **Quantify the practice made to conserve farm animals in Ethiopia**

Unfortunately, except for limited activities that are meant to maintain pure stocks of 3 cattle breeds and 1 sheep breed, no conservation activities of farm AnGR have so far been In Ethiopia, information for sustainable utilization and conservation of the farm animal genetic resources are very limited and, if available, are full of gaps (IBC, 2004) cited in kefyalew,2013. Conservation of Farm animal genetic resources (FAnGR) refers to all human activities including strategies, plans, polices, and actions undertaken to ensure that the diversity of FAnGR is maintained to contribute to food and agricultural production and productivity, now and in the future (ILRI, 2006). For example, Indigenous breeds of sheep and goats may produce less milk or meat than improved breeds. But they usually fulfill a wider range of functions for their owners and are much easier to manage. Many marginal areas can be exploited only by locally (IRLI, 2006).

## **Opportunity and constraints farm animal conservation in Ethiopia**

**Opportunity:** In situ conservation of livestock breeds is primarily the active breeding of animal populations and their continued use as part of an ongoing livelihood strategy (Solomon et al., 2008). Village-based breed improvement programs must be complementary to in situ livestock conservation objectives with the concept of conservation through sustainable utilization. In such a context, it can be viewed as part and parcel of a comprehensive conservation plan, and not as a separate genetic improvement activity, that entails significant additional costs. In the meantime, there are more feasible conservation methods at hand under the current circumstances including in vivo conservation. In vivo conservation includes in situ and ex situ methods. Ex situ in vivo conservation is the maintenance of pure-bred nucleus flocks in organized government farms or research farms which can form a repository of the pure breed. A conservation-based breeding program should be based on broader breeding objectives that incorporate the needs and perceptions of the community and maintenance of genetic diversity. Such as adaptation traits. Involvement of the farmers in the design and implementation of the breeding program in line with the principles of in situ conservation of genetic resources is one of the options which must be considered.

Trypanotolerant traits of Shako (Workneh et al., 2004; Stein et al., 2011) and Nuer/Abigar cattle breeds and the adapted breeds or species. For example, camels are the only livestock in areas with less than 50 mm of rainfall. If these animals die out, it will no longer be possible to use large areas of arid lands to produce food. Additionally, the genetic diversity they embody enables breeders to respond to changes in production, marketing and the natural environment. The adaptation of different species and breeds to a broad range of environments provides the necessary variability that offers opportunities to meet the increased future demands for food and provide flexibility to respond to changing markets and needs. However, currently, there is a threat of loss of genetic diversity in livestock populations to the extent that some breeds may be approaching extinction. This calls for strong conservation activity which is important, among others, in maintaining genetic diversity to meet the needs of current and future utilization; providing options for adaptation to changing

environmental conditions; and preserving cultural and historical values (Gibson et al., 2006). The first step in conservation is to know which breed to conserve (characterization).

Basically, conservation is categorized into ex situ and in situ conservation. The combined use of live animals and frozen semen appears to be the best strategy. For instance, a pure breeding strategy is necessary for breed conservation and it may be accompanied by a well-organized community based breeding program supported by a nucleus herd of purebred Sheko animals (Stein, 2011). Conserving the Ethiopian Boran in Borana lowlands of Ethiopia will secure the future use of the Borana genetic material at very little costs per animal (Kerstin, 2006). Priority should be given to breeds that have reached critical or endangered status, genetically diverse stocks, breeds with unique characteristics and Stocks with high overall economic merit. Some researches in Ethiopia like analysis of genetic diversity and conservation priorities for six north Ethiopian cattle breeds (Zerabruk et al., 2007) and Ethiopian sheep breeds (Gizaw et al., 2008) can also provide valuable information on conservation program.

According to Workneh et al., (2004), there are encouraging developments from FAO (DAD-IS) and ILRI (DAGRIS) as part of their global research programs for characterization, documentation and conservation of Farm animal genetic resources. Potential candidate institutions in Ethiopia for characterization and conservation are MOA (Ministry of Agriculture), IBCR, EIAR (Ethiopian Institute of Agricultural Research) and Academic institutions. Governments are sufficiently concerned about the erosion of livestock breeds to issue a Global Plan of Action for Animal Genetic Resources (FAO 2007a). This contains recommendations on monitoring the loss of breeds, their sustainable use and development, their conservation, and policies, institutions and capacity building to manage animal genetic resources. Supporting livestock keepers to add value to their traditional breeds also contributes to achieving two of the eight Millennium Development Goals (Goal 1 and 7) (UNDP, 2000). The Convention on Biological Diversity (Article 8 and 10) obliges governments to support traditional life styles, biological diversity and cultural practices – of which local breeds and species are an integral part (CBD, 1992).

**Constraints:** In Ethiopia, information for sustainable utilization and conservation of the farm animal genetic resources are very limited and, if available, are full of gaps (IBC, 2004). It would no doubt be of interest to future generations of animal breeding specialists, as well as to interested laymen, if it were possible to maintain representative samples of some of the once important animal breeds especially in ex situ conservation, which are now on the verge of disappearing (FAO, 1990). Practical and economic needs ought to be the most important reason for conservation in future; one could perhaps argue that, farmers with economically competitive breeds or genetic types should take care of their own preservation. Numerous attempts made to introduce 'improved' breeds with poor success in terms of achieving genetic potential. Fertility and longevity of introduced breeds so poor that continual importation of exotic breeds necessary. Rare breeds often crossed with 'improved' breeds due to small population, dilution of breed characteristics and creation of gene pool from which it is then difficult to identify and utilize favorable local breeds genetic characteristics are also the main threats to animal genetic resources in Ethiopia. Unfortunately, the situation is more complex.

The economic and environmental conditions are changing and genetic types which are superior under one set of conditions may be inferior under a different set of conditions. As the changes are gradual and different breeds or types are not generally compared under exactly the same conditions, the individual breeder or leader of a breeding program has usually no interest in, or possibility of, conserving for future use animals which, at any given time, considers slightly inferior to those selected for breeding (Rendel, 1975). However, sustainable use of genetic resources should effectively deal with semen and embryos preservation as part of the ongoing utilization and improvement programe.

Climate change has the potential to drive gradual changes in production systems (e.g. affecting the availability of feed resources), to cause more frequent climatic disasters, and to increase the exposure of breed populations to unfamiliar epidemic diseases. Other cross-cutting threats include lack of awareness of the significance of AnGR among decision-makers and lack of consultation with livestock keepers and other relevant stakeholders (FAO, 2009a), both of which contribute too

many threats arise because of policy and management decisions.

In Ethiopia, indiscriminate breeding, disease, feed shortage and agro-chemicals are some causes of threats to maintenance of animal genetic diversity. (IBCR, <http://www.abc.gov.et/biodiversity/conservation>). Feed shortage and disease burden exacerbated by climate change. Livestock health problems such as the high prevalence of Trypanosomiasis in the lowlands are among the challenges that affect livestock fertility.

## II.CONCLUSION

Population genetics is the study in theory, in the laboratory and in the field of genes and chromosomes in members of populations. Effective management of farm animal genetic resources (FAnGR) requires comprehensive knowledge of the breeds 'characteristics, including data on population size and structure, geographical distribution, the production environment, and within- and between-breed genetic diversity.

The major threats to the livestock genetic resource are feed shortage, agro-chemicals (pesticides/herbicides) for honeybees, diseases, pests and predators, inbreeding and interbreeding. Conservation a mandate for all but, still not practiced and managed well in the whole or all over the country except for few breeds.

### Recommendations

- Farm animal genetic resources conservation must be practice by well-designed management, support by national breeding policy strategy and economic wise to attain the food security of many societies finally to the GDP of the country as the whole.
- Farm animal genetic resource information should be accessible available for every interested specially for animal keepers and other stakeholders as well.
- Perceptions and thought s of the community should include during design and implementation of animal conservation in the country.
- Animal conservation must have support by government and non-government organization to facilitate ,fund and other related aspects

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# Over Pressure Layer Chromatography a Novel Technique

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## ABSTRACT

OPLC is coming of age. Its rudimentary beginnings, some 25 years ago, have led to innovative, high-performance products. The commercialization of modern, high efficiency columns has eliminated the need for analysts to prepare their own from TLC plates, making the technique even more accessible. Optimum performance laminar chromatography (OPLC) is a pumped flow chromatography technique that combines the user-friendly interface of HPLC with the capacity of flash chromatography and the multidimensionality of thin-layer chromatography. This review will describe the basis of modern OPLC instrumentation and the separation techniques, as well as give a brief account of some recent applications in OPLC.

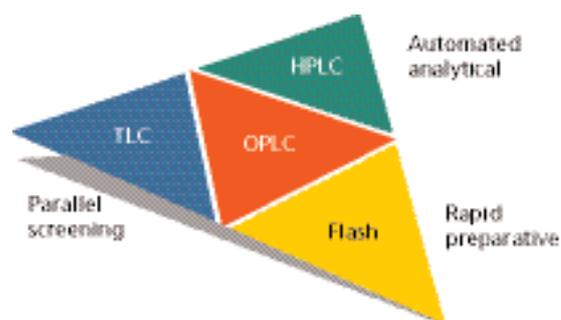
**Keywords:** OPLC, Instrumentation, Application.

## I. INTRODUCTION

Optimum performance laminar chromatography (OPLC) is a pumped flow chromatography technique that combines the user-friendly interface of HPLC with the capacity of flash chromatography and the multidimensionality of TLC. This review will describe the technique, as well as give a brief account of recent applications for which OPLC has found favour <sup>1</sup>.

The acronym OPLC describes a technique that dates back to the late 1970s.<sup>2</sup> The instruments at that time used nuts and bolts to compress a modified TLC plate between metal or glass sheets. There was little control over the pressure applied to the layer, as this was a purely manual technique. The TLC plate was also modified by the user with the addition of a sealant to the edges of the plate. To distribute the solvent homogeneously across the layer, it also was necessary to scrape a horizontal line of silica from the plate directly below the solvent inlet. These aspects, and others not mentioned here, hindered the development of the technique in modern analytical laboratories. Three very important factors contribute to an optimized homogeneity of lateral and laminar flow inside the 200-mm thick sorbent bed. First, the introduction of electronic controls provides better control of the chromatographic conditions and the quality of the

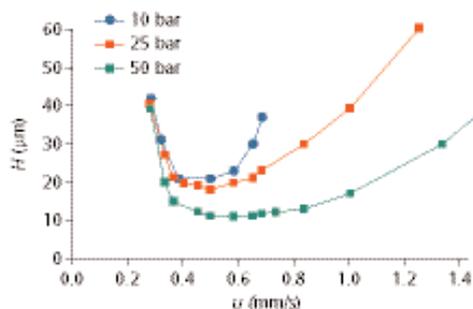
separation <sup>3</sup>. Second, factory-made flat columns have a high precision positioning and a more regular seal. Finally, the flowing eluent wall technology dramatically reduces disturbances at the layer edge and the inlet site (see text that follows and Figure 1). The technology described in recent articles demonstrates the jump in progress that justifies a new terminology introduced in 2000: optimum performance laminar chromatography <sup>4</sup>. This review will describe the basis of modern OPLC instrumentation and the separation techniques, as well as give a brief account of some recent applications in OPLC.



**Figure 1:** See text for details.

Instruments and Columns OPLC instruments: The basis of OPLC is similar to that of other chromatographic techniques in that a pump is used to force a liquid mobile phase through a stationary phase such as silica or a bonded-phase media (that is, C8, C18, amino or nitrile).

The uniqueness of OPLC lies in its column- housing structure, which allows flat planar columns to be used in the same way as cylindrical glass or stainless steel ones. An OPLC development chamber is composed of three basic structures: a planar column; a cassette constructed of a machined PTFE sheet and an aluminum support; and an electronically controlled hydraulic press. The planar column itself is a uniform sorbent bed layered on a glass or aluminum backing with an elastomeric polymer seal located at the periphery. The column is placed silica face-up in the cassette, in direct contact with the PTFE sheet. This in turn is placed inside the hydraulic press of the development chamber. Upon pressure, the PTFE sheet is pressed onto the sorbent bed and compresses the seal, creating a solvent- tight system ready for chromatographic separations. Mobile phase then is pumped through connections on the development chamber to the sorbent bed. The machined PTFE sheet is responsible for distributing solvent to particular zones on the column and recovering it from the sorbent bed as a chromatographic separation is performed. The first instruments, marketed some 25 years ago, applied relatively low pressures to the sorbent bed. Higher pressures, achieved in modern instruments, provide a better compaction of the sorbent bed and a more intimate contact of PTFE sheet and silica, which results in a noticeable increase in efficiency at a higher optimal flow rate (see Figure 2).



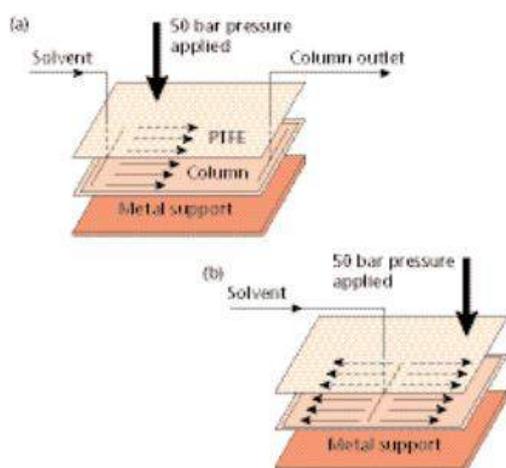
**Figure 2:** Plate height versus linear flow velocity as a function of applied pressure in OPLC for HPTLC layers (5-mm particle size) b) Instrument of OPLC

## II. METHODS AND MATERIAL

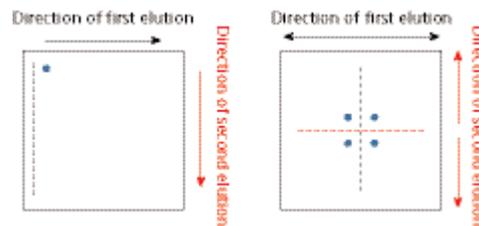
### A. Cassettes

The cassette has two main functions: creating solvent-tightness under compression and determining solvent flow within the sorbent bed. A cassette comprises an aluminum base plate and a PTFE cover. The PTFE sheet has 1-mm holes that allow flow to the sorbent bed. Microchannels (250- $\mu\text{m}$  wide, 350- $\mu\text{m}$  deep) on the underside of the PTFE sheet direct and recover solvent from predetermined zones on the stationary phase. Different cassette designs provide different separation modes. With two standard cassettes, it is possible to perform basic 1-D separations, as well as bidirectional, 2-D, and simultaneous four- sample 2-D development. Figure 3 shows schematics of both mono- and bidirectional elution cassettes. Cassette (a) is used for one-sample on-line separations or for the off-line separations of 1–50 samples in a single run (on- and off-line separation techniques are described later). This cassette format delivers mobile phase evenly over the entire width at one end of the column with recovery of eluting solvent at the other end. Samples are placed directly on the column 1 cm from the inlet. Twice as many samples can be separated on the same column using a bidirectional elution cassette (Figure 3b), which introduces solvent over the midline of the column. This cassette type also serves as a rinsing cassette when changing solvent systems. With this cassette, separation occurs out- wards in two directions toward the extremities of the column. Here, samples are deposited 1 cm to either side of the inlet trough, and the separation distance is only half the length of the column. 2-D separations can be achieved with mono directional cassette formats. The sample is placed in one corner of the column, 1 cm from the inlet trough. After development in the first direction, the column is removed, turned 90° such that the developed line is parallel to the inlet trough, reinserted and a second development is performed. Similarly, parallel four- sample, 2-D analyses are run using a bidirectional cassette format, starting from the central corner of each quadrant of a 20 3 20 cm flat column. Again, each separation is performed over half the column length.

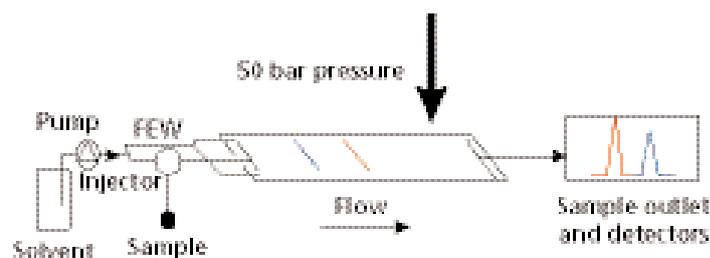
Each of the four samples migrates outwards from the center over a 10 x 10 cm area of the flat column (Figure 4). While other designs exist (such as radial, stacked parallel, or serial elution), these are less common in routine use. Specific cassettes are available for different column widths. More often, method development is performed on the smallest column, 5 cm wide, while screening and semi-preparative work is often done on intermediate and full-width columns (from 10 to 20 cm). Recently, a new technology has been introduced, known as flowing eluent wall (FEW) for on-line and semipreparative purposes<sup>5</sup>. Conceptually, FEW is a stream of sample-free eluent that is pumped to the lateral wall of the column, near the elastomer seal. This confines the sample to a central portion of the column and eliminates direct contact of the sample stream and the wall, improving band (peak) shape. The FEW does not require any additional equipment. The solvent stream simply is divided upstream of the injector: one stream goes to the injector and onto the portion of the column where the sample is to be separated, while the FEW stream is taken directly to the lateral walls of the column (Figure 5). Figure 6 shows a comparison of the separation of dyes injected on both a modern OPLC system with FEW and an older non-FEW version of the same instrument. We can see that flow is affected at the edges in the non-FEW instrument. We also can see that the sample band is deformed at its centre in the non-FEW version. This has been corrected in the FEW system by a modification of the inlet structure of the cassette.



**Figure 3:** OPLC cassette design for (a) linear one-dimensional and (b) bidirectional chromatography separations steps.



**Figure 4:** 2-D development on a planar OPLC column. Dotted lines correspond to solvent inlet microchannel. Blue corresponds to sample, and white, black, and red are the sequential development



**Figure 5:** Concept of FEW with the distribution of solvent at sample channel and flowing walls.



**Figure 6:** Close-up of dye molecules at column wall during a chromatographic separation with (a) non-FEW and (b) FEW instruments.

## B. Columns:

Presently, flat columns are made of irregularly shaped, preparative-grade silica (5 and 11 mm; 500 m<sup>2</sup>/g)<sup>6</sup> placed on an aluminum or glass backing (that is, HTSorb<sup>TM</sup> columns [Bionisis, Le Plessis Robinson, France]). They are 20 cm in length and come in three different widths: 5, 10, and 20 cm depending upon the capacity desired. Standard thicknesses are 200 μm and 500 μm. An elastomeric seal is placed at the periphery of each flat bed column. Normal silica and standard bonded phases are available, including C8, C18 nitrile, chiral, and amino phases. Plate height is independent of the solvent-front migration distance and achieves a minimum value for high-performance silica layers of about 10 μm at a mobile phase velocity of 0.5 mm/s.<sup>7</sup> This is sufficient for some analytical methods and is very acceptable for micro preparative isolation (20–200

mg). In addition, the possibility to observe residual non migrating products on the column provides a higher degree of confidence in the final analytical result.

### C. Basic Operation

A basic OPLC unit can be configured into an HPLC system and can either be used on-line, in the same way as a standard cylindrical HPLC column, or off-line, as with a high performance TLC development. In general, one uses the term “mode” to describe the way in which samples are applied and detected: off-line means that the column is handled outside the development chamber while on-line indicates that the column is left inside the chamber and is connected to the pumping and/or detection modules. A good technical description of the relationships between retention time and migration distance in OPLC has been developed by Siouffi and Mincsovic<sup>8</sup>.

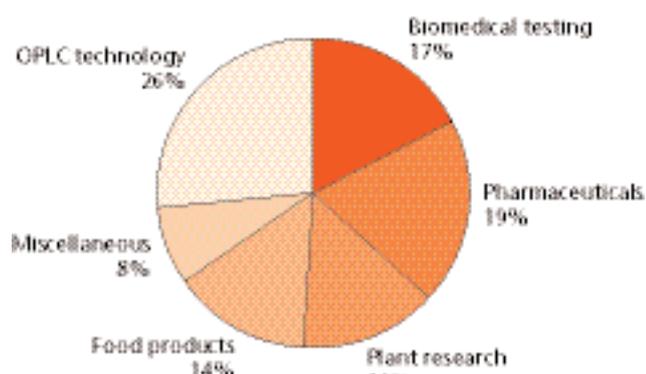


Figure 7: Published articles on OPLC technology and applications in various domains of research.

### D. Fully on-line mode:

The fully on-line mode has very few examples to date, mainly because a dedicated unit for this type of work only recently has become available. In brief, the OPLC unit is installed in an HPLC system much like an HPLC column. The programmable workstation of the HPLC system ensures column equilibration, sample injection, detection, and monitoring of the separation; that is, UV, mass spectrometry (MS), nuclear magnetic resonance (NMR), refractive index (RI), and evaporative light scattering (ELS). On-line mode can be used as an analytical or semi-preparative method<sup>9</sup>. FEW technology gives better peak shape in on-line separations.

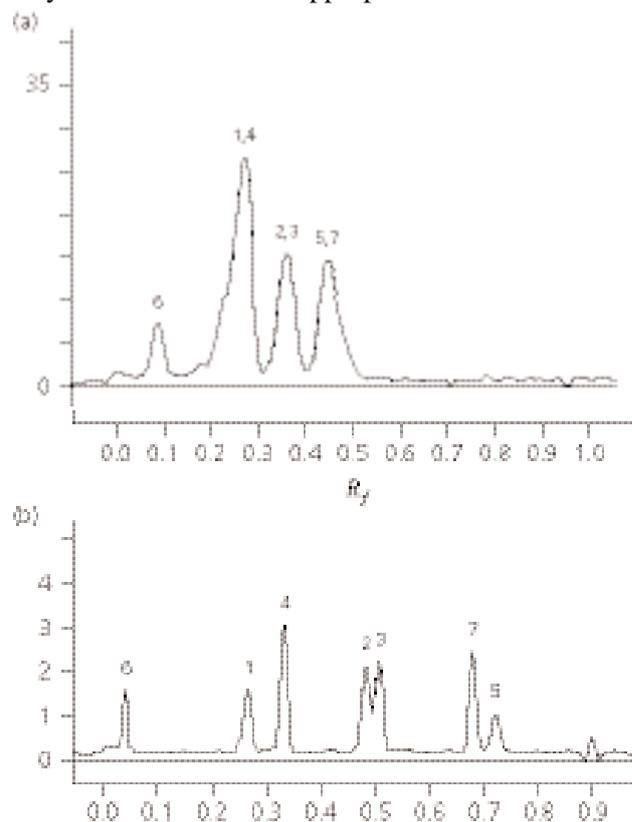
### E. Fully off-line mode:

This mode corresponds to application of the sample and the subsequent analysis of the separation directly on the stationary phase. The different steps of sample application, separation, and detection are effectively decoupled in off-line mode. Formulated products or crude extracts can be applied directly on the stationary phase without prior sample preparation steps. In many instances, the active ingredients can be determined among the formulating agents<sup>10</sup>. The possibility of depositing a sample on the column is an advantage, particularly when the sample is in a solvent that is not miscible with the eluting solvents, or when increased sample loading is desirable. The quantity of sample applied is not limited by a development<sup>11</sup>.

Otherwise, it is possible to flush the air from the column with an a polar solvent that does not cause migration of the products, then change solvents to provide the desired separation. A second phenomenon, known as solvent demixing, occurs when using a solvent mixture as the mobile phase and silica as the stationary phase. Demixing describes depletion of the more polar components of the mobile phase as a result of their strong interaction with a non-equilibrated (dry) stationary phase.<sup>12</sup> Resaturation with the solvent will reduce this effect dramatically and could represent a solution to the problem.<sup>13</sup> However, demixing can be useful. For a two-solvent system, two migrating fronts are observed: the total wetness front, as discussed previously, and a b-front resulting from demixing. Above this b-front, the mobile phase is almost devoid of the polar component of the mixture, while below it, the mobile phase is complete. In general, a small amount of a highly polar solvent will give a b-front at low R<sub>f</sub>, while a larger fraction of a less polar solvent will give a b-front at a higher R<sub>f</sub> value. By properly adjusting the amount and nature of the polar modifier, it is possible to create zones of “defined” polarity at predetermined areas on the column. Properly done, this can provide higher peak capacity for complex sample mixtures. Maintaining the sample components behind all secondary fronts is necessary when OPLC serves as a precursor to HPLC method development. Elution should be continued beyond one column volume of solvent. Samples either can be maintained injection loop as in HPLC. Sample components are not eluted from the sorbent bed, as the results of the separation are observed directly on the

column. Much longer time can be spent in the detection mode when it effectively is decoupled from the separation technique, because detection is not limited by the rate of elution. In some instances, this can lead to significant gains in the limits of detection, particularly with radio labeled metabolites<sup>14</sup> A fluorescent indicators (254 nm) can aid detection, or one could choose to derivative with a spray-on reagent solution to visualize the sample components on the column<sup>15</sup>. Semi quantitative screening and sample-comparison assays typically are performed with multiple samples on the same column, including calibration standards. Analytical determinations of UV-active substances are obtained with a full-spectrum scanning densitometric instrument, or more simply with a single-wavelength desktop scanner<sup>16</sup>. Other types of detection that have been used in conjunction with OPLC include radio-detection, Raman spectroscopy<sup>17</sup> and bioautography. Finally, the column is removed, stored with experimental records, and if necessary, retrieved for analysis later or at another location. With off-line OPLC, there is less waste (from 10 to 100 times less solvent consumption and fewer disposables used) as sample preparation is reduced to a bare minimum and column equilibration and regeneration are not necessary. In some instances, applying crude sample mixtures to the column can lead to improved reproducibility as the number of sample pretreatment steps is limited, and all of the sample components actually reach the stationary phase where the separation is performed. From a practical standpoint, fully off-line separations are more complex than on-line separations, and this can be disconcerting for uninitiated users. First, it is necessary to program a rapid solvent injection at the start of an off-line experiment, particularly for large column widths. This flash volume establishes a homogeneous flow over the width of the column, a requirement for linear bands and good peak shape. As the solvent passes into the sorbent bed, air is displaced. If the column outlet is open to allow gas to escape from the column, two solvent fronts can be observed: a faster eluting partial wetness front and a slower total wetness front. By simply closing the outlet on the OPLC, a pressure build-up occurs within the separation chamber, preventing vaporization of the eluting solvent and causing the air in the column to dissolve in the solvent. The upper pressure limit of the pump is set automatically by the instrument so that the pump stops when the development is complete. In this manner, the partial wetness front is not observed in full

column on the stationary phase and observed directly or they can be eluted to an appropriate detector.



**Figure 8:** Densitometric analysis of nandralone, analyzed by (a) TLC and (b) MD-OPLC. Reproduced from reference 13 with permission

## F. Other on-line and off-line techniques:

When OPLC is used as a tool for HPLC method development, it is possible to examine the column as development occurs to see how a particular method performs. If the conditions are inadequate for a satisfactory elution of the products, it is possible to put the column back into the OPLC, change the solvent conditions, and continue with the new elution method. Even when a method has been optimized for a particular product, the analyst always can recover the column to verify that the entire sample has been eliminated from the column. Mass balance analysis in drug-stability testing is just one application for which this mixed-mode technique finds an added advantage relative to HPLC. If non migrating products are observed on the column, it is possible to recover them by extraction and identify them using another method. Recent developments in the field of TLC-MS are pertinent for structural determination directly from the OPLC stationary phase<sup>18</sup>. Further improvement in resolution

can be obtained by using multiple development techniques.<sup>19</sup> Multiple developments is a series of development and drying cycles that cause solvent to flow over the sample several times during the course of a separation. Multiple developments takes advantage of the peak compression that occurs as the mobile phases pass over products on a dry sorbent bed and can provide very high resolution. Multiple development-TLC, which has been known for years, is an under- exploited high-performance TLC technique, mainly because it requires a long analysis time. With the pumped-flow of an OPLC, multiple developments become more accessible and has been applied successfully in pharmaceutical analysis for difficult separations<sup>20</sup>.

### III. RESULT AND DISCUSSION

#### A. Features and Benefits

- High chromatographic efficiency.
- Flexibility in monitoring the progress of the separation by either on-line or off-line detection.
- Simple scale-up from analytical to semi-preparative purification of up to 200 mg.
- Visualization of compounds retained on the stationary phase prevents any loss of information.
- The Personal OPLC-50 is a standalone unit that comes with its own pump.
- Inexpensive, disposable sorbent beds, low solvent consumption (up to 1000x less than other LC techniques) and fast separations (5-20 minutes) make this technique particularly economical.

#### B. Typical Applications

- i OPLC is a general separation technique that has been successfully applied to problems such as:
- ii QC of pharmaceutical products.
- iii Determination of impurities in drugs and reaction mixtures and cleaning validation of manufacturing vessels.
- iv Natural products: Extraction of compounds of pharmacological interest from natural products.
- v Drug metabolism: Isolation of metabolites in biological fluids.
- vi Sample preparation: Purification of reaction mixtures to extract the compounds of interest for additional studies (e.g. for NMR or MS).

6) Optimization of HPLC methods: determination of optimal solvent system for HPLC to eluate all compounds out of the column thanks to the possibility to inspect the flat column for retained compounds.

- vii Oligomers and synthetic polymers: Separation of oligomers of natural (e.g. peptides) and synthetic polymers (e.g. polystyrene).
- viii Toxicology: Determination of toxins in foodstuffs (e.g. aflatoxins in wheat).

#### C. Applications

OPLC technology has integrated several disciplines including pharmacognosy (plant research), pharmaceutical development, drug metabolism, drug abuse assays and others (see Figure 7). At this stage, in the development of OPLC, journal articles related to OPLC technology outnumber specific applications. However, many specific applications have been explored over the past 20 years. These include the detection of drugs and metabolites in animal tissues (homogenates, urine), potentially active ingredients in plant extracts,<sup>21</sup> formulating agents in cosmetics (lipsticks, shampoos and creams),<sup>22</sup> toxins in food stuffs, as well as chemical substances in crude reaction mixtures. Detection limits can reach 0.1–1000 ng depending on the product and the detection techniques employed. The aspects that make OPLC a preferred technique for many of these assays include limited sample preparation, the semi- disposable nature of the column, high capacity suitable for semi-quantitative analysis and micro-preparative scale-up, multiple parallel samples in a single run, and the possibility of direct on-column detection. The following paragraphs highlight four application types to exemplify a few aspects of the technique and the flexibility it offers.

#### D. Screening In Forensic Science

The Finnish group of Pelander has developed an OPLC technique for high-confidence identification of drug abuse substances in urine samples.<sup>23</sup> The authors take an innovative 2D approach to the problem, developing each sample twice: once in an acidic solvent and then in a basic solvent mixture. Fifteen samples and Rf correction standards are applied to each column.<sup>24</sup> After scanning densitometry of each separation, two corrected Rf values

are used to identify a particular drug substance from among more than 200 library compounds. A graphical 2D representation of their data is given in Figure 9, together with chromatograms from the analysis of a urine sample containing codeine. In zones on the map where two or more products show nearly identical Rf data, it is possible to differentiate between them by their UV spectra using scanning UV densitometry on the column. The success of this technique relies on the high peak capacity of the columns and the choice of two solvent systems with a low mutual correlation between them. In clinical and forensic toxicology the use of this low cost, rapid screening technique allows the group to perform routine high-throughput analysis, while more sophisticated instrumentation (LC-MS and GC-MS) is dedicated to other tasks (i.e., low-dose target compounds). Other specific assays for opiates, alkaloids (poppy)<sup>27</sup> and cannabinoids<sup>28</sup> have also been developed using OPLC.

#### **E. Preparative Isolation:**

OPLC is also semi-preparative chromatography allowing the isolation of more than 200 mg per run on a 20 × 20 HTSorb™ column.<sup>25</sup> It has been shown that the injection volume can attain more than 20% of the column volume while still maintaining adequate resolution for fractionation. OPLC has also been used for fractionation of metabolites of a radiolabelled pharmaceutical from tissue extracts during ADME studies.<sup>26</sup> Preparative OPLC columns 20 × 20 cm and 500 μm thick have only recently become available (11 μm particle size). Method development performed on a 5 cm wide column can be scaled directly to a 20 cm wide preparative column by simply adjusting the flow-rate. OPLC is also being explored as a substitute for solid-phase extraction prior to NMR studies.<sup>27</sup> The interest lies in the possibility to visualize the sorbent bed and to know when the molecules of interest have been eluted from the column. This is not a trivial matter in this application, as the molecules are eluted with deuterated solvents.

#### **F. Bioactive Molecule Research:**

Bacterial and fungal toxins and their metabolic by-products in food products are known to cause serious health problems in humans and animals and, therefore, require regulatory monitoring. Several publications

relate methods for the detection and assay of mycotoxin metabolites in food stuffs,<sup>29</sup> such as rice,<sup>30</sup> wheat<sup>31</sup>, fish<sup>32</sup> and corn.<sup>33</sup> The authors propose a screening method for aflatoxins with a detection limit below 0.1 ng which requires minimal preparation, high sample throughput and low operating costs.<sup>26,32</sup> Similarly, OPLC methods have also been developed for some peptide cyanobacterial toxins (microcystins and nodularins).<sup>34</sup> Bioautography is a particular on-column detection method that has also been applied in combination with OPLC in the search for bioactive substances from plant sources (pharmacognosy) and in toxic substance detection.<sup>35</sup> The OPLC column serves a double purpose: as the separation medium and as a mechanical support for cell culture. If a bioactive substance is present on the column, cell growth is modified (i.e., antibiotics inhibit proliferation, leaving cell-free zones on the column). The active substance can then be isolated and characterized. It is interesting to note that Tyihak et al. have taken this OPLC-bioautography technique a step further to explore cellular defence mechanisms.<sup>36</sup> OPLC, with its open planar column format and high peak capacity, is the only high-resolution, forced flow chromatographic technique capable of rapid, high-throughput screening with bioautography.

### **IV. CONCLUSION & FUTURE DEVELOPMENTS**

#### **Future Developments/Perspectives in OPLC**

OPLC is coming of age. Its rudimentary beginnings, some 25 years ago, have led to innovative, high-performance products. The commercialization of modern, high-efficiency columns has eliminated the need for analysts to prepare their own from TLC plates, making the technique even more accessible. The open-heart column in OPLC provides several unique possibilities for product detection, including standard on-line techniques such as UV, radiation, ELS, ESI-MS and NMR. Off-line detection methods include densitometry via a fluorescent indicator, via the UV chromophores of molecules on the column or via the addition of colorimetric reagents to reveal the presence of specific molecules (particularly those that do not have a chromophore). ESI-MS and MALDI-MS have been demonstrated with TLC and, therefore, should also be applicable to OPLC columns. Today, practical solutions are available for several sample types. An increasing number of publications in the field show that several

groups have adopted the technique, more often as a complement to HPLC, as it has all the required properties for preparative isolation, semi- quantitative screening and product profiling (fingerprinting). FEW technology has recently been integrated into routine instruments. This is also a fundamental part of a unique multiple sample injection/detection system which allows simultaneous, parallel purification of 4 or 8 samples in a single run using the un segmented flat columns presented here.<sup>28</sup> This opens the avenue for new applications in the field of high- throughput screening, combinatorial chemistry and microscale preparative isolation after parallel synthesis. 2D protein analysis could also become a viable application for proteomics research on these 2D flat columns; active research is underway in this direction. These and many other exciting possibilities are motivating innovations that only flat column technology can provide. For these and many other reasons, OPLC merits its place in modern research laboratories next to classic column chromatography instruments.

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# Herbomineral Formulations - A Review

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## ABSTRACT

Ayurveda has earth-shattering in audience of worldwide on virtue of its holistic approach of life and its fewer side effects. Ayurvedic herbal and herbo-mineral preparations are used for the treatment of chronic and degenerative diseases without any side-effect. Herbo mineral formulation uses the metals and minerals for chronic disorders in different combinations, dosage forms and at various levels of purities. Hence it is very essential to prepare it in a proper way. As per the reported data, there are so many herbo-mineral formulations available in market which is useful in anaemia, diabetes, cancer, liver diseases, skin diseases etc. This review is an attempt to emphasis on the benefits and problems associated with it.

**Keyword:** Cumulative toxicity, Ayurveda, Bhasma, Heavy metals, Herbo-mineral

## I. INTRODUCTION

Ayurveda is the science of life which deals with maintenance of health of healthy persons and cure of ailing humanity as its [01]main ambition .Ayurvedic doctrine of treatment is based on Hetu, (etiological factor) linga (symptom/manifestation) and Aushadh (medicine) and all these stand on concept of Tridosha (three fundamental humours), Panchamahabhuta (five basic of existence of cosmos), saptadhatu (seven vital [02] functionaries of human physiology). Ayurvedic physicians put into practice these all factor with excellence of their individual skill to find out understanding of Dosha-Dushaya-Samurchana (aetiology and pathology of disease) and SampraptiVighattan (breaking of pathology of disease to get healthy state of functions). Ayurveda viewed health as a state of many-sided equilibrium, and disease as its reversal. Ayurvedic practice of medicine aimed at the restoration of equilibrium and, in that process, represented more than the sum of dietary regimen, procedures and medications.

As quoted in ChikitsaChatuspad after the physician, medicine is said to be the second most important part and it is considered as the main tool by which one performs his duty [03] of treating the patient .In general we may categorise all Ayurvedic drugs in two group i.e. Kasthoushadhies ( herbal preparation ) and

Rasaoushadhies (Herbo-bio-mineral metallic preparation ).

Rasashastra may be defined as a branch of Ayurveda which deals with the various pharmaceutical processes of Shodhana (purification/potentiation), Marana (incineration/calcinations), Jarana (polling), Murchana (aprocedure by which substances specially mercury is transformed for therapeutic application) and other detailed description of metals, minerals, poisonous herbal drugs and animal products used therapeutically in practice of Ayurveda.[04].The innate qualities of Rasasadhies like quick action, lesser dose, tastelessness, prolonged self-life, better action, lesser dose, tastelessness, prolonged self-life, better well as pharmaceutical proprietors. [05].

## II. METHODS AND MATERIAL

### A. Herbomineral Formulations

#### Rasayana Tantra

The word Rasayana is composed of two words, rasa and Ayana. Rasa means dhatus responsible for sustenance of life. Ayana means specific measures used for obtaining rasa and responsible for longevity. Rasayana is a well-developed concept in Ayurveda. Rasayana means the augmentation of rasa, the vital fluid produced by the

digestion of food, which sustains the body through the strengthening of the dhatus [6].

In modern term, the study and practice of Rasayana is referred as rasavidya (alchemy). Rasa ausadhis are known as metallic preparation which includes Bhasma and sindoor. Metals like gold, silver, copper, lead, tin and iron, sand, lime and minerals like red arsenic, germs, salts and red chalk are indicated as drugs in Ayurveda. There are more than 200 plants mentioned in Rasashastra which uses mineral and metals as medicinal plant or a desirable chemical property [6].

## B. The Aim and Types of Rasayan

Rasayana therapy enriches rasa with nutrients to help one attain longevity, memory, intelligence, health, youthfulness, excellence of luster, complexion and voice, optimum development of physique and sense organs, mastery over phonetics, respectability and brilliance.

### Types of Rasayana[7]

1. Kamyas Rasayanas are promoters of normal health. These boost body energy levels, immunity and general health.
  - o Pranakamyas – Promoter of vitality and longevity
  - o Medhakamyas – Promoter of intelligence.
  - o Srikamyas – Promoter of complexion.
  - o Naimittika Rasayanas help to fight a specific disease.

In pursuit of these matters, herbal prescriptions with many herbal substances, preserved in ghee and honey are given. Chyawanprasha is one of the traditional rasayanas.

Specific adaptogenic herbs are also included in rasayanas including amla, shilajit, ashwaganda, holy basil, guduchi and shatavari. Several rasayana herbs have been tested for adaptogenic properties:

The whole, aqueous, standardized extracts of selected plants (*Tinosporacordifolia*, *Asparagus racemosus*, *Emblicoefficialis*, *Withaniasomnifera*, *Piper longum* and *Terminaliachabela*) were administered orally to experimental animals, in a dose extrapolated from the human dose, following which they were exposed to a

variety of biological, physical and chemical stressors. These plants were found to offer protection against these stressors, as judged by using markers of stress responses and objective parameters for stress manifestations. Using a model of cisplatin induced alterations in gastrointestinal motility; the ability of these plants to exert a normalizing effect, irrespective of direction of pathological change was tested.... All the plant drugs were found to be safe in both acute and sub-acute toxicity studies. Studies on the mechanisms of action of the plants revealed that they all produced immunostimulation. The protection offered by *Tinosporacordifolia* against stress induced gastric mucosal damage was lost if macrophage activity was blocked. *Emblicoefficialis* strengthened the defence mechanisms against free radical damage induced during stress. The effect of *Emblicoefficialis* appeared to depend on the ability of target tissues to synthesize prostaglandins. Recent data obtained with *Tinosporacordifolia* have led researchers to suggest that it may induce genotypic adaptation, further opening the arena for more research and experimentation.[8]

## C. Rasayana Formulae

Puri [9] has given detailed account of Classical formulations such as AmritRasayana, BrahmRasayana, JawaharMohra, KamdugdhaRas, Laxami Vilas Ras, Laxman Vilas Ras, MadanodayModak, Makrdhawajvati, ManmathRas, MuktaPanchamritRasayana, NariKalyan Pak, NavjeevanRas, NavratnaRas, NavratnakalpAmrit, PanchamritRas, ParadiRas, RamchuramniRas, Rattivalbh Pak, ShukarAmritVati, SmritisagarRas, SuvarnMaliniVasant, Suvarn VasantMalti, Swapanmehtank, VasantKusmakarRas, VishaRasaayana, VrihdaVangeshwar Rasa.

These classical Rasayan formulas, contain a large number of ingredients, including minerals, pearl, coral and gems, and include a specially processed (samskara) mercury (the word *Ras* indicates mercury as an ingredient). Because of negative publicity and cost factor, the use of the classical rasayana formulas has declined considerably, and most of the preparations available now have herbal ingredients with a couple of mineral and animal products. The non-availability and wild life protection act has made the use of musk, amber, and parts of wild-life animals nearly impossible. The current Rasayan formulas are based on such

ingredients as amla (Emblicaofficinalis which has very high stable Vitamin C, Vitamin E, and proteins), Terminaliabelerica, Terminaliachebula, Shilajit (a mineral exudate high in fulvic acid), Long pepper, Black pepper, Ginger, processed Guggul, Guduchi, Ashwaganda, Shatavari and similar ingredients.

Rasayan has meanings beyond healthful substances. RasayanShastra in Ancient India was much less developed than today. Nevertheless, the use and practice of Rasayan was widespread in Ancient India, and some examples of applied rasayan include paints used in the caves of Ajanta and Ellora, Maharashtra, the steel of Vishnustambha (literal meaning: the tower of Vishnu), and a processed wood sample in the Kondivade caves near the Rajmachi fort in Maharashtra.

#### D. Rasayana (Fruit Squash or Juice)

In many Indian homes, Fruit squash, juices are prepared and served as drink, desert or as accompaniment to meals. In Tulunadu region of India, Banana and Mango Rasayana are made by mixing of fruit pulp with cow's milk or water with thick consistency. This rasayana may be drunk as juice by diluting with water or milk. With thick consistency it is used as accompaniment to Dosa, Chapati or meals. Rasayana is also known as lassi. Many believe this rasayana helps to beat the heat of Indian summer.

#### E. Bhasma

Bhasma is an ash obtained by calcinations of metals. It is a unique preparation of Ayurveda with extracts of herbs and metals in combination which functions best when converted from their original metals to metal oxide forms.[10]. It is prepared by calcinations of metals in a closed crucible in pits and with cow dung cakes (puta).

Bhasmas are biologically produced nano-particles and are taken along with milk, butter, honey or ghee which makes these elements easily assailable, eliminating their toxic effects and enhancing their biocompatibility.[11]

#### F. Preparation of BHASMA[12]

It is prepared from purified minerals, metals, marine and animal products. The process of purification is called Shodhana.. It is aimed to remove harmful impurities present in the drug or sometimes modification of

undesirable physical properties of the drugs. It helps in enhancement of the therapeutic action and thereby increases the potency of drug. Shodhana is of two – Samanya Shodhana applicable to a large number of metals or minerals and Visesa Shodhana applicable to certain drugs and in certain preparation. After Shodhana, second stage is the Marana which means metals and minerals are made into paste with various drugs and juices. It is essentially the burning process or calcination. It is subjected to fire treatment in a measured manner for reducing them to ashes. The objective of doing Marana Process is to reduce the size of the drug to its finest particles so that it absorbs easily into the system and produce their desired effects without producing harmful side effects.

#### G. Types of BHASMAS[13]

Bhasmas are classified based on their colour and appearance. Scientifically they are classified based on their dominant metal and mineral groups, such as rajata bhasma (silver), tamra bhasma (copper), loha bhasma (iron), pravala bhasma (shells) etc. .bhasmas are generally yellowish, black, dark, white, grey, reddish black and red, depending upon the predominant drug as well as the other drugs used in the process of marara. They are stored in air tight container (glass or earthen) and maintain their potency indefinitely

#### Herbomineral Formulations

Company name	Marketed formulation of Bhasma
Shree Siddhashram Pharmacy, Jaipur, Rajasthan	<i>Abhrak Bhasma, Bang Bhasma, Kashish Bhasma, Ropya Bhasma, Tamra Bhasma, Swarna Bhasma</i>
Shree Baidyanath Ayurved Bhawan (P) Ltd. Kolkata	<i>Abhrak Bhasma, Akik Bhasma, Vaikrant Bhasma, Godanti (Harital) Bhasma</i>
Dindayal Aushadhi Pvt. Ltd. Dindayal City Mall, Gwalior	<i>Kashish Bhasma, Ropya Bhasma, Tamra Bhasma, Swarna Bhasma, Naga Bhasma</i>
Gurukul Kangri Pharmacy, Haridwar	<i>Swarna Bhasma, Ropya Bhasma, Tamra Bhasma</i>
Unjha Ayurvedic Pharmacy, Unjha, Gujarat	<i>Abhrak Bhasma, Bang Bhasma, Kashish Bhasm, Dhanwantary Prasad, Ropya Bhasma, Tamra Bhasma, Swarna Bhasma</i>

#### H. Evaluation Test of BHASMA[14]

1. Nischandrika – No chandrika or metallic luster
2. Rekhapurita – When taken between the index finger and thumb, it should be so fine as to get easily into the finger lines.

3. Varitara – Small quantity is spread on cold and still water, it should float on the surface.
4. Apurnabhava It should not revert to the original state.

## I. SINDOORA[12]

It is more potent preparation than bhasma and prepared by the elaborate process of sublimation. The preparation of sindoora process is called kupipakwavidhi in which sublimed mineral obtained on the neck of the sublimation glass flask is called sindoora. Characteristics and preparation of sindoora is similar to Bhasma.

## J. SHODHANA

It is a process of purification and detoxification by which physical and chemical blemishes and toxic materials are eliminated and substances are subjected for further processings[16][17]

### Procedures of Shodhana:

Following procedures are performed for the purpose of shodhana as per physico-chemical characters of substances.

1. Abhisheka (sprinkling) e.g. Mandura (Iron oxide) Shodhana

2. Achushana (absorption) e.g. Bhallataka (Semicarpusanacardium) Shodhana .
3. Atapa/Agni Shoshana (drying) e.g. Shilajatu (Black bitumen) Shodhana.
4. Bharjana (frying or roasting) e.g. Gairika (Ochre) shodhana.
5. Bhavana (levigation) e.g. Hingula (Cinabar) Shodhana .
6. Nirjalikarana (evaporation of water) e.g. Sphatika (Alum) Shodhana .
7. Mardana (trituration e.g. Parada (Mercury) Shodhana

## K. MARANA

The process which converts the purified metals and minerals into Bhasma (fine powder) after subjecting them to levigation and incineration is called as Marana.

## L. BHAVANA

Required amount of substances and liquid media are levigated smoothly for specific period and is shaped as requirement, often in flat shape and if pressed between finger tips it should be soft to touch, this is considered as indication of proper completion of process.

## Herbomineral Formulations

Compound name	Reference	Mineral ingredients	Sneha dravya, Madhura dravya and Drava Dravya for Bhavana
Ashtadashanga Lauha (Kiratadi Mandura)	BR-Pandu 12/34-36	LB (50%)	Honey, Cow's Ghee
Yograj	C.S Chi. Pandu 16/78-86, BR-(Pandu) 12/109-15	LB (12.8), SMB, Rajata Makshika Bh, Shilajit	Sugar candy, Honey
Maha Shwasari Lauha	BR-(Hikka Shwasa) 16/39-41	LB (15.3%), AB, Vamsalochana	Sugar candy, Honey
* <sup>4</sup> Saptamrita Lauha	BR-(Shula) 30/130 64/234-36	LB (50%)	Honey, Cow's Ghee
Yakshhari Lauha	BR-(Rajayakshma) 14/83	LB (50%), SMB, Shilajit	Cow's Ghee
Shularaja Lauha	BR-(Shula) 30/131-36	KLB (3.7%), AB	Sugar candy, Honey
Trikatrayadi Lauha	BR-(Pandu) 12/38-43	KLB (9%), MB	Honey, Cow's Ghee, five types of Sharkara (sugars)
*Pippalyadi Lauha-1	BR-(Hikkashwasa) 16/42-3	LB (50%)	Honey, water
Sarvatobhadra Lauha	BR (Amlapitta) 56/42-53	LB (16%), TB, AB, Kajjali (Dviguna), SMB, Manahshila, Shilajit	Honey, Cow's Ghee

## M. VIBHUTI

In certain circumstances Bhasma, 'Vibhuti' (Sanskrit) and 'Thiruneeru' (Tamil) are synonymous.

### Toxicity

Modern medical science finds that mercury is inherently toxic, and that its toxicity is not due to the presence of

impurities. While mercury does have anti-microbial properties, and formerly was widely used in Western medicine, its toxicity does not warrant the risk of using it as a health product in most circumstances. The Centers for Disease Control and Prevention have also reported a number of cases of lead and mercury poisoning associated with rasa shastra containing Ayurvedic medicines.

### III. RESULT AND DISCUSSION

#### A. Advantages of Herbomineral Formulations

Herbo mineral formulation uses the metals and minerals for chronic disorders in different combinations, dosage forms and at various levels of purities. Hence, it is very essential to prepare it in a proper way. As per the reported data, there are so many herbo-mineral formulations available in market which is useful in anaemia, diabetes, cancer, liver diseases, skin diseases etc. Table-5 shows the list of herbo-mineral formulation and its uses in common diseases as per WHO guidelines Traditional medicines in Asia [18] Prakash et al [19] reported Prak-20 (judicious combination of nineteen herbs and MandoorBhasma) that is a potent hepato-protective herbo- mineral formulation used in treatment of liver ailments in CCl<sub>4</sub> induced toxicity in rats. Prak - 20 treated animals had minimum necrotic changes after seven day treatment and further studies are required to understand its mechanism of action. Chronic urticaria (Refractory skin diseases) often creates a therapeutic challenge. Herbal treatment is gaining popularity in the treatment of chronic urticaria. Azad Hussain et al [20] has reported that Unaniherbo-mineral formulation is effective and safe for the treatment of chronic urticarial. It has produced significant effect on itching, wheals, and erythema without causing any untoward effect or adverse reactions. Scabies (Skin diseases) is very common skin disease found in urban population of Gadap town area or Karachi, Pakistan. Sultan Ayaz et al [21] has explained the safety and efficacy of herbal coded medicine, ScaNeem (Herbal coded medicine), for the treatment of scabies. The test drug was found to be an economical, safe and effective in treatment of scabies. Infertility is a very common medical problem associated with male person. Rajeev Kumar et al [22] has reported the use of herbo- mineral supplement – Addyzoa® (Charak Pharmaceuticals, Mumbai India contains Withaniasomnifera and Emblicaofficinalis as its

principal constituent) in men with idiopathic oligoasthenoteratospermia (iOATs). The study concluded that treatment with Addyzoa is effective in improving the semen quality by increasing the sperm count and sperm motility in men with iOATs after 3 months of therapy. There was no change in the sperm concentrations, ROS, DFI or TAD levels. Ajitvaze [23] has described the use of Addyzoa herbo-mineral formulation in oligospermia in comparison with Ubiquinone (COENZYME Q 10). A double-blind, randomized controlled trial was conducted and study concluded that treatment with Addyzoa is effective in improving semen quality by increasing the sperm count and sperm motility. The results are comparable with that of Ubiquinone on semen parameters. Lauhabhasma is the most widely used herbo-mineral formulation used in iron deficiency anaemia. Verma P. R. P. and Prasad [24] have reported the use of Lauha bhasma in anaemia. It is better absorbed in GIT and devoid of the usual side effects associated with administration of the allopathic iron preparation. Pattonder R. K. et al [25] has reported the standardization and quality control of herbo-mineral drug AgnimanthaBhavitaShilajatu- ABS (contains Agnimantha and Shilajatu) and TrivritYuktaNavakaGuggulu- TYNG (contains Shunthi, Maricha, Pippali, Haritaki, Vibhitaki, Amalaki, Chitrak, Nagarmotha, Vayavidanga, Siddha Guggul and Trivia). Both ABS and TYNG were prepared using authentic raw materials and subjected for physicochemical analysis which provides objective parameters to fix up the standards for quality control of finished drugs. Pawar R. K. and et al [26] has reported the physico-chemical standardization and development of HPTLC method for the determination of Plumbagin in KalmeghNavayasaLoha. The proposed HPLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of KalmeghNavayasaLoha. The powder. Herbo-mineral formulation showed remarkable improvement in quality of life of various cancer patients who have been found to be refractory or poor responders to modern chemotherapy and radiation treatment. Jayawardhane N. D. N. et al [27] has stated herbo-mineral treatment as an adjuvant in the treatment of Hepatocellular carcinoma (HCC) patient. Herbo- mineral formulations (Hemabhraka, TamraSindoora, Vanda Bhasma, and Manikyapistietc) are safe, cheap and effective in management of HCC and have developed a new clinical application in treatment of Cancer. Saba Sheikh et al

[28] has reported a novel herbo-mineral formulation Las 01 (which contains a number of herbs and different types of inorganic minerals like mercury), to be effective as a potent anticancer drug in the human cell lines, the MCF-7 and Hela cancer cell lines. It is devoid of toxicity both in animals as well as human which is the main drawback of chemotherapeutic agents used in modern system of medicine. BadariNath and MadhusudhanaChetty [29] have studied the effect of a herbo-mineral tablet (containing green tea aqueous extract and Sodium selenite) for chemoprevention of lung cancer in tobacco severe adductors. The prepared tablets meet the Pharmacopoeia requirements and are more comfortable in use. Hypertension (High blood pressure) is a world's leading contributory case of death. It has estimated to have caused 7.6 million premature deaths and contributed to 92 million disability adjusted life years worldwide in 2001. Ruchika Nanda et al [30] has found the use of herbo-mineral medicine Rakatchaphar (contains Sarpganha, Shankhpushpi, Jatamansi, JaharMohrakhataiPishti, MotiPishti and Rassindoor) in essential hypertension. It has offered an efficacious and safe combination of natural product available for treatment of hypertension. Non-insulin diabetes mellitus is one of the major diseases speared worldwide and over 85% of diabetes mellitus is associated with high incidence of mortality and morbidity. Maji D. and Singh A. K. [31]has reported a D-400 herbomineral preparation (contains Eugenia jambulana, Pterocarpusmarsupium, Ficusglomerulata, Gymnemasylvestre, Momordicacharantia, Ocimum sanctum and Shilajeet) is effective in treatment of Diabetes mellitus. They have studied the effect of D-400 on blood sugar level, serum cholesterol, triglycerides, LDL, HDL, blood urea, serum creatinine levels and the effect of long-term diabetic complications. D-400 is shown to have beneficial effects as regards the long-term complication and can serve as an important adjuvant in the treatment of diabetes. The ancient Indian (Asian) Ayurvedic medicinal system uses herbomineral drugs to treat arthritis. Arvind Chopra et al [32] has tested RA-11 (ARTREX, MENDAR), a standardized multiplantAyurvedic drug (Withaniasomnifera, Boswelliaserrata, Zingibero officinalis, and Curcuma longa) in treatment of arthritis. It has been reported that RA-11 is effective in the symptomatic treatment of osteoarthritis knees.

## **B. Problems Associated with Ayurvedic Formulation**

Ayurveda is one of the world's oldest traditional medicinal systems and is experiencing revitalization among the consumers throughout the word. However the major drawback associated with Ayurveda is the lack of evidence based on its standard profile and quality and safety aspects of Ayurvedic formulations. There is a lack of data supportingthe efficacy of clinical trials in traditional medicines. Other major problem associated with Ayurvedic formulation is the loss of genetic biodiversity or risk of annihilation [33]. Heavy metal (HM) toxicity is a major safety issue in Ayurvedic formulations and it is essential to evaluate them for their content. Table-1 provides data of Ayurvedic formulations containing heavy metals (Lead, Mercury, and/or Arsenic) [34].Ayurvedic literature emphasizes the use of heavy metals in their formulation due to their particular biological properties for curing. Ayurveda has described specific physiochemical processes like sublimation, heating etc to detoxify the metals and to avoid its toxicity Heavy metals in Ayurvedic medicines include not only lead and mercury but other metals such as chromium, iron, zinc, nickel, cadmium, arsenic and tungsten. Amount of heavy metals and trace elements in Ayurvedic preparation depends on the geographical location, varying according to the quality of the soil, water or air pollution [35]. The American medical research community has sounded a heavy metal warning against Ayurvedic cures. Herbal products from the Indian system of medicine sold in the US contain dangerous levels of lead, mercury & arsenic [36]Environmental impact of heavy metals [37, 38] such as Cd, Pb, Hg and as it causes serious concern on the health of individuals.In Japan, Itai-itai disease [39] and Mina Mata disease [40] are caused due to the consumption of rice containing Cd and eating methyl mercury contaminated fish respectively. Table-2 gives information about most important disaster occurred with heavy metals [41].Current levels of toxic metals in some environmental compartments may be high enough to constitute a threat to human health. Table-3 provides data of global emission of trace metals into atmosphere, water and soil. Environmental exposure to low levels of lead has been allied with a wide range of metabolic disorders and neuropsychological deficiencies. Toxicity of lead at metabolic and cellular level in asymptomatic children include - Impairments in haemoglobin, vitamin

D and red blood cell nucleotide metabolism, - Trepidations of calcium homeostasis in the hepatocytes, bone cells and brain cells, - Neurological damage Dangerous level of cadmium in environment leads to intoxication of the kidney like tubular proteinuria ad renal dysfunction. High amount of arsenic in environment contaminates drinking water and causes skin cancer, peripheral vascular disorders (known as black foot disease), hyper-pigmentation and keratosis [42]. Table-4 provides data of most commonly used metals, its toxic effect on human and their treatment [43]. Medicinal plants grows in nature can accumulate heavy metals at certain extent which depends on its individual properties and the concentration of HM in soil, air and water [44]. Heavy metal contamination is due to accidental contamination during manufacturing process such as grinding, use of lead releasing equipment's or other manufacturing utensils [45]. Due to its hazardous effect, heavy metal content in plants and foodstuff must be limited and GMPs procedures should be implemented during manufacturing of Ayurvedic formulation [46]. Environment factors can be controlled by implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing medicinal products from herbal or natural sources. The public belief that herbal and natural products are safer than synthetic medicines can only be ascertained by imposing regulatory standards on herbal products that should be manufactured using good practises. [47].

### C. Cumulative Toxicity: Caused In Herbomineral Formulations [48]

Most of the Ayurvedic preparations prescribed contain herbal, minerals and metals. These preparations are believed to be fast acting and disease specific. Non purified heavy metals have been known for their toxicity. The possible heavy metal related toxicity arising from the use of herbo mineral preparation is the subject of interest. Historically there is no consensus on a scientifically validation. Regarding toxic effect of heavy metals used in herbomineral preparation. There is a layman tendency, unsupported by facts to assume that all the so called heavy metal and their compounds are highly toxic or have eco-toxic properties. The confusion keeps on increasing, when you find the most referred book on toxicology. All metals are present in earth's

crust and enter our bodies continuously at lower levels. The multimillion ayurvedic drug industries are attracting so many pharmaceutical companies and thousands of products are being marketed per year. Unfortunately, none of them have given emphasis on the toxic effect of herbomineral preparation. This lead to decrease in the projected efficacy, even though they are combination or modification of classical formulations. This may be due to following reasons: improper purification of heavy metals used in herbomineral preparation. The selected formulation may not be suitable for a specified clinical condition of that person .inappropriate form for the finished product. Method of modification for a classical drug or method of preparation of new drug may be incorrect. Failure of determining the accurate dose. Out of the above said factors the improper purification plays a key role in developing toxicity in formulations containing mineral drugs. The incidence of cumulative toxicity is related to the concentration of toxic chemicals in the body. In the living organism, local, systemic, short term, delayed, reversible or irreversible toxicity may be precipitated. Many chemicals are toxic because they are bio transformed into more toxic chemicals. In this situation effective measures should be taken to inhibit biotransformation, to de- crease the toxicity of compound preparations.

### D. Causes of Toxicity In Herbomineral Preparations

1. **Improper identification of crude drugs-** Ayurvedic products most commonly has multiple ingredients. Before taking any crude drug to a formulation they should properly get identified. If the drug is wrongly identified then this may lead to unwanted effects.
2. **Adulteration/ substitution-**This is another serious problem associated with Ayurvedic formulation. Most of the Ayurvedic manufacturers are purchasing the crude drugs from commercial suppliers. Some times the price of crude drug may be very high. It is here, that the profit motive commercial suppliers may increase the bulk of the drug by adding adulterants or sometimes entirely re- placing the crude drug with some other substances.
3. **Manufacturing under unhygienic conditions-** In India apart from few big companies most of the Ayurvedic manufacturers are from small scale unit. The hygienic conditions of these units

are very poor. Many times the store rooms are the thriving areas of many rodents and they will make the raw material further unhygienic.

4. **Lack of technically qualified persons**-like other manufacturing industries Ayurvedic manufacturing units also require technically qualified persons, unfortunately not common in India.
5. **Improper quality control checks**- At least at the state level of government laboratory should be established for the exclusive testing of Ayurvedic drugs.
6. **Inadequacy of the present laws**-The laws relating to manufacturing of Ayurvedic products are very weak and it enables a simple way to get license for their manufacturing. These laws should be made strict so that no product should be marketed without proper standardization.

#### **E. Factors of Cumulative Toxicity**

The cumulative toxicity is attributed to the drug due to some extrinsic and intrinsic factors

##### **Extrinsic factors:**

Pesticide residue e.g. Agro chemicals. Fumigants e.g. Methyl bromide Adulteration of accidental mixing with more toxic plant

##### **Intrinsic factors:**

Chemical constituents such as toxalbumin, Hydrocyanic acid, certain alkaloid, saponins, glycosides etc. Compound preparation mostly causes cumulative toxicity. The toxicity of medicines not only arises by combination but by antagonistic surroundings namely: Drug growth in different seasons (Kaa laviruddha). Discrepancies in its collection (Sampathvirudha) Blemishes on its processing (Samskaravirudha and paakaviruddha). Faults in dose fixation (Maatravirudha). Deficiency in preservation (Sampath virudha)

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# Formulation and Evaluation of Fast Dissolving Tabletes of Candesartan using Natural Excipients for the Treatment of Hypertension

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## ABSTRACT

Candisartan is used commonly for the treatment of hypertantion. Candesartan shows, has a long duration of action. It has half-life of 9 hrs where asT max is 3-4 hr so rapidly achieve desired plasma concentration and stands for long time, so once daily dose is enough for onset of clinical effect, which is also convenient to the patient. Pharmaceutical products designed for oral delivery and currently available on the prescription and over-the-counter markets are mostly the immediate release type, which are designed for immediate release of drug for rapid absorpition. Provides convenience to whom that has trouble in swallowing tablets. The objective of the present study was to prepare the mouth dissolving tablet of candisartan using different superdisintegrants. .The tablets were evaluated for hardness, friability, weight variation, wetting time, thickness, water absorption ratio, disintegrating time, uniformity of content and in-vitro drug release.

**Key words:** Sodium Starch Glycolate, Plantago ovata, Superdisintragate, Candisartan

## I. INTRODUCTION

A solid dosage form is drug delivery system that includes tablets, capsules, sachets and pills as well as a bulk or unit-dose powders and granules. Among the various dosage forms oral solid dosage forms have greater importance and occupy a prime role in the pharmaceutical market.

The U.S food and drug administration center for drug evaluation and research (CDER) defines an ODT as “a solid dosage form containing medicinal substances, which disintegrates rapidly usually within a matter of seconds, when placed upon the tongue. The most desirable formulation for use by the elderly is one that is easy to swallow and easy to handle. Taking these requirements into consideration, attempts have been made to develop a rapid dissolving tablet. Since such a tablet can disintegrate in only a small amount of water in the oral cavity, it is easy to take for any age patient, regardless of time or place. For example, it can be taken anywhere at any time by anyone who do not have easy access to water. It is also easy to dose the aged, bed-ridden patients, or infants who have problems

swallowing tablets and capsules. Recently, many companies have researched and developed various types of fast-disintegrating dosage form technologies with the potential to accommodate various physicochemical, pharmacokinetic and pharmacodynamic characteristics of drugs. These dosage forms disintegrate within 30sec with very less quantity of water. This can be achieved by addition of various superdisintegrants like Croscarmellose sodium, Crospovidone, sodium starch glycolate [1, 2].

Some 600 million people worldwide have high blood pressure and nearly 3 million die every year as a direct result. Yet seven out of every 10 people with hypertension are not being treated adequately, according to WHO and the International Society of Hypertension (ISH).

The tablet is the most widely used dosage form because of its convenience in terms of self administration, compactness, and ease in manufacturing. For the past one decade, there has been an enhanced demand for more patient- friendly and compliant dosage forms. As a result, the demand for developing new technologies has

been increasing annually. Since the development cost of a new drug molecule is very high, efforts are now being made by pharmaceutical companies to focus on the development of new drug dosage forms for existing drugs with improved safety and efficacy together with reduced dosing frequency, and the production of more cost-effective dosage forms[3]

Compare to other ARBs Candesartan shows, has a long duration of action. It has half-life of 9 hrs where as  $T_{max}$  is 3-4 hr so rapidly achieve desired plasma concentration and stands for long time, so once daily dose is enough for onset of clinical effect, which is also convenient to the patient. Pharmaceutical products designed for oral delivery and currently available on the prescription and over-the-counter markets are mostly the immediate release type, which are designed for immediate release of drug for rapid absorption. Disintegrating agents are substances routinely included in tablet formulations and in some hard shell capsule formulations to promote moisture penetration and dispersion of the matrix of the dosage form in dissolution fluids. Superdisintegrant improve disintegrant efficiency resulting in decreased use levels when compared to traditional disintegrants. Traditionally, starch has been the disintegrate of choice in tablet formulation, and it is still widely used. For instance, starch generally has to be present at levels greater than 5% to adversely affect compatibility, especially in direct compression. Drug release from a solid dosage form can be enhanced by addition of suitable disintegrants. [4]

anism of Disintegrants:

- 1) High swellability
- 2) Capillary action and high swellability
- 3) Chemical reaction

In this work; an attempt is made to formulate immediate release tablets of Candesartan to increase patient compliance by reducing dosing frequency and to achieve even plasma concentration profile.

## II. METHODS AND MATERIAL

### Materials

Candesartan was obtained as a gift sample from Ranbaxy Labs Ltd. All solvents were pure analytical grade purchased; Double distilled water was used throughout the experiment.

## Methods

### Determination of absorption maxima of candesartan cilexetil[5]

Candesartan cilexetil (100mg) was accurately weighed, transferred to 100ml volumetric flask and dissolved in small quantity of ethanol. The volume was made up with ethanol to get a concentration of 1000 $\mu$ g/ml. From this 10 ml was withdrawn and diluted to 100ml in HCl pH1.2/pH 6.8 phosphate buffers to get concentration of 100 $\mu$ g/ml. From this solution, 1 ml was withdrawn and added to the 10 ml volumetric flask. Finally, the standard solution (1 $\mu$ g/ml) of Candesartan cilexetil was scanned between 200-400 nm on UV-visible spectrophotometer to record the wavelength of maximum absorption ( $\lambda_{max}$ ). The  $\lambda_{max}$  was found to be 224nm from UV spectrum of candesartan in ethanol; Absorbance was measured at 224nm against ethanol as blank spectrophotometrically. Shown in fig no 1.

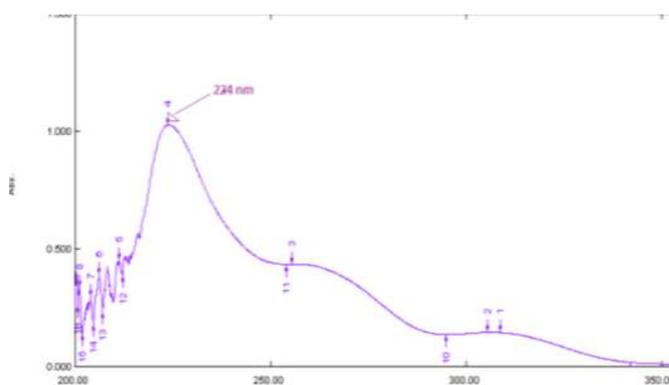


Figure 1: Absorption maxima of candesartan cilexetil in phosphatebuffer pH= 6.8

### Calibration curve of Candisartan celexetil [6]

#### 1) Preparation of standard solution:

Candesartan cilexetil (100mg) was accurately weighed into 100ml volumetric flask and dissolved in small quantity of ethanol. The volume was made up with ethanol to get a concentration of 1000 $\mu$ g/ml. From this 10 ml was withdrawn and diluted to 100ml in HCl pH1.2/pH 6.8 phosphate buffers to get concentration of 100 $\mu$ g/ml.

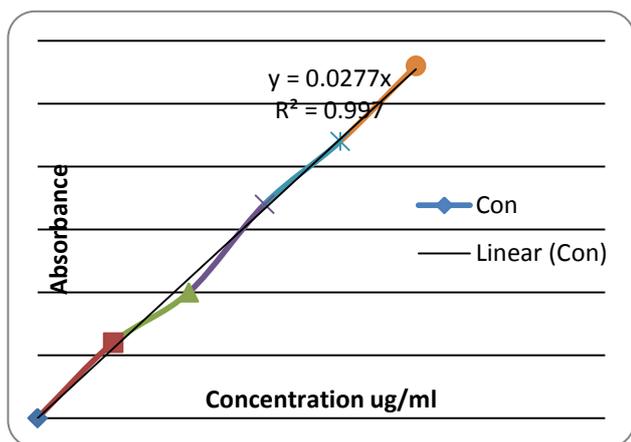
#### Preparation of working solutions

From the standard stock solution aliquots 2ml, 4ml, 6ml, 8ml and 10ml were pipetted out into 100ml volumetric flask. The volume was made up with phosphate buffer

pH6.8 and HCl pH1.2 to get final concentration of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10µg/ml respectively. The absorbance of each concentration was measured at 224nm. Absorbance was measured at 224nm against ethanol as blank spectrophotometrically. Shown in table no 1

**Table 1:** Linearity data of candesartan cilexetil in buffer pH=6.4

1. Calibration curve: Concentration (µg/ml)	Average Absorbance
0	0
2	0.06
4	0.10
6	0.17
8	0.22
10	0.28



**Physicochemical property of drug[7] [8]:**

**1. Loose Bulk Density (BD)**

25 g of drug was weighed accurately, which was previously passed through 30 # sieve and transferred in 100 ml graduated cylinder. Carefully level the powder without compacting, and read the unsettled apparent volume (V0). Calculate the apparent bulk density in gm/ml by the following formula

$$\text{Bulk density} = \text{Weight of powder} / \text{Bulk volume} \dots\dots(1)$$

**2. Tapped bulk density (TD)**

25 g of drug was weighed accurately, which was previously passed through 30 # sieve and transferred in

100 ml graduated cylinder. Then mechanically tap the cylinder containing the sample by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of 14± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped for 500 times initially and tapped volume (V1) was measured to the nearest graduated units. Tapping was repeated an additional 750 times and the tapped volume (V2) was measured to the nearest graduated units. The tapped bulk density was measured in gm/ml by the following formula

$$\text{Tapped Density} = \text{Weight of powder} / \text{Tapped volume} \dots\dots(2)$$

**3. Carr's Index**

The Compressibility Index of the powder blend was determined by Carr's compressibility index. The formula for Carr's Index is as below:

$$\text{Carr's Index (\%)} = [(TD-BD) \times 100] / TD \dots\dots(3)$$

**4. Hausner's Ratio**

The Hausner's ratio was determined by the following equation

$$\text{Hausner's Ratio} = TD / BD \dots\dots(4)$$

**5. Melting point of drug:**

Melting point of the drug was determined as per USP method by DBK prog. Melting point apparatus. Melting point of Candesartan cilexetil was found to be 164°C, which is in the range as given in literature (158-166°C). Hence the drug can be stated as pure.

**6. Solubility profile:**

Solubility studies were conducted by placing an excess amount of Candesartan (approximately 200 mg) in a 2 ml microtube containing 1 ml of each buffer. Then, the mixture was vortexed and kept for 3 days at 37°C in a shaking water bath to facilitate the solubilization. The samples were centrifuged at 10,000 rpm for 10 min to remove the undissolved candesartan. The supernatant was taken, diluted with ethanol upto 10 times and filtered through Whatman filter paper for quantification of drug by UV spectroscopy at 224 nm.

## Preparation of Candesartan Matrix Tablet

Direct compression was followed to manufacture the gas generating floating tablets of Candesartan. All the polymers selected, drug and excipients were passed through sieve no. 40 before using into formulation. Polymers selected with formulation is Shown in table no 2

## Evaluation of Candesartan Cilexetil Tablet

**1 Description of reference product:** The reference product was orange to pink colored, round shaped, uncoated tablets.

**2 Physical characterization of reference product:** The reference product bloopress was physically characterized. Shown in Table no

**Table 3:** Physical characterization of reference product

Average Weight (mg)	150 ± 3%
Thickness (mm)	2.4 ± 0.03mm-2.8 mm ± 0.03mm
Hardness (Kp)	4-5±0.5kp
Friability (% w/w)	Nil
disintegration time (min.)	17-18 min

## 3 Acceptance Criteria for Final Product:

**Table 4:** Acceptance criteria for final product

Test Parameter	Acceptance Criteria
Appearance	Orange to pink colour, round shaped
Average weight	150% mg
Uniformity of weight	Average weight 5 %
Hardness	40.5-50.5 kp
Disintegration time	17-18 min
Thickness	2.4± 0.03mm-2.8 mm ± 0.03mm.
Friability	NMT 1%
Dissolution	NLT 85.0 % is dissolved in 15-30 min.
Assay	NLT 99%.& NMT 101% of label claim

## Evaluation of Candesartan cilexetil immediate release tablet:

**General Appearance:** Any variation in tablet thickness within the particular lot of tablets or between manufacturer's lots should not be apparent to unaided eyes for consumer acceptance of the product. In addition thickness and diameter must be controlled to facilitate packaging. Thus thickness and diameter of tablets were important for uniformity of tablet size. Ten tablets were taken and their thickness and diameter were recorded using vernier caliper.

**Average weight and weight variation:** For weight variation test JP procedure was followed. Twenty tablets were taken and their weight was determined individually and collectively using electronic balance. The average weight of the tablets was determined from collective weight. From the individual tablets weight, the range and percentage standard deviation was calculated. Not more than 2 tablets should deviate from the average weight of tablets and the maximum percentage of deviation allowed. In direct compression of tablet, uniform weight of tablets represents appropriate powder flow and uniform die filling.

## Disintegration test

Disintegration time is considered to be one of the important criteria in selecting the best formulation. To achieve correlation between disintegration time in-vitro and in-vivo, several methods were proposed, developed and followed at their convenience. One tablet was placed into each tube and the assembly was suspended into the 1000ml beaker containing water maintained at 37±2oc and operated the apparatus for 15 minutes. The assembly was removed from the liquid and the tablets were observed. If one or two tablets fail to disintegrate completely, repeat the test on 12 additional tablets. The requirement is met if not less than 16 of the total of 18 tablets tested are disintegrated.

## Dissolution test

**Dissolution:** It is the amount of the solid substance that goes into the solution per unit time under standard conditions of the temperature and pressure.

**Method:** dissolution media was taken as 6.8pH, 900ml was placed in the vessel and the USP apparatus –II (paddle Method) was assembled. The medium was allowed to equilibrate to temp of 37 + 0.5°C. Tablet was placed in the basket and placed in the vessel; the

apparatus was operated for 15min at 50 rpm. At definite time intervals, 5 ml of the fluid was withdrawn; filtered and again 5ml of the fluid was replaced. Suitable dilutions were done with the dissolution fluid and the samples were analyzed using UV

### III. RESULT AND DISCUSSION

**FTIR studies-** Fourier transform infrared (FTIR) spectra of candesartan cilexetil and physical mixture of drug and excipients were recorded using potassium bromide KBr mixing method on FTIR instrument as depicted in Figure 1 and

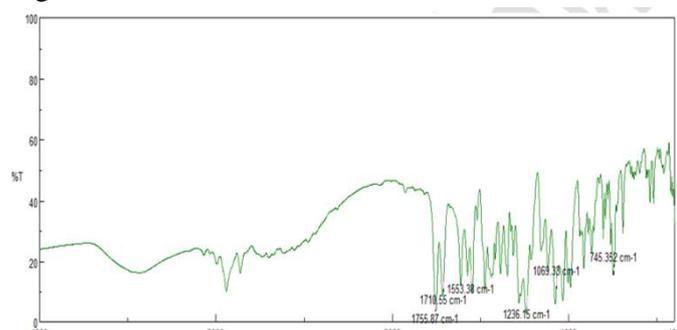


Figure 2: FTIR Study of Candisartan

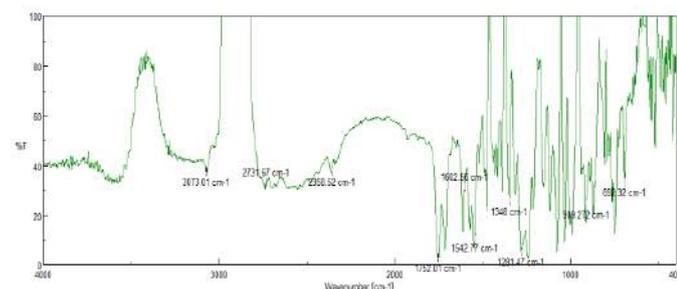


Table 5: Precompressional parameters of Candesartan cilexetil

Batch	Bulk density (g/cm <sup>3</sup> )	Tapped density (g/cm <sup>3</sup> )	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F01	0.401±0.004	0.510±0.002	21.37	1.27	20.07±1.43
F02	0.420±0.003	0.515±0.002	18.44	1.22	21.32±1.32
F03	0.430±0.002	0.524±0.002	17.73	1.21	22.23±1.22
F04	0.428±0.004	0.543±0.002	21.17	1.26	24.12±1.72

Table 6: Evaluation parameters of Candesartan tablets

Evaluation parameters of Candesartan tablets	Avg. Tab Wt. (mg)	Thickness (mm)	Hardness (Kp)	Fria bility (%)	D.T. (min)
F01	150 ± 3%	2.5-2.7± 0.03mm	4.4 ±0.05	0.47	16-17
F02	150 ± 3%	2.5 -2.6 ± 0.03mm.	4.54 ±0.05	0.40	16-17
F03	148 ± 3%	2.4 -2.7 ± 0.03mm.	4.57 ±0.05	0.37	16-17
F04	149 ± 3%	2.5 -2.8 ± 0.03mm.	4.78 ±0.05	0.38	15-18

#### **In vitro dissolution test for tablet formulations**

*In vitro* dissolution studies for all batches of tablets were carried out using the USP paddle method in 900 ml of 0.05M phosphate buffer pH 6.5 containing as dissolution media, maintained at 37±0.5 at 50 rpm. 5 ml of aliquots

#### **Figure 3: FTIR Study of candisartan and Excipients**

FTIR studies revealed that there was no physico-chemical interaction between Candesartan cilexetil and other excipients. The pure drug Candesartan cilexetil showed characteristic absorption at 2731 cm<sup>-1</sup>, 1752cm<sup>-1</sup>, 1714cm<sup>-1</sup>, 1614 cm<sup>-1</sup>. This absorption peak at 3073 cm<sup>-1</sup> was due to stretching of C-H bond, the peaks at 1752cm<sup>-1</sup> and 1714cm<sup>-1</sup> were due to two CO bonds (carbonyl group) and peak at 1614cm<sup>-1</sup> was due to C-N bond. These peaks were present in IR scan of all formulations, so it was conformed that, presence of undisturbed drug in the formulations. Hence there were no drug-excipient interactions. As shown in fig 2.

#### **PRE COMPRESSION PARAMETERS**

The physicochemical properties of all the formulation were observed and recorded in the table no.5 and the evaluation of In-process parameters were determined and recorded in table no.5 Tablet was evaluated for hardness, friability, weight variation, thickness, disintegration time, wetting time, water absorption ratio, drug content and stability study. The Pfizer hardness tester and roche friabilator were used to test hardness and friability loss respectively. Results are shown in Table no 5and 6.

were withdrawn at 10, 20, 30, 40, 50 and 60 minutes from the basket and replaced by 5 ml of fresh dissolution media. The collected samples were analyzed after suitable dilution at 254 nm using UV- Visible spectrophotometer against the blank. The results of *in vitro* dissolution test is shown in table no 7

**Table 7:** *In vitro* dissolution tests for tablet (Batch F1-F4)

Time (min)	F01 (%)	F02 (%)	F03 (%)	F04 (%)
0	0	0	0	0
2	13.3±0.3	15.5±0.11	16.1±0.71	25.1±0.55
4	21.3±0.3	18.5±0.11	17.1±0.71	25.1±0.55
6	43.6±0.71	38.2±0.31	35.7±1.34	45±0.42
8	<b>65.1±1.33</b>	<b>52.4±0.42</b>	<b>49.8±1.54</b>	<b>60.3±1.32</b>
10	67.9±1.51	65.7±0.32	64.4±1.90	74.1±1.54
12	<b>71.1±1.8</b>	<b>70.5±0.45</b>	<b>69.5±1.76</b>	<b>75.6±1.9</b>
15	<b>72.9±0.64</b>	<b>80.3±0.34</b>	<b>76.9±1.25</b>	<b>78.7±1.97</b>
17	<b>74.2±0.70</b>	<b>88.89±0.74</b>	<b>79.2±1.24</b>	<b>80.78±0.54</b>
20	<b>77.60±0.64</b>	<b>94.14±0.44</b>	<b>82±0.74</b>	<b>83.87±0.34</b>

#### IV. CONCLUSION

Tablet formulation F2 showed a higher rate of dissolution and acceptable stability. Formulation was quick when compare to other formulations. It can be concluded that fast dissolving tablets with improved Candisartan cilexetil dissolution could be prepared by using natural Excipients like Kyran T, Plantago ovata.

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Science and Technology**

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