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

Techno Science Academy
The International Open Access Publisher

IJSRST

National Conference on Research and Developments in Synthetic Organic Chemistry

Organised by

Department of Chemistry

AGASTI ARTS, COMMERCE AND DADASAHEB RUPWATE SCIENCE COLLEGE

Akole District, Ahmednagar-422601, Maharashtra, India

VOLUME 3, ISSUE 10, NOVEMBER-DECEMBER-2017

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH IN SCIENCE & TECHNOLOGY

Email : editor@ijsrst.com Website : http://ijsrst.com

National Conference on Research and Developments in Synthetic Organic Chemistry

In Association With

International Journal of Scientific Research in Science and Technology

Print ISSN: 2395-6011 Online ISSN : 2395-602X
UGC Approved Journal [Journal No : 64011]

Volume 3, Issue 10, November-December-2017

International Peer Reviewed, Open Access Journal

Organised by

Department of Chemistry

AGASTI ARTS, COMMERCE AND DADASAHEB
RUPWATE SCIENCE COLLEGE
Akole District, Ahmednagar-422601, Maharashtra, India

Sponsored By

Savitribai Phule, Pune University, Pune, Maharashtra, India

Published By

Technoscience Academy
(The International Open Access Publisher)

[www.technoscienceacademy.com]

PATRONS

- ✓ Hon. Shri. J. D. Ambre Patil (President, ATES, Akole)
- ✓ Hon. Shri. Madhukarrao Sonawane (Vice- President, ATES, Akole)
- ✓ Hon. Shri. Yashwantrao Abhale (Secretary, ATES, Akole)
- ✓ Hon. Adv. Bhausaheb Godase (Joint- Secretary, ATES, Akole)
- ✓ Hon. Shri. S. P. Deshmukh (Treasurer, ATES, Akole)

ADVISORY COMMITTEE

- ✓ Professor S. P. Gejji (Head, Department of Chemistry, SPPU, Pune)
- ✓ Professor Dr. R.D. Pokharkar (Emeritus Professor, UGC New Delhi)
- ✓ Prin. Dr. V. B. Gaikwad (KTHM College, Nashik)
- ✓ Prin. Dr. Sambhaji Pathare (Arts, Commerce and Science College, Rahuri)
- ✓ Dr. S. P. Lavande (Shree Chatrapati Shivaji Mahavidyalay, Shrigonda)
- ✓ Prin. Dr. B. K. Karale (Secretary, Rayat Shikshan Sanstha, Satara)
- ✓ Prin. Dr. K. K. Deshmukh (Sangamner College, Sangamner)
- ✓ Prin. Dr. B. H. Zaware (New Arts, Commerce and Science College, Ahmednagar)
- ✓ Prin. Dr. S. S. Jadhav (Shri Dhokeshwar Mahavidyalaya, Takali Dhokeshwar)
- ✓ Prin. Dr. D. D. Patil (Radhabai Kale Mahila Mahavidyalaya, Ahmednagar)
- ✓ Dr. Dilip Kotkar (Senior General Manager, Lubes and Coats, Grauer and Weil (India) Ltd.)

ORGANIZING COMMITTEE

- ✓ Prin. Dr. Sanjay Takate (Convener)
- ✓ Prof. Suresh Malunjkar (Co- ordinator)
- ✓ Prof. Sandesh Kasar (Organizing Secretary)

MEMBERS

- ✓ Prof. Mahendra Banait
- ✓ Prof. Dr. Sampatrao Sonawane
- ✓ Prof. Mrs. Sadhana Bangal
- ✓ Prof. Pankaj Naikwadi
- ✓ Prof. Arun Wakchaure
- ✓ Prof. Mrs. Jyoti Shinde
- ✓ Prof. Varsharani Shete

Advisory/Editorial Board

International Advisory/Editorial Board

CONTENTS

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Antioxidant Potential of Roots of Clerodendrumserratum(Linn.)

Vaishali D. Murade[1], Sonali Dichayal[2]

[1]Padmashri Vikhe Patil College, Pravaranagar, Rahata, Ahmednagar, Gujarat, India
[2]S. N. Arts, D. J. M. Commerce and B. N. Sarada Science College, Sangamner, Ahmednagar, Gujarat, India

ABSTRACT

The objective of this study was to determine the antioxidant capacity of polyphenols extracted from the roots of the Clerodendrumserratum plant. Extracts CSRC, CSRA and CSREA were found to have high percentage of total phenolic and flavonoid content and strong 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity. The results suggested that CSRC, CSREA and CSREAhas significant potential as a natural antioxidant to promote health and to reduce the risk of disease.

Keywords: Antioxidant, polyphenols, Clerodendrumserratum.

I. INTRODUCTION

The ClerodendrumserratumLinn.(CS) is a small perennial woody shrub of the family Verbenaceae and it is commonly known as Bharangi (Patel et al., 2014). It is native of India and growing in moist deciduous forests of Western Ghats of India (Manjunatha et al., 2004). Traditionally the various parts of this plant were used in the treatment of asthma, inflammation and infectious disorders (Patel et al., 2014). It has been reported that, this plant shows wide range of pharmacological activities including hepatoprotective (Vidya et al., 2007;Agrawal et al., 2013), Analgesic (Saha et al., 2012), Antioxidant (Bhujbal etal.,2009 b;Mohamedetal.,2012), antiinflammatory (Narayanan et al.,1999), antibacterial(Rashid et al, 2013; Vidya et al.,2010),anticancer(Zalke et al., 2010;Chinchali et al.,2011), and anti-asthmatic activity (Bhujbal et al.,2009a;Thalla et al.,2012).

The earlier study revealed the presence of pharmacologically active constituents include flavonoids(Bhujbal et al., 2010d; Fan et al., 2007), phenyl propanoids (Yang et al., 2000a; Fan et al., 2007; Wei et al., 2000a), Iridoids (Yang et al., 2000c; Wei et al., 2000a), terpenoids (Banerjee et al., 1969; Yang et al., 2000b; Raju et al., 2008; Vidya et al., 2007) and sterols (Banerjee et al., 1969;; Fan et al., 2007).A free radiacal is a very reactive species resulted from metabolic reaction at cellular levelwhich leads to various types of diseases like ageing, neurodegenerative diseases, CNS related disorders, mutagenic disorders etc. The antioxidants are the compounds which scavenge these radicals and prevent damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Kalita et al., 2013).The current study aims to identify the potent phytoconstituents present in CSRH, CSRC, CSREA and CSRA extractsof CS roots and further evaluation of their In- vitroantioxidant potential.

II. MATERIAL AND METHODS

Plant material

For this study, the roots of Clerodendrumserratum(CSR)were collected from the different localities of Paithan,They were identified by Dr. K. J. Salunke of the Department of Botany, PadmashriVikhe Patil College of Arts, Science and Commerce, Pravaranagar, India.The fresh roots of the plant were cleaned and powdered coarsely and stored for further use.

Extraction of crude drug

The 500 g powdered roots were subjected to the maceration in 4.5 L absolute ethanol (99.9%) at 50°C for 3days (1.5×3). The crude extract was filtered and thenallowed to concentrate on rotary under reduced pressure. The obtained crude extract was dark brown in colour and the percentage yield was 5.53 %.

The crude extract (23 gm) was suspended in 10 ml distilled water and successively fractionated with n-

hexane, chloroform, ethyl acetatesolvents and yields CSRH, CSRC, CSREAand residual aqueous extract CSRAs respectively.

Estimation of total phenolic content

The total phenolic content (TPC) was estimated by Folin-Ciocalteu reagent using previously reported method (Khatoon et al., 2013). TPC of variousextracts of CS roots were determined using calibration curve of standard gallic acid (GA). Methanol was used as blank and GA as a standard. All determinations were carried out in triplicates. TPC was determined from standard calibration curve produced with GA and it was expressed as GA equivalent per milligrams (µg GAE/mg) of extracts.

Estimation of total flavonoid content

The total flavonoid content (TFC) of different extracts were estimated by using aluminium chloride colorimetric method (Maddan et al., 2011; Saeed et al., 2012). TFC of various extracts was determined using calibration curve of standard rutin. Alldeterminations were carried out in triplicates. The TFC was expressed in terms of rutin equivalent per milligrams (µg RE/mg) of extracts.

In-vitro antioxidant activity

DPPH radical scavenging activity

In the DPPH˙radical scavenging assay, the methods described inKhatoon et al.(2013) and Saeed et al.(2012) were followed with minor modifications. A 10 mg mL^{-1}stock solution of various extracts were prepared by dissolving the extract in DMSO and sample concentrations of 0.812 to 100 µg mL^{-1}were prepared in methanol.A sample solution was mixed with 0.004% freshly prepared DPPH˙methanol solution. The reaction mixture was shaken and kept in dark at room temperature. After that, the absorbance of the mixture was measured immediately at 517 nm using a Systronic1203 UV/Vis Spectrometer. The DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_o - A_S)/ A_o \times 100]$. Where $A_o$and $A_s$are the absorbance of control and standard or tested sample respectively. The experiment was performed in triplicates and the mean values were recorded

Statistical analysis

All experiments were carried out in triplicate and results are reported as mean ±SD. Data was analyzed with one-way ANOVA.

III. RESULTS

Total phenolic and flavonoid content

The total phenolic content (TPC) of various extracts of CS roots were determined by using Folin-Ciocalteu reagent and it is reported as micrograms per milligrams (µg/mg) of gallic acid equivalent (GAE) by reference to gallic acid standard curve (y= 0.1041x and r2= 0.9917). All extracts contained a considerable amount of phenolic content and it was found that, of all theextracts, the CSRCextract had the highest total phenolic content (139.74±2.41) µg of GAE/mg followed by CSRA (99.35±1.46), CSREA (53.20±1.46) and CSRH(6.41±2.22) µg of GAE/mg respectively.

The total flavonoid content (TFC) was expressed as µg/mg of rutin equivalents (RE) by reference to rutin standard curve (y=0.0326x and r2= 0.9976). It was found that, the CSREA extract had the highest TFC (86.45±4.77) µg of RE/mg followed by CSRA (72.91±1.80), CSRC (15.72±0.18) and CSRH (Nil) µg/mg of rutin equivalent respectively.

Table 1. Estimation of total phenolic and flavonoid content of extracts of *Clerodendrumserratum*

Extract	TPC	TFC
CSRH	6.41±2.22	0.0
CSRC	139.74±2.41	15.72±0.18
CSREA	53.20±1.46	86.45±4.77
CSRA	99.35±1.46	72.91±1.80

DPPH free radical scavenging activity

DPPH radical scavenging ability ofvarious extract was determined using standard antioxidant ascorbic acid (AA). The % inhibition of standard and extract of *Clerodendrumserratum* roots are in the order of CSRC>CSRA >CSREA>CSRH (Fig. 1.). The IC_{50} values of standard Ascorbic acid and extracts are

presented in Table 2. The IC_{50} values of AA and extracts (CSRA,CSREA, CSRC and CSRH) were 1.24, 3.36, 10.61, 165.58µg/mL respectively.

Table 2. An effect of extracts of CS roots on free radicals by DPPH method

Extract code	IC_{50} (µg/ml) DPPH
AA	2.48
CSRH	165.98
CSRC	10.61
CSREA	3.36
CSRA	1.24

AA, Ascorbic acid; CSRH, hexane extract; CSRC, chloroform extract; CSREA, ethyl acetate extract; CSRA, alcohol extract.

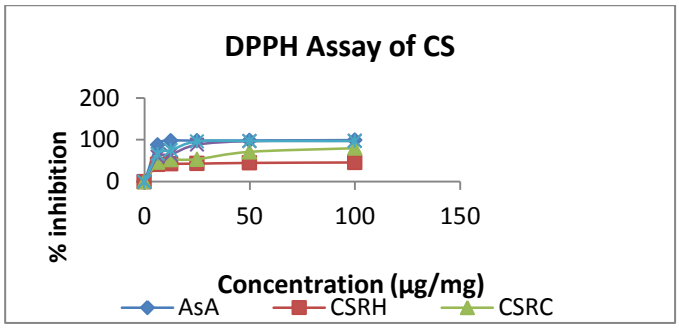


Figure 1. In vitro antioxidant activity of extracts of CS roots in DPPH scavenging assay

IV. DISCUSSION

The plant phenolic compounds and flavonoids have been reported to show strong antioxidant activity in biological systems, acting as oxygen radical and free radicals scavengers (Rice-Evans et al., 1997; Jorgensen *et al.*, 1999; Halliwell et al., 1995) due to ability of benzene rings to transfer electrons (Brown, 1995).

Present study revealed that relatively highest phenolic content are found in chloroform and hydro alcoholic residual extract, moderate in ethyl acetate extract, while lowest in n-hexane extract and highest flavonoid content in ethyl acetate and hydro-alcoholic extracts and absent in n-hexane extract. These results may arise due to higher solubility of phenolic and flavonoid compounds in ethyl acetate, chloroform and hydro-alcohol extract and thus it suggests the potent antioxidant property.

A number of way have been published in order to evaluate the antioxidant and the free radical scavenging properties of natural products out of which, the DPPH assay has provided information on a great deal of plants and is commonly used. DPPH• is a stable and organic nitrogen centered radical bearing no similarity to the highly reactive and transient peroxyl radicals implicated in a variety of oxidative processes *in vivo* (Biapa et al., 2011; Basma et al., 2011). The scavenging ability of DPPH radical is related to the inhibition of lipid peroxidation and it was determined by decrease in intensity of violet colour. Lower IC50 value indicates higher antioxidant activity (Rekka and Kourounakis, 1991). Among all extracts, CSREA and CSRA showed the lowest IC50 value with highest antioxidant activity. Hence when more antioxidants occur in the extracts, the more DPPH reduction will occur and it relates to high scavenging capability of respective extracts. In this assay, the antioxidant activity of CSREA and CSRCextracts on DPPH radical may be attributed to a direct role in trapping free radicals by donating hydrogen atom.

V. ACKNOWLEDGEMENTS

The author is grateful to the Principal, PadmashriVikhe Patil College, Pravaranagar, MS.

VI. REFERENCES

[1]. Agrawal SK, Jat RK, Chhipa RC. Pharmacological evaluation of hepatoprotective activity of Clerodendrumserratum. International Journal of Pharmacology and Toxicology. 2013; 3(2): 67-70.

[2]. Banerjee SK, Chakravarti RN, Sachdev KS, Vasavada SA. Constituents of rootbark of Clerodendrumserratum. Phytochemistry, 1969; 8: 515.

[3]. Basma AA, Zakaria Z, Latha LY, Sasidharan B. Antioxidant activity and phytochemical screening on methanol extract of Euphorbia hirtaL.Asian Pacific Journal of Tropical Medicine,2011; 4: 386-390.

[4]. Bhujbal SS, Kewatkar SM, Kumar D, Mudgade SC, Patil MJ.In-vivo and in-vitro anti-asthmatic studies of Clerodendrumserratum Linn.Pharmacologyonline, 2009a; 2: 745–752.

[5]. Bhujbal SS, Kewatkar SMK, More LS, Patil MJ. Antioxidant effects of roots of

Clerodendrumserratum Linn. Pharmacognosy Research, 2009b; 1: 294–298.
[6]. Bhujbal SS, Nanda RK, Deoda RS, Kumar D, Kewatkar SM, More LS, Patil MJ.Structure elucidation of a flavonoid glycoside from the roots of Clerodendrumserratum (L.)Moon, Lamiaceae. BrazilianJournalofPharmacognosy, 2010d ; 20:1001–1002.
[7]. Biapa N, Oben J, Ngogang J. Scavenging radical kinetic and antianaemic screening properties of some medicinal plants used in Cameroon. Int J Appl Res Nat Prod2011; 4: 29-35.
[8]. Brown RH. Free radicals, programmed death and muscular dystrophy. Current Opinion in Neurology, 1995; 8: 373-378.
[9]. Chinchali JF, Sanakal RD, Kaliwal BB. Evaluation of anticarcinogenic activity of Clerodendrumserratum leaf extract on liver and kidney of 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis in mice. European Journal of Experimental Biology, 2011; 1: 130–141.
[10]. Fan,Ju-di,Long,Qing-de,Yang,J.,Luo,Xi-rong,Studies on the chemical constituents of Clerodendrumserratum (L.)Moon.Medical Journal of Chinese People's Health, 2007; 19:423.
[11]. Halliwell B. Antioxidants and human disease: a general introduction. Nutrition Review, 1997; 55: 44-49.
[12]. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO, Skibsted LH. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. Free Radical Research, 1999; 30: 207-220.
[13]. Kalita P, Barman TK, Pal TK, Kalita R. Estimation of total flavonoid content and Antioxidant activities of methanolic whole plant extract of BiophytumSensitivum Linn. Journal of Drug delivery and Therapeutics, 2013; 3 (4): 33-37.
[14]. Khatoon M., Islam E, Islam R, Rahman AB, KhurshidAlam AHM, Khondkar P, Rashid M, Parvin S. Estimation of total phenol and in vitro antioxidant activity of Albiziaprocera leaves. BMC Research Notes, 2013, 6, 121.
[15]. Maddan R, Bansal G, Kumar S, Sharma A. Estimation of total phenols and flavonoids in extracts of Actaeaspicata roots and antioxidant activity studies. Indian Journal of Pharmaceutical Sciences, 2011: 73, 666-669.
[16]. Manjunatha BK, Krishna V, Pullaiah T. Flora of Davangere District, Karnataka, India.Regency Publications, New Delhi, India.
[17]. Mohamed AJ, Mohamed EAH, Abdalrahim FAA, Ameer OZ, Ismail Z, Ismail N, Shah AM, Abdulmajid MZA, Yam MF. Antioxidant, antiangiogenic and vasorelaxant activities of methanolic extract ofClerodendrumserratum (Spreng.) leaves. Journal of Medicinal Plants Research, 2012; 6: 348–360.
[18]. Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of Clerodendrumserratum roots in experimental animals. Journal of Ethnopharmacology, 1999; 65: 237–241.
[19]. Patel JJ, Acharya SR, Acharya NS. Clerodendrumserratum(L.)Moon.-A review on traditional uses, phytochemistry and pharmacological activities. Journal of Ethnopharmacology, 2014; 154: 268-285.
[20]. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. Analytical Biochemistry, 1999: 269, 337-341.
[21]. Raju R, Akoni JL, Subban R. Chemical constituents from Clerodendronserratum. Journal of Asian Natural Products Research, 2008; 10: 652–655.
[22]. Rashid MT, Yadav AS, Bajaj A, Lone SA. An Assessment of antibacterial potency of aqueous leaf extract of Clerodendrumserratum Linn. against pathogenic bacterial strains. Indo American Journal of Pharmaceutical Research, 2013; 3(2): 1637-1644.
[23]. Rekka E, Kourounakis PN. Effect of hydroxyethylrutosides and related compounds on lipid peroxidation and free radical scavenging activity of some structural aspects. Journal of Pharmacy and Pharmacology,1991; 43: 486-491.
[24]. Rice-Evans C, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants in vivo.Free Radical Research 1997; 26: 381-398.
[25]. Saeed N, Khan MR, Shabbir M.. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts TorilisleptophyllaL. BMC complementary and Alternative Medicine, 2012: 12, 221.
[26]. Saha D, Talukdar A, Das T, Ghosh SK, Rahman H. Evaluation of analgesic activity of ethanolic extract of Clerodendrumserratum Linn.leaves in rats. International Research Journal of Pharmaceutical and applied sciences, 2012; 2(6): 33-37.

[27]. Thalla, S, Tammu J, Pentela B, Thalla SR. Antiasthmatic activity of alcoholic extract of Clerodendrumserratum induced by ovalbumin. International Journal of Chemical and Pharmaceutical Sciences 2012; 3:83–85.
[28]. Vidya SM, Krishna V, Manjunatha BK, Mankani KL, Ahmed M, Singh SDJ. Evaluation of hepatoprotective activity of Clerodendrumserratum L. Indian Journal of Experimental Biology, 2007; 45: 538–542.
[29]. Vidya SM, Krishna V, Manjunatha BK, Rao RB. Antibacterial activity of Clerodendrumserratum L. Electronic Journal of Environmental, Agricultural and Food Chemistry, 2010; 9: 1059–1063.
[30]. Wei XM, Zhu QX, Chen JC, Cheng DL. Two new iridoidglucosides from Clerodendrumserratum. Chemical Journal of Chinese Universities, 2000a; 21: 1675–1678.
[31]. Yang H, Hou AJ, Mei SX, Peng LY, Sun HD. A new phenylpropanoid glycoside: Serratumoside A from Clerodendrumserratum. Chinese Chemical Letters, 2000a; 11(4): 323-326.
[32]. Yang H, Jiang B, Na Z, Guo YP, Sun HD.Two new iridoidglucosides from Clerodendrumserratum. Chinese Chemical Letters, 2000c; 11: 231–234.
[33]. Yang H, Mu Q, He YN, Sun HD. A new triterpenoidsaponin: Se-saponin A. Chinese Chemical Letters, 2000b; 11: 333–336.
[34]. Zalke AS, Kulkarni AV, Shirode DS, Duraiswamy B. In-vivo anticancer activity of Clerodendrumserratum (L)Moon. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1: 89–98.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Synthesis and Antimicrobial Activity of Thiazolidinones Derivatives

N.K. Halikar[*1], G. P. Sadawrte[2], M. J. Gawande[1]
[1]Department of Chemistry, Dr. Shantilal Dhanji Devsey College Wada, Palghar, Maharashtra, India
[2]Department of Chemistry, B.P. S.M.A. Science and K.K.C. Commerce College Chalisgaon, Jalgaon, Maharashtra, India

ABSTRACT

A new synthesis of Schiff base 3a-e was prepared from naphthalene 1 amine. All the naphthalene 1 amine Schiff base were refluxed with thioacetic acid in presence of anhydrous zinc chloride and solvent N,N, dimethyl formamide to form Thiazolidinones 4a-e. All synthesized Thiazolidinones were screening their antimicrobial activity.

Keywards: Benzaldehyde, thioacetic acid, ZnCl2, Antimicrobial Activity.

I. INTRODUCTION

In heterocyclic chemistry hetero atom possess interesting pharmacological properties but Thiazolidinones are possess good biological activities [1-2]. antibacterial [3-5], anticancer [6-7], antitubercular [8-11], antifungal [12], anti-inflammatory [13], antiviral [14,15], and analgesic [16,17]. Due to this vital role it was thought to synthesize 4-thiazolidinone derivatives and study for their antimicrobial activities.

II. EXPERIMENTAL

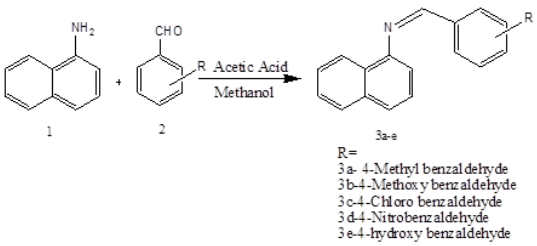
Material and methods

All the chemicals used in present study are of analytical grade purchased from Himedia Chemical Co. All the reactions monitored by thin layer chromatography (TLC) on 0.2 mm silica gel-C plates using iodine vapors for detection. Infrared spectra were recorded in nujol or as potassium bromide pellets on infrared spectrophotometer. 1H NMR spectra were obtained on Brukner advance spectrophotometer 400. MHz mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV. Melting points of synthesized compounds were determined by a Kofler micro melting point apparatus and were uncorrected. All the reactions were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN-O rapid analyzer.

General Procedure

Substituted Naphthalene 1 amine Schiff bases (3a-e)
A mixture of Naptahlene 1-amine (1) (0.005mol) and 4-methyl benzaldehyde, 4-methoxy benzaldehyde, 4-chloro benzaldehyde, 4-nitro benzaldehyde and 4-hydroxy benzaldehyde (0.005mol) in 10 ml methanol was refluxed independently on water bath for 3-4 hrs. The reaction mixture was allowed to cool and separated solid was filtered, washed with water dried and recrystallized from ethanol to give products 3a-e. **(Tabel-1)**

Substituted 2-phenylthiazolidin-4-one(4a-e)
A mixture of 3a-e, (0.01 Mol) was refluxed with thioacetic acid (0.01mol) in 10 ml of N,N-dimethyl formamide in presence of anhydrous zinc chloride were refluxed for 5-6 hrs. After complete heating reaction mixture was cooled, which was then poured in ice water, the solid obtained, filtered, dried and recrystallized from ethanol to give fine solid products 4a-e. (Table 1)



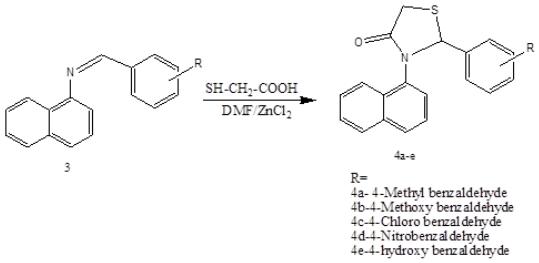


Table 1. Physical data of compounds (3a-e)

Sr.No	Compounds	M.P(^{0}C)	Yield
1.	3a	72	73
2.	3b	86	78
3.	3c	106	84
4.	3d	166	64
5.	3e	156	88
6.	4a	190	68
7.	4b	184	80
8.	4c	165	75
9.	4d	180	73
10	4e	196	85

III. RESULT AND DISCUSSION

The compound naphthalene 1 amine were condensed with 4-methyl benzaldehyde, 4-methoxy benzaldehyde, 4-chloro benzaldehyde, 4-nitro benzaldehyde and 4-hydroxy benzaldehyde to afforded Schiff bases 3a-e.

The structure were 3,3a-e,4a-e were supported by their spectral data. The IR KBr spectra showed the presence of absorption band in the region 1495-1525 cm^{-1} due to –CH=N- stretch. Mass spectra of these products exhibit molecular ion peaks at M^{+2} and M^{+} which corresponds to their molecular weight.^{1}H NMR (DMSO-d6) spectra of these compounds revealed signals in the region δ 7.1-7.8 (m, due to Ar-H), and 8.1-8.4 (s, due to N=CH). All the newly synthesized compounds gave satisfactory C,H and N analysis and spectral data.

The IR in KBr showed absence of absorption in the region 1495-1525 cm^{-1} due to –CH=N-and presence of absorption band in the range 1725-1730cm^{-1} due to C=O of thiazolidinone. ^{1}H NMR (DMSO-d6) signals appeared at δ 3.1-3.9 ppm due to $–CH_2$- of 4-thiazolidinone.

Antimicrobial Activity:

All the tested compounds were found their antimicrobial activity using disc diffusion technique against *S.aureus, B.substilis*, *S. Typhi*, *E.Coli*. These compounds were dissolved in dimethyl sulphoxide. Incubation period for bacteria was 24 hours. The newly synthesized compounds shows zone of inhibition 7-18 mm in diameter where as standard streptomycin exhibit zone of inhibition 18-22 mm in diameter against *S.aureus, B.substilis*, *S. Typhi* and *E.Coli.* Compounds 4b, 4c, 4d were found moderate to best active against *S.aureus, B.substilis*, *S. Typhi and E.Coli* respectively.

Compounds	Zone of inhibition in mm			
	S.aureus	***B.substilis***	***S. Typhi***	***E.Coli***
4a	12	14	10	06
4b	10	09	07	09
4c	18	15	12	11
4d	18	13	11	13
4e	12	07	09	10
Positive control	22	20	18	18

IV. ACKNOWLEDGMENT

Authors are grateful to principal M.V. Bildikar, B.P. S.M.A. Science and K.K.C. Commerce College Chalisgaon, dist. Jalgaon for providing laboratory facility and to the Director, Indian Institute of Chemical Technology, Hyderabad for providing spectral data.

V. REFERENCES

[1]. V.M. Sherekar, S.E. Bhandarkar, Der Pharma Chemica, 2015, 7(3), 1-4.
[2]. Vartale SP, Pawde AV, Halikar NK, Kalyankar ND, Pawar YD, RJPBCS 1(4) Page No. 1061.
[3]. J. Matsumoto and S. Minami, J. Med. Chem., 1975,18, 74.
[4]. N. Mont, J. Teixidó, J. I. Borrell and C. Oliver Kappe, Tetrahedron Lett., 2003 44, 5385.
[5]. V. Oakes and H. N. Rydon, J. Chem. Soc., 1956 10, 4433.
[6]. J. Bhatt, B. Shah, H. Shah, P. Trivedi, N. Undavia, N. Desai, Ind. J. Chem., 1994, 33(B), 189-192.
[7]. A. Sawale, R. Bendre, P. Patil, RJPBCS, 2012, 3(3), 415-420.
[8]. P. Sah, C. Gharu, JCPR, 2012, 9(1), 44-48.

[9]. V. Dave, M. Thakor, J. Curr. Chem. Pharm. Sci., 2015, 5(3), 99-104.
[10]. K. Swathi, M. Sreenivasulu, N. Pramod, R. Prema, G. Mahaboob Basha, IJBPR, 2014, 5(11), 843-847.
[11]. K. Waghmode, J. Chem. Pharm. Res., 2014, 6(5), 1101-1105.
[12]. s p. vartale, n d. kalyankar and nk. halikar, Int. J. Chem. Sci.: 11(2), 2013, 1164-1172
[13]. R. Yadav, S. Srivastava, S. Srivastava, Ind. J. Chem., 2005, 44(B), 1262-1266
[14]. M. Abdullah, Zan. J. App. Sci., 2014, 26(3), 1-12.
[15]. N. Seelam, S. Shrivastava, Bull. Korean Chem. Soc., 2011, 32(11), 3996-4000.
[16]. S. Ramachandran, P. Shanmugapandiyan, C. Nalini, IJPSR., 2011, 2(6), 1564-1568.
[17]. D. Pareek, M. Chaudhary, P. Pareek, K. Ojha, A. Pareek, Int. J. Pharm. Pharm. Sci., 2010, 6(4), 438-455.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Synthesis of Sulfonyl Azides from Sulfonic Acids using Cyanuric Chloride

S. S. Gholap[1], N. R. Pawase[2]

[1,2]P.G. Department of Chemistry, Padmashri Vikhe patil College, Pravaranagar (Loni kd.) Rahata, Ahmednagar, Gujarat, India

ABSTRACT

A mild, efficient and convenient method for synthesis of sulfonyl azides from sulfonic acids is described. In situ sulfonyl chlorides are prepared from sulfonic acids. The sulfonyl chlorides were then further allowed to react with excess NaN3 in the same pot.

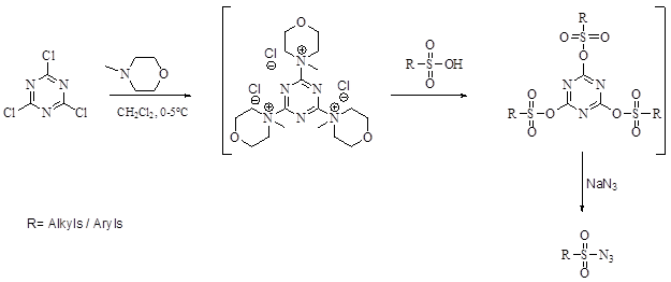
Keywords : Sulfonyl Azides, Sulfonyl Chlorides, NaN3, N-Methyl

I. INTRODUCTION

Sulfonyl azides are valuable synthetic intermediate in organic chemistry. They can be prepared from sulfonyl chlorides. In situ sulfonyl chlorides can be prepared from corresponding thiols using number of methods, generally by bubbling Cl_2 gas into aqueous acids or a biphasic mixture containing thiols. Use of excess oxidant or aqueous acids was required in this methodology. (1) Sulfonyl azides are valuable reagent for variety of chemical transformation, they have been used as a valuable reagent for synthesis of α-diazocarbonyl reagents, (2,3,5,6) hydroazidation of olefins,(7) the aziridation of olefins, (8,9) the radical amination (4,10,11) and metal catalysed coupling reaction (8) owing to a wide range of applications.

Preparation of sulfonyl azides involves the reaction of sulfonyl chlorides with sodium azides (12). Sulfonic chlorides are difficult to prepare and handled. Generally sulfonyl chlorides are prepared by treating sulfonic acid with chlorinating agents like $SOCl_2$ (13), $POCl_3$ (14), PCl_5 (15), triphosgene (16) and cyanuric chloride (17). One pot synthesis of sulfonyl azides from sulfonic acids was reported (18).Preparation of sulfonyl azides from thiols with chloramine-T, Bu_4NCl and water was reported (19). A mild, efficient and general method for the preparation of acyl azides from carboxylic acids and sodium azide using Cyanuric acloride was performed (20). One pot process for preparation of various sulfonyl azides by treating sulfonic acids with triphenyl phosphine/ trichloroisocyanuric acid/ sodium azide at room temperature was described. A wide range of arenesulfonyl and alkanesulfonyl azides was obtained in excellent yield under mild conditions. Cyanuric chloride and its derivatives like 4,6-dimethoxy derivative, 2-chloro-4,6-dimethoxy-1,3,5-triazine derivative found considerable applications in organic chemistry. Sulfonic acids can be activated with cyanuric chloride and subsequently react with 18-crown-6 in acetone or NEt_3 in acetone (17). We now report the use of cyanuric chloride for the direct conversion of sulfonic acids to sulfonyl azides. (scheme1)

The mechanism proceeds through activation of sulfonic acid by cyanuric chloride in presence of N-Methyl morpholine in dichloromethane. Various aryls, heteroaryls, alkylaryls, and alkyl sulfonic acids on reaction with cyanuric chloride in presence of NaN_3 and N-Methyl morpholine undergoes smooth conversion to the corresponding sulfonyl azides in excellent yield (Table 1). Cyanuric chloride is inexpensive reagent and safe to handle in comparison to the recently reported use of hazardous and expensive triphosgene, thionyl chloride, and phosphrous pentachlorides.



Scheme 1.

II. RESULTS AND DISCUSSION

In this Strategy reactivity of Sulfonyl group enhances using 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride). The sulfonic acid was first allowed to react with 2,4,6-trichloro-1,3,5-triazine in dichloromethane in presence of N- Methylmorpholine. The resulting reaction mixture containing activated sulfonic acid was further sodium azide at room temperature gives sulfonyl azide in good to excellent yield. The results are summarized in table 1.

In conclusion, we have developed a mild efficient and general procedure for conversion of sulfonic acids to corresponding sulfonyl azides.

Table1. Synthesis of sulfonyl azides from sulfonic acids using cyanuric chloride.

Entry	Sulfonic acid	Sulfonyl azide	Time (min)	Yield (%)a,b	Ref. c
1	$PhSO_3H$	$PhSO_2N_3$	40	88	_d
2	p-me[C_6H_4]SO_3H	p-me[C_6H_4]SO_2N_3	55	83	_e
3	p-Ome[C_6H_4]SO_3H	p-Ome[C_6H_4]SO_2N_3	50	74	18
4	p-Cl[C_6H_4]SO_3H	p-Cl[C_6H_4]SO_2N_3	180	77	18
5	2,4-Cl_2[C_6H_3]SO_3H	2,4-Cl_2[C_6H_3]SO_2N_3	180	82	21
6	p-NO_2[C_6H_4]SO_3H	p-NO_2[C_6H_4]SO_2N_3	190	76	18
7	p-Br[C_6H_4]SO_3H	p-Br[C_6H_4]SO_2N_3	190	78	21
8	C_6H_5–CH_2SO_3H	C_6H_5–$CH_2SO_2N_3$	40	81	18
9	1-Napthalenesulfonic acid	1-Napthalenesulfonyl azide	50	76	21
10	2-Napthalenesulfonic acid	2-Napthalenesulfonyl azide	55	75	21
11	CH_3SO_3H	$CH_3SO_2N_3$	60	80	21
12	CH_3–CH_2–SO_3H	CH_3–CH_2–SO_3H	65	72	21
13	p-$NHCOCH_3$[C_6H_4]-SO_3H	p-$NHCOCH_3$[C_6H_4]-SO_2N_3	65	74	21
14	m-$NHCOCH_3$[C_6H_4]-SO_3H	p-$NHCOCH_3$[C_6H_4]-SO_2N_3	60	81	21

[a]Yield of pure isolated product. [b]Products were characterized by their m.p. or b.p. with authentic samples. [c]Published physical and spectral properties. [d]Spectroscopic data for 1 and 2 is given in experimental section.

General procedure for synthesis of sulfonyl azides-
To a solution of cyanuric chloride (3 mmol) in dichloromethane (25ml) N-Methyl morpholine was added at 0- 5°C with continuous stirring. A white suspension is formed. To this suspension sulfonic acid (9 mmol) in 10 ml dichloromethane was added and the stirring was continued for 3 Hrs. at room temperature and completion of reaction was checked by TLC. After completion of reaction mixture was washed with saturated solution of $NaHCO_3$ (3X10ml) and then with water (3x10ml). Organic layer was dried with Na_2SO_4, passed through short a silica gel, column and the solvent removed under reduced pressure to give pure sulfonyl azide.

Data for selected compounds. Benzenesulfonyl azide (Entry1, Table1): IR(cm^{-1}):1088, 1168(SO_2), 1372(SO_2), 2128(N_3), 3070, 3095. ^{1}H-NMR(300MHz, $CDCl_3$): 7.50 (t, 2H, J=7.3 Hz), 7.58 (t, 1H, J=7.3 Hz), 7.72(d, 2H, J=7.6 Hz)

p-Toluenesulfonyl azide (Entry2, Table1): IR(cm^{-1}):1085, 1170(SO_2), 1371(SO_2), 2127(N_3), 2920, 3059. ^{1}H-NMR (400MHz, $CDCl_3$): 2.49 (s, 3H), 7.43 (d, 2H, J=8 Hz), 7.86(d, 2H, J=8.5 Hz)

III. REFERENCES

[1]. I.B. Douglas, T.B. Johnson, J. Am. Chem. Soc. 1938, 60, 1486; A. Meinzer, A. Breckel, B.A. Thaher, N. Manicone, H.H. Otto, Helv. Chim. Acta 2004, 87, 90; H.I. skulnick, P.D. Johnson, P.A. Aristoff, J.k. Morris, K.D. Lovasz, W.J. Howe, K.D.Watenpaugh, M.N. janakiraman, D.J. Anderson, R.J. Reischer, T.M. Schwartz, L.S. Bannitt, P.K. Tomich, J.C. Lynn, M.M. Horng, K.T. Chong, R.R. Hinshaw, L.A. Dolak, E.P. Seest, F.J. Schwende, B.D. Rush, G.M. Howard, L.N. Toth, K.R. Wilkinson, T.J. Kakuk, C.W. Johnson, A.L. cole, R.M. Zaya, G.L. Zipp, p.L. Possert, R.J. Dalga, W.Z. Zhong, M.G. Williams, K.R. Romines, J. Med. Chem. 1997,40,1149; E. Vedejs, C. Kongkittingam, J. Org. Chem. 2000,65,2309; K.M. Youssef, E.Al-Abdullah, H. Ei-Khamees, Med. Chem. Res. 2001, 10, 404.

[2]. A.M.Harned AM, W.M. Sherril, D.L. Flynn, P.R.Hanson, Tetrahedron. 2005, 61, 12093-12099.

[3]. E.D. Goddard- Borger, R.V.Stick, Org Lett. 2007, 9, 3797-3800.

[4]. P. Panchaud , L. Chabaud , Y.Landais , C. Ollivier, P.Renaud,S. Zigmantas, Chem Eur J. 2004, 10, 3606-3614.

[5]. A.B.Charette , R.P. Wurz , T. Ollevier, J. Org. Chem. 2000, 65, 9252-9254.

[6]. D.F. Taber, R.E. Ruckle, M.J. Hennessy, J. Org. Chem. 1986, 51, 4077-4078.

[7]. J. Waser, H.Nambu, E.M.Carreira, J. Am. Chem Soc. 2005, 127, 8294-8295.

[8]. J.V. Ruppel, J.E Jones, C.A.Huff, R.M. Kamble, Y. Chen, X.P. Zhang, Org Lett. 2008, 10, 1995-1998.

[9]. J. Waser, B.Gaspar, H. Nambu, E.M. Carreira, J. Am. Chem Soc. 2006, 128, 11693-11712.

[10]. D.S. Masterson, J.P. Shackleford, Synlett. 2007, 8, 1302-1304.

[11]. P. Panchaud, P. Renaud. Adv Synth Catal. 2004, 346, 925-928.

[12]. R.A. Ei-Sayed, Phosphrous Sulfur Silicon Relat. Elem. 2004, 179, 237; N.G. Anderson, P.D.

Ramsden, D. Che, M. Parvez, B.A. Keay, Org. Lett. 1999, 1, 2009; R.E. Harmon, G. Wellman, S.K. Gupta, J. Org. Chem. 1973, 38, 11; J.E. Leffler, Y. Tsuno, J. Org. Chem. 1963, 28, 902.
[13]. J. Humlian, S. Gobec, Tetrahedron Lett. 2005, 46, 4069.
[14]. S. Fujita, Synthesis 1982, 423.
[15]. A. Barco, S. Benetti, P. Pollini, R. Tadia, Synthesis, 1974, 877; M. Frankel, P. Moses, Tetrahedron 1960, 9, 289.
[16]. R. C. Reynolds, P.a. Crooks, J.A. Maddry, M.S. Akhtar, J.A. Montgomery, J.A. Secrist, J. Org. Chem. 1992, 57, 2983.
[17]. G. Blotny, Tetrahedron Lett. 2003, 44, 1499.
[18]. J. G. Kim, D.O. Jang, Synthesis 2008, 2885.
[19]. B. Maleki, s. Hemmati, R. Tayebee, S. salami, Y. farokhzad, M. Baghayeri, F.m. Zonoz, E. Akbarzadeh, R. Moradi, A. Entezari, M.R. Abdi, S.S. Ashrafi, F. Taimazi, Helvetica Chimica acta. 2013, 96, 2147-2151.
[20]. B.P. Bandgar, S.S. Pandit, Tetrahedron Lett. 2002, 43, 3413-3414.
[21]. A. Kiani, B. Akhlaghinia, H. Rouchi- Saadabad, M. Bakavoli, Journal of Sulfur Chem. 2014, 35, 119-127.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

One Pot Synthesis of Octahydroxanthene-1, 8-dione Derivatives using Magnesium Vanadium Oxide

Vijay V Dabholkar*, Sandip Gulve, Shrikant Anpat, Karthik Krishnan
Organic Research Laboratory, Department of Chemistry,Guru Nanak College, G.T.B Nagar, Mumbai, Maharashtra, India

ABSTRACT

A simple and efficient one pot synthesis of the 9-aryl substituted octahydroxanthene-1,8-diones. The reaction of aromatic aldehyde and Cyclic 1,3 diketone in ethanol using magnesium vanadium oxide nanocatalyst an efficient heterogeneous solid catalyst which can be easily synthesized and can be recovered from the reaction .This method has many advantages such as catalyst recoverable, recyclable, eco-friendly and simple work up procedure with high yield.

Keywords: Aromatic aldehydes, Cyclic 1,3 diketone, Catalyst.

I. INTRODUCTION

The substance that enhances the reaction rate is called a catalyst. The science and technology of catalysis is of great significance as it affects our daily life. Four major sectors of the world economy is petroleum-energy production, chemicals-polymer production, food industry and pollution control, involve catalytic processes. Now a day's surface active metal oxides are generally used to prepare heterogeneous catalysts material in the field of electronics and nanotechnology. Metal oxides have several applications because of its important and interesting properties such as large expansion coefficient, low magnetic transition temperature, high specific heating and low saturation magnetic moment. These properties are extensively used in catalysis, magnetic properties, sensor etc. [1-2].

In general catalytic property of metal nanoparticle are a function of their size, crystal lattice parameter. The development of new catalyst in nano-range has emerged as a fertile field for innovation and research. Owning to unique and novel properties like reusability, ability to generate clean product, high surface area and non-corrosiveness, recently $Mg_3V_2O_8$ heterogeneous solid nanocatalyst is applied as powerful catalyst for several organic transformation these facts encouraged us to use $Mg_3V_2O_8$ nanocatalyst for the efficient and green synthesis of octahydroxanthene-1,8-diones $Mg_3V_2O_8$ (orthovanadate) have been successfully prepared by the citrate method reported in earlier literature [3]. XRD spectra of the Mg vanadate phases after calcination at 550°C they are in agreement with the standard spectra [4,5,6].In present work, we demonstrated nanoparticle of magnesium vanadium oxide, a heterogeneous mixed oxides have several applications. Xanthene are of importance as they have various industrial, pharmaceutical and biological applications. Xanthene derivative 1,8-dioxo-octahydroxanthens are of importance as they have various industrial, pharmaceutical and biological applications[7-12]. For example, these compounds have been applied as dyes in laser technology [7], and as pH sensitive fluorescent materials for visualization of biomolecules [8].Moreover, xanthenes derivatives have been used as antibacterial [9], antiviral [10], antitumor [11] and anti –inflammatory agents[12]. Some solvents and catalysts have been applied silica supported SiO2 (SPNP),2,4,6- trichloro-1,3,5-triazine (TCT),p-dodecylbenzenesulfonic acid (DBSA), Tetrabutyl ammonium bromide (TBAB), $BiVO_4$, Tri methylsilyl chloride (TMSCl), Saccharin sulfonic acid (SaSA), SiO2-Cu(0)for thesynthesis of 1,8-dioxo-octahydroxanthenes involves the one-pot multi-component reaction of dimedone (5,5-dimethyl-1,3-cyclohexanedione) (2 mole) with aldehydes (1 mole) [13-22].But ,many of the above methods suffer from limitations such as prolonged reaction time, high temperature and tedious work-up processes, low yield, hazardous reaction conditions are environmentally unacceptable from green chemistry view point

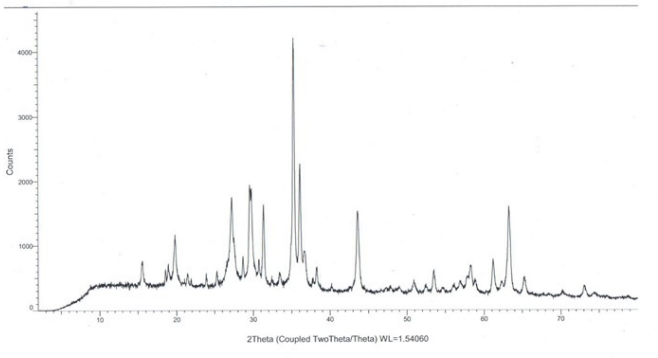
Having the above subjects in mind, we report here a new, highly efficient and simple method for the one-pot synthesis for the formation of 1,8-dioxo-octahydroxanthene in low temperature ,shortened reaction time , selectivity, recyclability and excellent yields by one-pot Knoevenagel condensation, Michael addition in the presence of magnesium vanadium oxide. The condensation of two equivalent of 1,3-diketones, such as dimedone(5,5-dimethyl-1,3-cyclohexanedione) with various aldehydes.

II. MATERIAL AND METHODS

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The purity of the compounds was monitored by thin layer chromatography on silica gel coated aluminium plates (Merck) as adsorbent and UV light as visualizing agent. 1H NMR spectra were recorded on BRUKER 400 MHz NMR Spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ ppm).

III. RESULT & DISCUSSION

$Mg_3V_2O_8$ nanoparticles were synthesized by citrate method. The structural characterization of $Mg_3V_2O_8$ nanoparticles were done by X-ray Diffraction using CuKα radiation (λ = 1.54059 Å) at 40 kV and 15 mA shown in Figure.



XRD patterns for $Mg_3V_2O_8$.nanoparticles sintered at 550°C.

Table 1. Synthesis of 9-aryl substituted octahydroxanthene-1,8-diones under different solvent systems at 35-40°C.

Entry	Solvent	Temperature°C	Yield of product (%)	Time (min)
1	H2O	35-40	79	60
2	EtOH/H2O	35-40	90	55
3	EtOH	35-40	94	40
4	DMF	35-40	85	55
5	DMSO	35-40	75	90
6	Without Solvent	35-40	35	120

Reaction conditions: Dimedone(1mmol),Benzaldehyde (0.5mmol), Mg3V2O8(0.03g), at 35- 40°C.

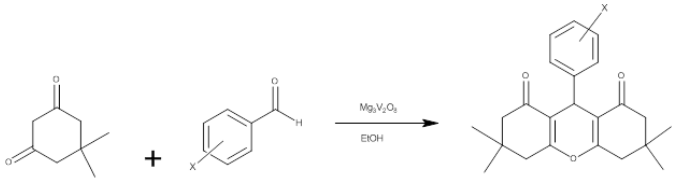
In addition to the above, the effect of catalyst concentration was also studied shown in (Table 2). Which indicated that 0.03g of the $Mg_3V_2O_8$ nanocatalyst was sufficient to catalyzed the reaction and increase the quantity of catalyst beyond this did not increase the yield.

Table 2. Effect of different quantity of catalyst on reaction.

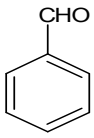
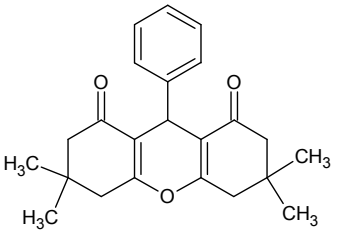
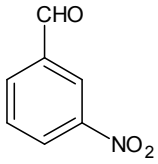
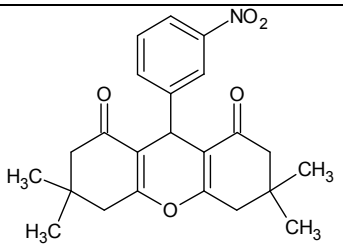
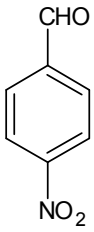
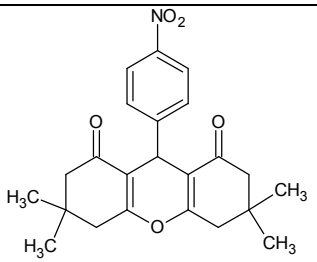
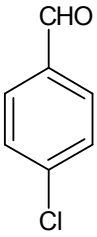
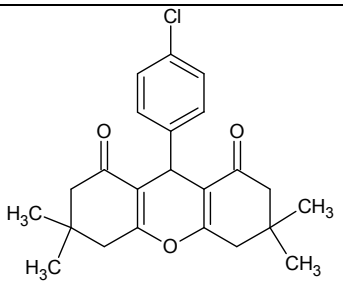
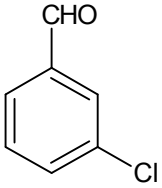
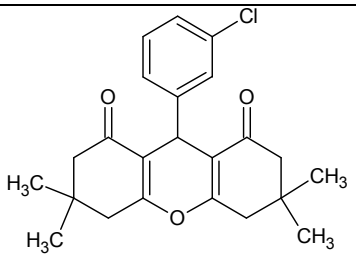
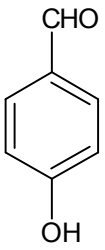
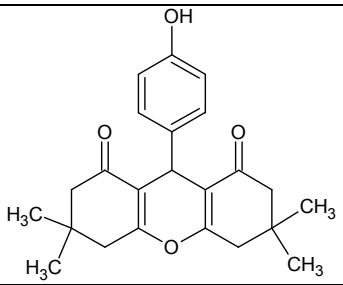
Entry	Catalyst quantity (g)	Yield of product (%)
1	0.01	60
2	0.02	80
3	**0.03**	**94**
4	0.04	94
5	0.05	91

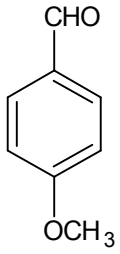
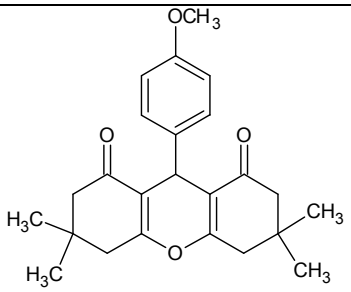
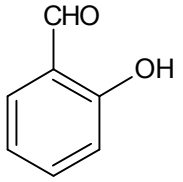
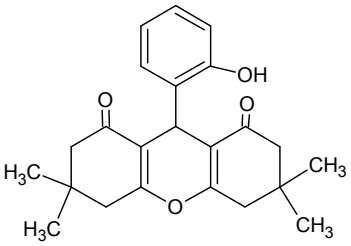
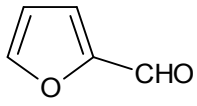
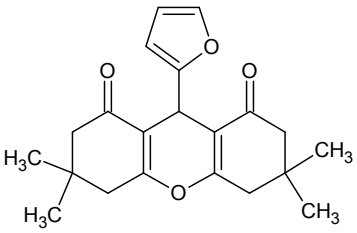
Reaction conditions: Dimedone (1mmol), Benzaldehyde (0.5mmol), Ethanol (5ml) at 35- 40°C.

After optimizing the reaction conditions, we applied this catalyst for the synthesis of substituted octahydroxanthene-1,8-diones by using substituted aromatic aldehyde. Almost, all the employed aldehyde resulted in good to excellent yield of the corresponding product.



Scheme 1. Synthesis of 9-aryl substituted octahydroxanthene-1,8-diones.

Sr.No.	Aldehydes	Product 3	Time/min	%Yield	M.P/°C Found	M.P/°C Reported
1		 a	45	92	199-201	203-205
2		 b	25	94	166-167	165-166
3		 c	30	94	226-229	228-230
4		 d	40	93	230-231	231-233
5		 e	45	91	182-184	183-185
6			50	90	244-247	244-246

		f				
7		 g	45	91	245-248	248-250
8		 h	55	91	239-240	-
9		 i	45	89	187-189	-

IV. EXPERIMENTAL

General procedure for the preparation of 1,8-dioxooctahydroxanthene derivatives :

A mixture of 5,5-dimethyl-1,3-cyclohexanedione (0.14 g,1 mmol), various aldehydes(0.5 mmol),ethanol (5ml) followed by NPs (0.03 g,0.1 mmol, 10 mol %) was stirred at 35-40°C for 30–40 min. reaction mixture was monitored by TLC. After completion of the reaction, EtOAc (10 mL) was added to the reaction mixture, stirred for 2 min and filtered. The catalyst was separated by filtration, dried and reused for subsequent reactions. filtrate was evaporated, obtained solid was recrystallized from EtOH to afford the pure product.

3,3,6,6-Tetramethyl-9-(3-chlorophenyl)-1,8-dioxo-octahydroxanthene.(4d)

IR (KBr, cm−1) ν max 3015, 2960, 1662, 1464, 1375, 1204, 1165, 795; 1H NMR (400MHz, CDCl3) δ 1.13 (6H, s, 2 × CH3), 1.25 (6H, s, 2 × CH3), 2.41 (4H, dd, , 2 × CH2), 2.49 (4H, s, 2 × CH2), 5.56(1H, s,CH), 7.11-7.25 (4H,m, ArH).

3,3,6,6-Tetramethyl-9-(2-hydroxyphenyl)-1,8-dioxo-octahydroxanthene.(8h)

IR (KBr, cm−1) ν max 3250,2915, 1640, 1454, 1370, 1202, 1166, 799; 1H NMR(400MHz,CDCl3) δ 1.04 (6H, s, 2 × CH3), 1.16 (6H, s, 2 × CH3), 2.30 (4H, dd, , 2 × CH2), 2.58 (4H, s, 2 × CH2), 4.67(1H, s,CH), 4.80(1H, s,OH), (7.01-7.28 (4H,m, ArH).

V. CONCLUSION

Results revealed that the $Mg_3V_2O_8$ catalyst exhibited excellent catalytic performance in the one-pot condensation of dimedone and aromatic aldehyde, at low temperature. Because of its small particle size and high specific surface area, the catalyst provides a favorable surface for the reactants and the reaction completes successfully in terms of excellent yield with short span

of time, this means that catalyst plays crucial role in this transformation. Finally we concluded that in comparison to existing methods, this method is the best alternative for the synthesis of 9-aryl substituted octahydroxanthene-1,8-dionesbiologically important compound in presence of $Mg_3V_2O_8$ as a solid base heterogeneous catalyst. Catalyst relatively non-toxic, environmentally safe, easy to recover and reuse.

VI. ACKNOWLEDGEMENT

Authors are thankful to the Management of Guru Nanak College, Mumbai- 37 for constant encouragement and for providing necessary facilities.

VII. REFERENCES

[1]. Delpiero G, Trifiro F and Vacari A. J. Chem. Soc. Chem. Commun. 1984, 10, 656.
[2]. Pradeep A, Priyadharsini P and Chandrasekaran G.. J. of Magn. Mag. Mater.,2008, 320, 2779.
[3]. Xingtao Gao, p. Ruiz, Qin Xin, Xiexian Guo and B. Delmon.Catalysis Letters.1994,321-337.
[4]. A. Nord et al, (ASTM file No. 37-35l), Chim. Scripta.1985,25, 212.
[5]. G. Clark and R.J. Morley, J. Solid State Chem.1976, 16,1429 .
[6]. Gao,XT Delmon,Bernard ruiz,Catalysis Letters-1994 , 23, 321-337.
[7]. Menchen S M, Benson S C, Lam J Y L, Zhen W, Sun D, Rosenblum B B, Khan S Hand Taing M, Chem Abstr., 2003, 139,
[8]. Sarma R J and Baruah J B, Dyes Pigm., 2005, 64, 91.
[9]. Qiao Y F, Okazaki T, Ando T, Mizoue K, Kondo K, Eguchi T and Kakinuma K, J Antibiot., 1998, 51, 282.
[10]. Jamison J M, Krabill K, Hatwalkar A, Jamison E and Tsai C, Cell Bio Inter Rep.1990, 14, 1075.
[11]. Rewcastle G W, Atwell G J, Zhuang L, Baguley B C and Denny W A, J MedChem.1991, 34, 217.
[12]. Poupelin J P, Ruf G S, Blanpin O F, Narcisse G, Ernouf G U and Lacroix R, Eur J Med Chem., 1978, 13, 67.
[13]. Das B, Thirupathi P, Reddy K R, Ravikanth B and Nagarapu L, Catal Commun.,2007, 8, 535.
[14]. Karthikeyan G and Pandurangan A, J Mol Catal A: Chem., 2009, 36,311.
[15]. Zhang Z-H and Tao X-Y, Aust J Chem., 2008, 61,77.
[16]. Jin T S, Zhang J S, Xiao J C, Wang A Q and Li T S, Synlett,2004, 866.
[17]. Kantevari S, Bantu R and Nagarapu L, J Mol Catal A: Chem.2007, 269, 53.
[18]. Bigdeli M, Chin Chem Lett.2010, 21, 1180.
[19]. Kantevari S, Bantu R and Nagarapu L, ARKIVOC, 2006,16, 136.
[20]. Javid A, Heravi M M and Bamoharram F F, E-J Chem, 2011, 8, 910.
[21]. Zhang Z-H and Liu Y-H, Catal Commun., 2008, 9, 1715.
[22]. Mohammadpoor-Baltork I, Moghadam M, Mirkhani V, Tangestaninejad S and Tavakoli H R, Chin Chem Lett., 2011, 22, 9.
[23]. Tavakoli, H. R., Zamani, H., Ghorbani, M. H, Iranian Journal of OrganicChemistry,2009, 2, 118-126.
[24]. Venkatesan, K., Pujari, S. S., Lahoti, R. J., & Srinivasan, Ultrasonics Sonochemistry, 2008, 15, 548-553.
[25]. Zhang, Z. H.& Lui, Y. H. Catalysis Communications,2008, 9, 1715-1719.
[26]. Poor Heravi, M. R. .Journal of the Iranian Chemical Society,2009,6, 483-488.
[27]. Karade, H. N. Sathe, M. & Kaushik, M,Catalysis Communications,2007, 14, 2-67.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Physico-Chemical Characteristics of soil in Sangmner Area of Irrigated Region

Sangita Dandwate
Department of Chemistry, S.M.BST College Sangamner, Ahmednagar, Gujarat, India

ABSTRACT

A physico-chemical monitoring of major rivers in Sangamner area was done during the month of June and July 2015. Sangamner area distribute into two regions Pravara basin and Mula basin. For this fifteen soil sampling points were selected from Sangamner irrigated area (Pravara basin).The analysis was carried out for the parameters namely pH, acidity, alkalinity, calcium carbonate, organic carbon, available nitrogen, phosphorous, potash. Due to increasing demand for cash crops the practice of monoculture cropping pattern have further helped to deteriorate water as well as soil quality. Continuous use of chemical fertilizers slowly changed soil properties ultimately the production in long run is reduced. It has resulted in leaching of chemical into the surface and ground water. Therefore, it is essential to evolve and adopt a strategy of integrated nutrient supply by using a combination of chemical fertilizers, organic manures and biofertilizers.

Keywords: Soil Physico -Chemical Properties

I. INTRODUCTION

Sangamner area is located in the northern part of the Ahmednagar district of Maharashtra State. The Sangamner tahsil lies between 180 36' N and 1901' N latitude and between 740 1' W and 740 56' W longitude. The area is drained by the Pravara river, which originates in the hilly region of Western Ghats at Ratangarh. Geologically, basalts underlay the Pravara basin. In general the climate is dry and hot the average maximum temperature during summer is as high as 42^0C in month of May and average minimum temperature falls up to 10^0C during the month of December. The area receives rainfall, chiefly from the south waste monsoon between June and September as the area falls under the rain shadow zone of Western Ghat and receives very low precipitation, annual rainfall ranging from 290 to 594 mm.

II. MATERIAL AND METHODS

In all 15 soil samples from study area were collected during summer 2015

While collecting soil samples the upper layer of vegetation, surface litter, stones stubble if any were cleared away and then layer of soil immediately below (0-15 cm) was collected in cotton cloth bag. The dried soil samples were pounded in wooden mortar and pestle and sieved through 2 mm sieve. The sieved soil samples were used for physical and chemical analysis. From all of fifteen soil sampling stations were selected and present study have been considered as follows:

Table 1

S. No	Irrigated Village Name
S1	Khandgaon
S2	Nimaj
S3	Sangamner Kh.
S4	Gunjalwadi
S5	Ghulewadi
S6	Sangavi
S7	Sukewadi
S8	Ashvi Kh.
S9	Jorve
S10	Kolhewadi
S11	Rahimpur
S12	Chinchpur
S13	Sadatpur
S14	Umbari balapur
S15	Kharadi

III. RESULT AND DISCUSSION

In general, the soil in the study area is medium to high in fertility. Pravara rivers bank soils were dominated by medium black to black cotton soil with sandy clay loam

texture known as garden soils. Chemical properties of soil pH varied from 7.90 - 8.50 with the average value of 8.21categorized alkaline in nature.(Table 2)

The electrical conductivity varies from 0.64 to 4.88 dSm^{-1}. Organic carbon content varies from 0.41 to 0.63 percent with an average value 0.52 percent indicating high productivity. Calcium carbonate ranges from 8.15 to 16.13 percent with average value 11.34 percent. The available nitrogen was in low category (less than 250 kg /ha)due to low mineralization . The available P_2O_5 classified in low class in all irrigated soil (10.41-38.6 kg /ha) while available potash content was in the very high range of class.

By the soil examination it has been concluded that the soils under the present investigations are enriched with potassium. Figure 1 shows Sangamner taluka fertility status of irrigated area - Pravara basin

Table 2. Chemical properties of soils from Sangamner area during summer 2009 (Average of 3 samples)- Pravara basin

Parameter	Irrigated area															
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	Mean
P^H (1:2.5)	8.30	8.40	8.19	8.40	8.02	8.40	8.50	8.21	8.20	8.31	8.41	7.91	7.92	7.90	8.10	**8.21**
EC dSm^{-1}	0.78	0.41	0.26	1.10	4.88	0.81	2.78	0.94	2.59	1.85	1.02	0.96	0.64	0.77	0.71	**1.36**
Ca^{++}meq%	13.4	14.4	20.32	19.01	20.42	23.01	12.61	11.6	28.49	22.94	18.14	18.30	14.60	15.20	14.21	**17.78**
Mg^{++}meq%	16.70	20.67	25.55	14.10	20.05	14.78	19.02	15.2	17.24	15.14	20.5	11.60	11.70	23.12	12.15	**17.74**
Na^{+}meq%	0.34	0.44	2.35	0.61	0.95	0.85	0.52	0.53	1.32	0.84	0.81	0.52	0.80	0.48	0.38	**0.78**
$CaCO_3$%	12.4	10.8	9.10	12.09	12.11	11.09	8.15	10.83	15.24	9.42	16.13	9.16	10.17	9.50	14.04	**11.34**
Organic carbon%	0.52	0.45	0.50	0.58	0.47	0.55	0.51	0.60	0.41	0.55	0.62	0.63	0.47	0.45	0.60	**0.52**
Organic matter %	0.89	0.77	0.86	0.82	0.81	0.94	0.87	1.03	0.84	0.94	1.06	1.08	0.81	0.77	1.03	**0.90**
Available N kg/ha	148.52	120.40	210.11	110.42	145.16	130.42	83.90	145.0	289.54	150.51	220.71	131.0	154.0	178.0	145.0	**157.51**
Available P kg/ha	16.04	10.41	17.24	33.0	28.0	38.6	31.8	12.59	16.0	31.6	33.0	31.6	30.8	35.1	12.0	**25.38**
Available K kg/ha	365.11	390.22	611.7	370.40	509.62	770.4	312.2	610.3	680.2	693.65	516.4	539.0	464.0	597.0	545.0	**531.68**

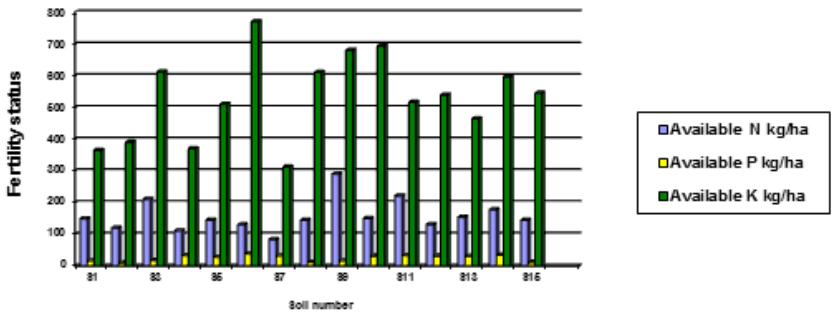


Figure 1. Sangamner taluka fertility status of irrigated area - Pravara basin

IV. REFERENCES

[1]. Bharambe,P.R. and Ghonsilkar, C.P. 1985. Physico –chemical characteristics of soils in Jayakwadi Command.J.Maharashtr agric.univ.10:247-249.

[2]. Bhattacharya, P.and ghose, G.2001.Phosphorous use efficiency in brinjal with FYM and Sulpher.J. Indian Soc.Soils Sci. 49(3):456-462.

[3]. Biswas, B.C., Yadav, D.S. and Maheshwari, S. 1985. Role of calcium and magnisium in Indian agriculture. A Rev., ferti. News, 30: 15-35.

[4]. Brar, B. S. and Bajwar, M. S.1996. Soil sodification as influenced by salinity and various ratios of bicarbonate to calcium and magnesium in irrigation water. J. Indian Soc. Soil Sci. 44(2): 353-356.

[5]. Challa, O.B., Bhaskar, P., Anantwar, S.G. and Gaikwad, M.S. 2000. Characterization and classification of some problematic vertisols in semi-arid ecosystem of Maharashtra plateau. J.Indian Soc. Soil Sci. 48 (1): 139-145.

[6]. Chopra, S.L. and Kanwar, J.S. 1991. Analytical agricultural Chemistry. Kalyani publi; New Delhi, Ludhiana

[7]. Dahama, A.K. 2002. Organic farming, an overview for sustainable agriculture. Second Enlarged Edition. Agrobios (India) Jodhpur.

[8]. Daji,J.A.1998.A textbook of soil Science. Media promoters and public. Bombay.pp1-365

[9]. Gaikwad, R.M. and Wani, P.V. 2001. Response of Brinjal (Cv Krishna) to phosphate solubilizing biofertilizers.j. Maharashtra agric.Uni 26(1):29-32

[10]. Kanwar, J.S.1997.J,Indian Soc.Soil Sci 45:417-428

[11]. Kaushik,B.D.1996. Consoildated Report,Mission Mode Project on technology Development and Demonstration of Algal Biofertilizer, IARI,New Delhi,India.

[12]. Sat Pal and Saimbhi , M.S. 2003. Effect of varying levels of nitrogen and phosphorous on earliness and yield of brinjal hybrids (Solanum melongena L.). Res .on crops.4 (2):217-

[13]. Singh, R. N. 1961. The role of blue green algae in nitrogen economy of Indian Agriculture. I. C. A. R., New Delhi, (India), pp. 175.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Review on some Thiazole Containing Heterocyclic Compounds and their Biological Activity

R. B. Toche[*1], Sanjay U. Deshmukh[2]
[1]Dadasheb Bidkar ASC College, Peth (Nashik), Maharashtra, India
[2]Department of Chemistry, KRT Arts, BH Commerce & AM Science College,Nashik, Maharashtra, India

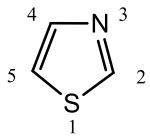
ABSTRACT

Heterocyclic compounds are very important due to their versatile industrial application. there are number of heterocyclic compounds use as medicine in different therapeutic targets. Thiazole is one of the important pharmacophore in drug discovery and development process. there are number of medicine and biologically active molecules those are thiazole substituted heterocyclic compounds covering wide range of therapeutics targets including Anti-Microbial, Anti- cancer, Anti-Imflametry , anti-HIV etc. These reviews collectively get idea about thiazole containing medicines and biological active compounds in recent medicinal chemistry research.
Keywords: Thiazole, Heterocyclic, Biological Activity.

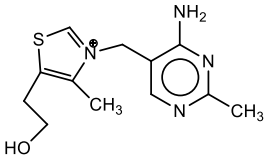
I. INTRODUCTION

Thiazole is five member organic heterocyclic compound containing N and S hetero atom's. thiazole is aromatic as delocalization of alone pair electron of Sulfur atom completing 6π electron to satisfy Huckel rule.

A Thiazole is found naturally in vitamin B1 (Thiamin), **Thiamin** is water soluble vitamin that helps the body to release energy from Carbohydrates during metabolism. and it's enzyme play vital role in decaboxylation of alfa keto acid and as an electron sink respectively. it also helps in the normal functioning of the Nervous system by its role in the synthesis of the acetylcholine a neurotransmitter.



Thiazole



Thiamin

There are number of medicine covering various therapeutic targets are thiazole substituted heterocyclic compounds. like antibiotic Cefidinir the third generation of cephalosporin and antibiotic Abafugin, HIV -1 protease inhibitor Ritonavir, drug use for efficient treatment of hyperuricemia in gout is Febaxostat, dehydrogenase anticancer drug Thiazofurin , anti-depressant drug Pramiperoxale, antineoplastic agent Bleomycin, antiasthamatic drug Cinalukast, antiulcer agent Nizatidine, non-steroidal immunomodulatory drug Fanetizole, antiimflamatory drug Meloxicin, etc.

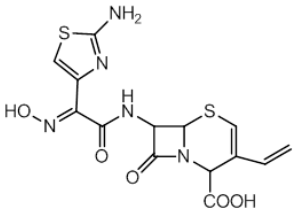
In resent research updates find out that thiazole containing heterocylic compounds are potent Bacterial DNA Gyrase inhibitors, Flavivirus envelope protin inhibitors, anticancer CDK9 inhibitors, p38-MAP, Pan-Src, Spleen-Thyrosin kinase inhibitors. along with that there are number of biologically active thiazole containing heterocyclic compounds show extensive application in medicinal chemistry research as antimicrobial, anti-inflamatory, antiviral, anti-HIV, anticancer, antitumour, antidiabetic, anti-convulscant,anti-depressant [1-28] etc.

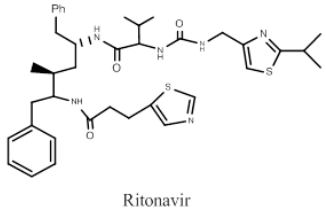
II. THIAZOLE CONTAINING BIOLOGICALLY ACTIVE HETEROCYCLIC COMPOUNDS.

A. Thiazole containing drugs

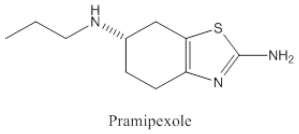
Cefidinir the semi- synthetic third generation cephalosporin, containing amino thiazole with ester and acid moiety it's an orally administrated antibiotic show antibacterial activity against both germ positive and germ negative bacteria. It shows excellent activity against Staphylococcus species.

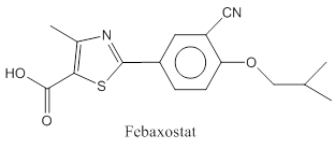
The HIV-1 protease inhibitor **Ritonavir** containing two different substituted Thiazole ring, which achive good bioavailability and long plasma half life in addition, H-bonding of 5-substituted thiazole to back bone of Asp-30 as compare to initially design pyridine ring. It's an earlear candidate for the treatment of AIDS.

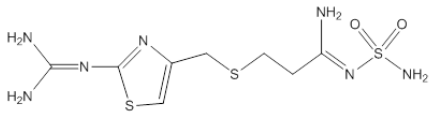

Cefidinir


Ritonavir

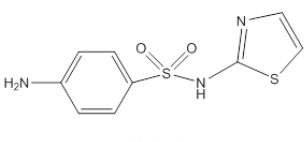
The Dopamine D_2-agoinst **Pramipexole** consist of fused bicyclic tetrahydrobenzothiazole with protected form of 4-amino cyclohexane. Pramiperoxle effectively use as antidepressant agent. **Famotidine** Is an H_2 - receptor antagonist which inhibit isoenzyme of hepatic CYP 450 system and has the additional side effect of increasing the amount of gastric bacteria such as nitrate reduing bacteria. It effectively use in ulcer treatment. It contains Thiazole substituted guanidine and sulfonyl amide. **Febaxostat** The Thiazole containing drug is given in the novel xanthine oxidase in non competitive fashion. Consequently , the amount of the oxidation produce uric acid is reduced. Thus it is an efficient treatment for hyperuricemia in gout.

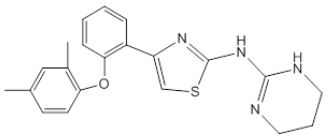

Pramipexole

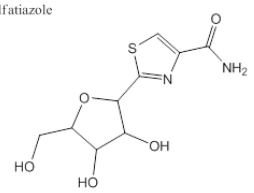

Febaxostat


Famotidine

Sulfatiazole The Thiazole containing sulphonamide effective antimicrobial agent. the drug **Abafugin** amino-thiazole substituted heterocyclic compound use as antibiotic. and **Thiazofurin** Thiazole containing inhibitor of IMP dehydrogenase anticancer drug.


Sulfatiazole

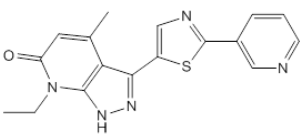

Abafugin

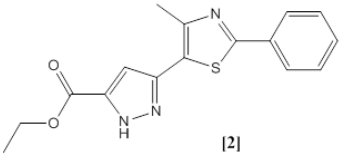

Thiazofurin

B. Thiazole containing biological active molecules in recent drug discovery and development process

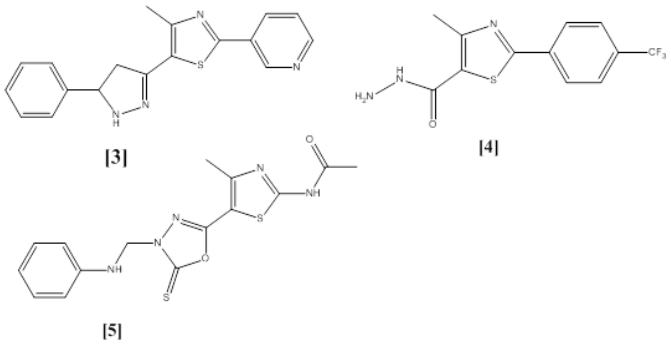
a. Antimicrobial activity.

Bacterial DNA gyrase is highly valuable target for antibacterial research, there are number of new biologically active molecules targating over Gyrase-B, DNA gyrase exist as A_2B_2 tetramer that binds dsDNA and catalyzes strand reformation that introducted in negative supercoils. Chemotypes bind to other regions of the protin complex make advantage of avoiding on-target fluroqunolone resistance, inhibition of ATPase activity of GyrB with bacterial DNA replication, resulting potential antibacterial activity. In resent update there are number of researcher find potential DNA Gyrase B inhibitors like- discovery of thiazole substituted pyrazolopyridones [**1]** show potential Gram-positive antibacterial activity and low resistance incidence against clinically important pathogens. thiazole substituted pyrazole esters **[2]** and benzthiazole substituted amid also show potential activity with DNA Gyrase-B.

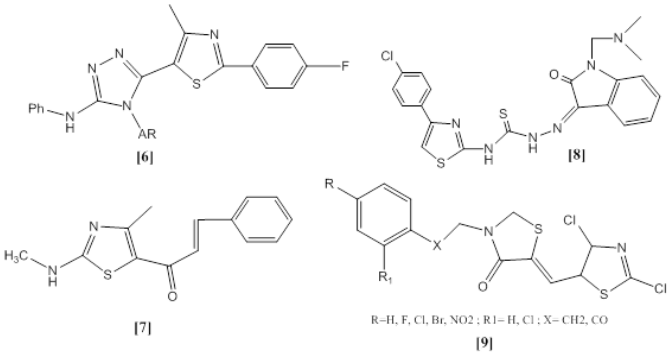

[1]


[2]

Synthesis of some new 2-(3-pyridil)-5 pyrazole substituted thiazole **[3]** act as potential antimicrobial agent. new hydrazones bearing thiazole scaffold **[4]** show efficient antimicrobial and antioxidant activity. novel thiazole clubbed 1,3,4-oxadizole with different aromatic substitution like 2F,3F-Ph **[5]** show potential antimicrobial and cytotoxic activity.

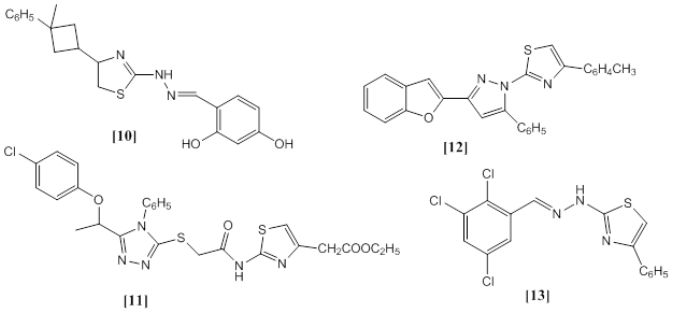


Alog with it number of researcher find various substitution on thiazole show potential antimicrobial activity. like-. Karale et al [6] present new series of Thiazolyl Triazoles analoges and their anti microbial activity. K Liarus et al **[7]** present Thiazole base chalcones as potent anti microbial agents. Pandeya et al **[8]** prepared a series of Schiff and Mannich bases derived from isatin derivatives find out potential antimicrobial activity.



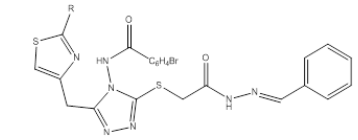
Dundar et al **[9]** presented a set of thiazolyl thiazolidine-2,4-dione derivatives and screened for them for their antibacterial and antifungal activities against methicillin resistant S.aureus, Ecoli and C albicans. All thecompound were found to be modereately potent against screened micro organism.

Cukurovali et al **[10]** reported a synthesis of thiazole substituted series of Schiff bases containing 2, 4 – disubstituted thiazole and cyclobutane rings and hydrazones and evaluated them for antibacterial and antifungal activities. the most effective compound providing a MIC value of 16 μ ml^{-1} was found to be against C..tropicallis and B .subtilis bacterial spaces. Zitouni et al **[11]** reported new thiazole derivatives of triazole and evaluated for antifugal and anti bacterial activity. Their antimicrobial activities Candida albicans,C.glabrata,E. coli,S. aureus,P. aeruginosa were investigated. Abdel – Wahab et al **[12]** synthesized various thiazole containing compounds which show good to modrate antibacterial and antifungal activities. Karegoudar et al **[13]** synthesized a series of novel Thiazole compound. The newly synthesized compounds were screened for their antibacterial and antifungal activities. Shiradkar et al **[14]** reported a series of thiazole substituted amide derivatives were tested for anti bacterial activity

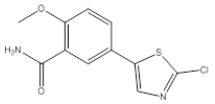


Xin et al **[15]** reported novel oxazolidinone thiazole analogues containing substituted thiazole all the compound were find antibacterial activities. Vicini et al **[16]** reported novel thiazolidinones and assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, they find potent agent against Gram positive bacteria. Narayana et al **[17]** prepared a series of 5-{2-[(N-substituted aryl) amino]-1, 3-thiazol-5-yl} 2-hydroxy benzamides The newly synthesized compounds were screened for their antifungal activity.

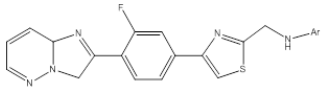
Chimenti et al **[18]** reported the synthesis of a novel series of 2-thiazolylhydrazone derivatives and the influence of the substituents on the thiazole ring on antifungal activity. All synthesized compounds were screened for their *in vitro* activities isolates of *Candida* sp., representing six different species, compared to clotrimazole as a reference compound. Some of the tested compounds were found to possess significant antifungal activity. Vicini et al **[19]** reported novel thiazolidinones and assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, yeast and mould. All the compounds exhibited potent against Gram positive bacteria



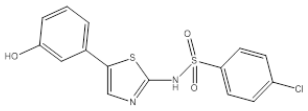
[14]



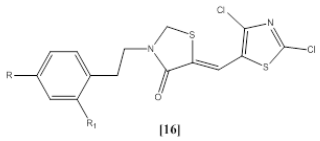
[17]



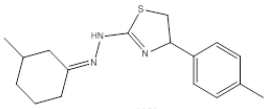
[15]



[18]



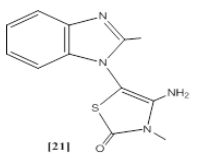
[16]



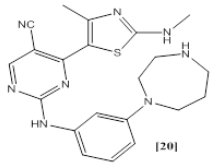
[19]

b. Anticancer activity

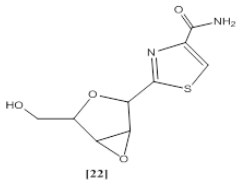
Synthesis of substituted 4-(thiazol-5-yl)-2-(phyenylamino)pyrimidines derivatives **[20]** with functional groupattached to the C5 positionof the pyrimidine and investigated there effects on CDK9 potency and selectivity, one of them inhibits CDK9 with IC_{50}= 7nM and shows 80 fold selectivity over CDK-2. Ramla et al **[21]** synthesized a variety of 1-substituted-2-methyl-5-nitrobenzimidazoles and evaluated them for anti-tumor activity. The anti-tumor effect of compound was found to be significant. Popsavin et al **[22]** reported a set of 2-(2, 3-anhydrofuranosyl) thiazole-4-carboxamide (2', 3'-anhydro tiazofurin) derivatives and screened them for their anti-tumor activity. The most active compound was found to be against K_{562} malignant cells, with IC_{50} ranging from 0.09-0.49 μM. Gulsory et al **[23]** presented a series of thiazole substituted arylidene hydrazides from [6-(4-bromophenyl) imidazol-3yl] acetic acid hydrazide. The synthesized compounds were evaluated one dose primary cytotoxicity assay. Compound demonstrated the most effective agents on a prostate cancer cell lines.



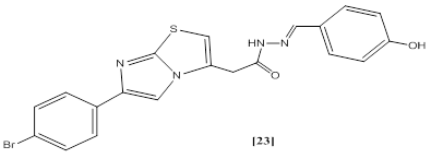
[21]



[20]



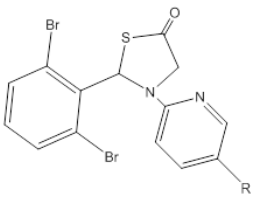
[22]



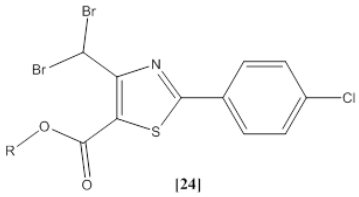
[23]

c. Anti-Viral activity

Synthesis of thiazol -2-(4-Clphenyl)-5-ethyl ester and relevant analogs **[24]** describe over shoe potential antiviral activity targating Flavivirus envelope protins. Many flaviviruses are arthropod-borne, human pathogen that can cause encrphalitis, hemorrhagic fever, shoch syndrome, and jaundice, example of pathogenic flaviviruses include dengue virus, yellow fever virus, west nile virus, tick-bone encephalitis virus, and Japanese encephalitis virus. Rawal et al **[25]** synthesized a series of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidine-4-one and evaluated as selective human immunodeficiency virus type-1 reverse transcriptase (HIV-1, RT) enzyme inhibitors. In vitro cell assay showed that effectively inhibited HIV-1 compounds having isothiourea or thiourea functional group showed high anti-HIV-1 activity.



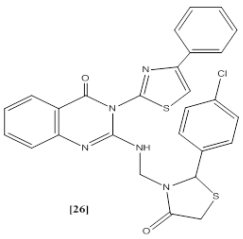
[25]



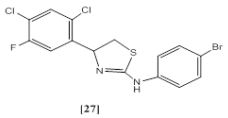
[24]

d. Anti-inflammatory activity

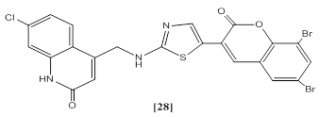
Kumar et al **[26]** synthesized a group of 3-[4'(p-chlorophenyl) thiazole-2'-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethyl]-6-bromoquinazolin-4-ones and screened them for anti-inflammatory and analgesic activities. They found that the presence of thiazolidinone ring have shown much better anti-inflammatory as well as analgesic activity. Holla et al **[27]** reported different series of arylaminothiazoles, and hydrazinothiazoles. screened them for their antibacterial and anti-inflammatory activities. Kalkhambkar et al [**28]** reported triheterocyclic thiazoles containing coumarin and carbostyril. The synthesized compounds were tested for their in vitro analgesic and anti-inflammtory activities.



[26]



[27]



[28]

III. REFERENCES

[1]. Jason B .Cross Discovery of Pyrazolopyridones as novel class of Gyrase-B inhibitors using structure guided design. ACS, Medicinal Chemistry letters 2016, 7, 374-378

[2]. Steven M.Ronnkin, discovery of pyrazole thiazoles as novel and potent inhibitors for bacterial Gyrase. Bioorganic Medicinal chemistry letters,2010, 20, 2828-2831.

[3]. Samir Bondock, synthesis of some new2-(3-pyridyl)-4,5-disubstituted thiazoles as potent antimicrobial agent. European Journal of Medicinal chemistry. Jan-2013.

[4]. Cristina Nastasa. New Hydrazones bearing thiazole scaffold: synthesis characterization , Antimicrobial and Antioxidant investigation. Molecules, 2015, 20, 17325-17338.

[5]. N. C. Desai. Synthesis antimicrobial and cytotoxic activity of some novel thiazole clubbed 1,3,4-oxadizoles. European Journal of Medicinal chemistry. 2013, 67, 54-59.

[6]. B. Karale. Syntesis and Biological Screening of novel thiazolyl triazoles and thiadizoles. Indian Journal of Chemistry 53B, 2014, 339-344.

[7]. K Liaras. Thiazole based chalcones as potential antimicrobial agent, synthesis and biological evaluation. Bioorganic and Medicinal Chemistry 2011, 19, 3135-3140.

[8]. Pandeya S N. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl) thiazol- 2-yl] thiosemicarbazide. European Journal of Pharmasutical Sci. 1999, 9, 25-31.

[9]. Dundar O. B. Ozgen O, Mentese A, Altanlar N, Ath O, Kendj E, Ertan R. Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4- dione derivatives. Bioorganic and Medicinal Chemistry. 2007, 15, 6012-6017.

[10]. Cukurovali A, Yilmaz I, Gur S, Kazaz C. Synthesis antibacterial and antifungal activity of some new thiazolylhydrazone derivatives containing 3-substituted cyclobutane ring. European Journal of Medicinal Chemistry. 2006, 41, 201-207.

[11]. Zitouni GT, Kaplancıklı ZA, Yıldız MT, Chevallet P, Kaya D. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1- phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. European Journal of Medicinal Chemistry. 2005, 40, 607–613.

[12]. Abdel-Wahab BF, Abdel-Aziz HA, Ahmed EM. Synthesis and antimicrobial evaluation of 1-(benzofuran-2-yl)-4-nitro-3- arylbutan-1-ones and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4-(aryl)-1,3-thiazol-2-yl]-1H-pyrazoles. European Journal of Medicinal Chemistry. 2009, 44, 2632-2635.

[13]. Karegoudar P, Karthikeyan MS, Prasad DJ, Mahalinga M, Holla BS, Kumari NS. Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents. European Journal of Medicinal Chemistry. 2008, 43, 261- 267.

[14]. Shiradkar M R, Murahari KK, Gangadasu HR, Suresh T, Kalyan CA, Panchal D, Kaur R, Burange P, Ghogare J, Mokale V, Raut M. Synthesis of new S-derivatives of clubbed triazole as anti Mycobacterium tuberculosis agents. Bioorganic and Medicinal Chemistry. 2007, 15, 3997-4008..

[15]. Xin Z, Yang-fang Z, Jia W, Wei J, Liang H, Ping G. Synthesis and antibacterial activity of novel oxazolidinone analogues containing substituted thiazole/fused bicyclic groups. Chem Res Chinese U. 2006, 22, 459-464.

[16]. Vicini P, Geronikaki A, Anastasia K, Incerti M, Zani F. Synthesis and antimicrobial activity of novel 2-thiazolyl imino-5-arylidene-4-thiazolidinones. Bioorganic and Medicinal Chemistry. 2006, 14, 3859-3864.

[17]. Narayana B, Vijaya Raj KK, Ashalatha BV, Kumari NS, Sarojini BK. Synthesis of some new 5-(2-substituted-1,3-thiazol-5-yl)-2- hydroxy benzamides and their 2-alkoxy derivatives as possible antifungal agents. . European Journal of Medicinal Chemistry. 2004, 39, 867–872.

[18]. Chimenti F, Bizzarri B, Maccioni E, Secci D, Bolasco A, Fioravanti R, Chimenti P, Granese A, Carradori S, Rivanera D, Lilli D, Zicari A, Distinto S. Synthesis and in vitro activity of 2-thiazolylhydrazone derivatives compared with the activity of clotrimazole against clinical isolates of Candida spp. Bioorganic Medicinal chemistry letters 2007, 17, 4635–4640.

[19]. Vicini P, Geronikaki A, Anastasia K, Incerti M, Zani F. Synthesis and antimicrobial activity of novel 2-thiazolyl imino-5-arylidene-4-thiazolidinones. Bioorganic Medicinal chemistr 2006, 14, 3859-3864.
[20]. Hao Shao. Substituted 4-(thiazol-5-yl)-2-(phyenylamino)pyrimidines are highly active CDK9 inhibitors: synthesis, X-ray crystal Structures, Strucure-Activity Relationship, and Anticancer activity. Journal of Medicinal Chemistry. 2013, 56, 640-659.
[21]. Ramla MM, Omar MA, El-Khamry AMM, El-Diwan HI. Synthesis and Antitumor Activity of 1-Substituted-2-methyl-5- nitrobenzimidazoles. Bioorganic and Medicinal Chemistry. 2006, 14, 7324-7332.
[22]. Popsavin M, Spaic S, Svircev M, Kojic V, Bogdanovic G, Popsavin V. Synthesis and Antitubercular Activity of New Tiazofurin Analogues Bearing a 2, 3-Anhydrofunctionality in the Furanose Ring. Bioorganic Medicinal chemistry letters. 2007, 17, 4123-4127
[23]. Gulsory E, Guzeldemirci NU. Synthesis and primary cytotoxicity evaluation of new imidazo [2,1-b] thiazole derivatives. European Journal of Medicinal Chemistry. 2007, 42, 320-326.
[24]. Mark Cushman. Design Synthesis and Biological Evaluation of thiazole targating Flavivirus Envelope protin. Journal of Medicinal Chemistry. 2011, 54, 1704-1714.
[25]. Rawal. R.K.. Design and synthesis of 2-(2,6-dibromophenyl)-3-heteroaryl-1as anti-HIV agent. European Journal of Medicinal Chemistry. 2008, 43,2800-2806
[26]. Kumar A, Rajput CS, Bhati SK. Synthesis of 3-[4'-(p-chlorophenyl) thiazol-2'-yl]-2-[(substituted azetidinone/thiazolidinone)- aminomethyl]-6-bromoquinazolin-4-ones. Bioorganic and Medicinal Chemistry. 2007, 15, 3089-3096.
[27]. Holla BS, Malini KV, Rao BS, Sarojini BK, Kumari NS. Synthesis of some new 2, 4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agent. European Journal of Medicinal Chemistry. 2003, 38, 313-318.
[28]. Kalkhambkar RG, Kulkarni GM, Shivkumar H, Rao NR. Synthesis of novel triheterocyclic thiazoles as anti-inflammatory and analgesic agents. European Journal of Medicinal Chemistry. 2007, 42,1272-1276.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Study of Removal of Heavy Metal Copper (II) from Aqueous Solutions by Punica Granatum Seed Powder by Adsorption

Shobha Borhade
Department of Drug Chemistry, S.M.B.S.T.College, Sangamner, Ahmedabad, India

ABSTRACT

Heavy metals contribute to a variety of adverse health environmental effects due to their acute and chronic exposure through air, water and food chain. Heavy metal toxicity can result indamaged or reduced mental and central nervous function, lower energy levels,and damage to blood composition, lungs, kidneys, liver, and other vital organs.Long-term exposure may result in slowly progressing physical, muscular, andneurological degenerative processes that mimic Alzheimer's disease. Copper is highly toxic due to its non biogegradable and carcinogenic. Punica granatum seed powder use as a natural adsorbent for effective removal of copper (II). The main parameters influence the adsorption of copper (II) as percentage recovery, initial metal concentration, effect of adsorbent dosage, metal ion concentration, effect of time and effect of pH was studied in batch process.The adsorption study were carried out isothermally at different temperatures. Freudlich isotherm and Langmuir isotherm were used to describe the equilibrium data and the result were discussed in details.The kinetic data well described by the pseudo first order kinetic model. The thermodynamic parameters such as standard free energy change, entropy change and enthalpy change were studied for Punica granatum seed powder. This values showed that the adsorption of copper (II) ion on Punica granatum seed powder was a spontaneous and endothermic process. The removal of copper (II) ion from waste water and the techniques will be applicable at low cost.

Keywords: Adsorption,.Punica Granatum Seed , Langmuir Isotherm, Freundlich Isotherm, Kinetic, Thermodynamics Data.

I. INTRODUCTION

Environmental pollution causes due to the heavy metals. Heavy metals are major toxic pollutants with severe health effects on humans. They are released into the environment from a variety of industrial activities. Cadmium, lead, zinc, chromium and copper are the most toxic metals of widespread use in industries such as tanning, electroplating, electronic equipment manufacturing and chemical processing plants. Heavy metals contribute to a variety of adverse health environmental effects due to their acute and chronic exposure through air, water and food chain The international community is beginning to recognize the adverse health effects of heavy metals [1] . Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Heavy metals are associated with myriad adverse health effects, includingallergic reactions (e.g., beryllium, chromium), neurotoxicity (e.g., lead), nephrotoxicity (e.g., mercuric chloride, cadmium chloride), and cancer (e.g.arsenic, hexavalent chromium). Humans are often exposed to heavy metals in various ways mainly through the inhalation of metals in the workplace or polluted neighborhoods, or through the ingestion of food (particularly seafood) that contains high levels of heavy metals or paint chips that contain lead [2]. It is therefore essential to remove heavy metals from industrial waste water and drinking water. Conventional treatment methods of metal removal are often limited by their cost and ineffectiveness at low concentrations. Adsorption has emerged as promising technique for metal removal. It is one of the most popular purification methods due to its high selectivity, low cost, high efficiency and easy post-treatment after adsorption. Adsorption can be a potential alternative to traditional treatmentprocesses of metal ions removal [3-

5].The process can occur at an interface between any two phases, such as, liquid liquid, gas-liquid, or liquid-solid interfaces [6]. Recently, a series of low-cost adsorbents derived fromagricultural waste or natural materials [7-8] have been investigated for toxic substances like fluoride removalfrom aqueous solutions, such as apple and orange juicing residue, waste tea, peanut hull, rice straw, and coconut shell. Adsorption can be a potential alternative to traditional treatmentprocesses of metal ions removal [6-8] . The phenomenon of adsorption has been described in a wide range of non-living biomass like potato peel waste [9], untreated Cocos Nucifara [10] , orange peel [11], crab shell [12] ,untreated coffee grounds [13] , as well as of living biomass like, microbialcell [14], moss [15], yeast [16] , fungi [17-18] , algae [19-20]. Adsorption has been proved to be an excellent way to treat industrial waste effluents, offering significant advantages like the low-cost, availability, profitability, easy of operation and efficiency [21-22].There is a large volume of literature relating to the performance of different biosorbentsfor the removal of variety of heavy metals [23-26].

Copper is highly toxic because it is non biodegradable and carcinogenic. The effect of copper, liver disease, renal dysfunction, fibromyalgia symptoms, muscle and joint pains, depression, chronic fatigue symptoms, irritability, tumor, anemia, learning disabilities and behavioral disorders, stuttering, insomnia, niacin deficiency, leukemia, high blood pressure [27].

The objective of this work is to study the adsorption behavior of respect to copper ion. The batch method was employed parameters such as percentage recovery, initial metal ion concentration, effect of addition of dose of adsorbent, effect of time, effect of pH, effect of temperature, Freundlich and Langmuir adsorption isotherm, Kinetic study and also thermodynamic were studied.

II. MATERIALS AND METHODS

Materials

Adsorbent

The *Punica granatum* seed common name, Pomegranate, was purchased from market. Then the material was washed with doubly distilled water to remove the free acid and then dried at 200 K for 12 hours. The resulting product was cooled to room temperature and grind into fine powder and sieved to the desired particle size. Finally, the product was stored in air tied bottle for use as a adsorbent.

Preparation of copper (II)

Copper (II) prepared by dissolving copper sulphate in double distilled water. The chemicals used were of analytical grade and used without further purifications. The solutions were prepared in distilled water. The prepared solutions were standardized as per literature.

Absorption Study (Batch Process)

The dried powder of *Punica granatum* seed powder of 1.0 gm was taken in stoppered bottle. The Copper (II) with initial concentration of 10 mg/dm^3, 20 mg/dm^3, 30 mg/dm^3, 40 mg/dm^3, 50 mg/dm^3. The mixture were well stirred on a shaker at 100 rpm at the temperature 298 K,303 K, 308 K and 313 K for 20, 40, 60, 80, 100 & 120 minutes until the equilibrium condition were reached. The content was filtered. The adsorbate and adsorbent were separated by filtration. The filtrate in the aqueous solution after adsorption was measured by using pH values of Copper (II) solution were determined by using pH 5 using pH meter. The percentage of adsorption was determined from initial and equilibrium concentration respectively.

The percentage of Copper (II) removal was calculated as

$$\text{The \% of Metal ion removal} = \frac{Ci-Cf}{Cf} \times 100$$

C_i & C_f= Concentration of metal ion before and after the treatment.

The adsorption isotherm studies and kinetic studies were also carried out and the calculation were performed to get the appropriate result as well as thermodynamic parameters were calculated.

III. RESULTS AND DISCUSSION

Effect of Adsorbent Dose

The effect of adsorbent on copper (II) removal was studied by batch adsorption process. The percentage of removal of copper (II) reaches about 86-87 % each. The dose required is nearly about 160 mg/ 25 ml for the initial concentration of 25 mg/L at pH 4.

Effect of pH

Effect of pH solution is very important in adsorption process of metal ion .pH of solution affect on the surface of adsorbent, solubility of metal and also the speciation of metal ion. Effect of removal of copper (II) ion using *Punica granatum* seed powder as an adsorbent. With increasing pH from 2 to 8 the percentage of copper (II) also increases.

Effect of Temperature

Temperature is very important factor for adsorption. Higher temperature increases the rate of the adsorbate and decrease in the viscosity of the solution. Change in the temperature changes the equilibrium capacity of the adsorbent for the particular adsorbate. A series of experiments were conducted at 298 K,303 K, 308 K , 313 K and 335 K to study the effect of temperature on the adsorbate time rate for 20, 40, 60, 80, 100 & 120 minutes. **Figure 1.**

Freundlich Adsorption Isotherm

Freundlich plot for the adsorption of copper (II) ion with *Punica granatum* seed powder shows that the values of adsorption intensity 1/n is less than 1. Indicates the applicability of Freundlich adsorption **Table 2.**

Langmuir Adsorption Isotherm

The value of Q_0 of Langmuir adsorption isotherm found to be comparable with commercial activated carbon. Value of b lies between 0 to 1 it indicate that the adsorption is favourable. It indicate that the applicability of Langmuir adsorption isotherm **Table 3.**

Adsorption Kinetics

Adsorption rate of copper (II) ion on *Punica granatum* seed powder was studied by first order kinetic rate equation. It is found that the initial concentration of copper (II) ion increases rate constant decreases it indicate that the adsorption does not follow the first order kinetics **Table 4.**

Thermodynamics Parameters

Adsorption depends on temperature. Increasing temperature mass of copper (II) ion per unit mass was increased. Thermodynamics parameters like change in Gibb's free energy, change in enthalpy and change in entropy was calculated. The change in enthalpy indicate the process is endothermic nature **Table 5.**

Table1. Summary of % Recovery of Adsorbent Capacity of adsorbent
Initial concentration 10 mg/LAdsorbent dose 1 mg/L

Sr.No.	Adsorbent	Heavy Metal	Final Conc (mg/L)	% Recovery	Q (mg/L)
1	*Punica granatum* seed powder(PGSP)	Copper (II)	57.48	87.04 %	26.43

Table 2. Freundlich Adsorption Isotherm of Copper (II) on PGSP

		Concentration	Freundlich Constant	
			K	1/n
1	Copper (II)	10 mg/L	2.9401	1.001
		20 mg/L	4.9265	1.632
		30 mg/L	7.3527	2.115
		40 mg/L	8.2573	3.920
		50 mg/L	9.2104	4.026

Table 3. Langmuir Adsorption Isotherm of Cu (II) on PGSP

Sr.No.	Heavy Metal	Concentration	Langmuir Constant	
			Q_0	B
1	Copper (II)	10 mg/L	42.18	0.041
		20 mg/L	52.89	0.059
		30 mg/L	67.23	0.072
		40 mg/L	71.25	0.091
		50 mg/L	74.09	0.099

Table 4. Adsorption Kinetics of Copper (II) on PGSP

Sr.No.	Heavy Metals	Concentration	First order rate constant (K_1)
1	Copper (II)	10 mg/L	4.382×10^{-3}
		20 mg/L	5.032×10^{-3}
		30 mg/L	6.193×10^{-3}
		40 mg/L	7.374×10^{-3}
		50 mg/L	8.215×10^{-3}

Table 5. Thermodynamic parameters for the adsorption of Copper (II) on PGSP

Sr.No.	Heavy Metals	T (^{0}K)	Change in Gibb's free energy (KJ/mole)	Change in enthalpy (KJ/mole)	Change inentropy (KJ/mole)	R^2
1	Copper (II)	298 K	-32.84	55.31	0.4892	0.0945
		303 K	-41.03			
		308 K	-50.11			
		313 K	-54.28			
		335 K	-67.36			

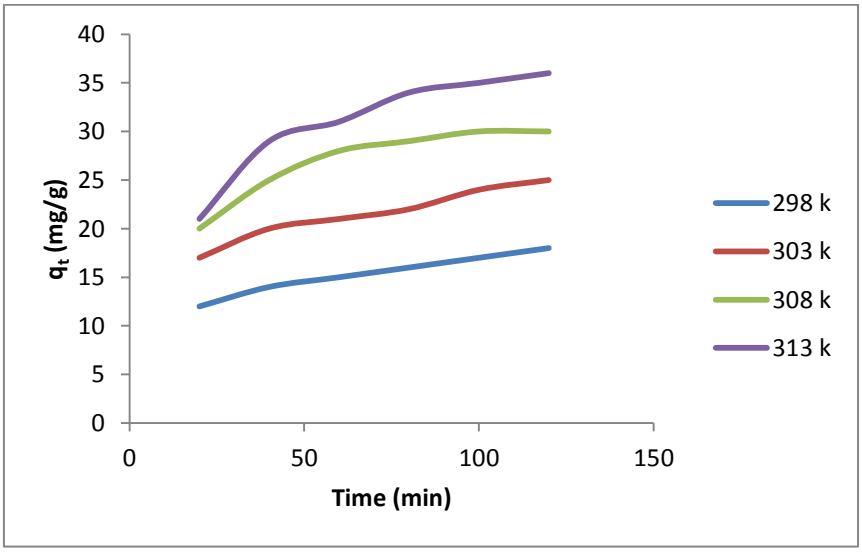


Figure 1. Effect of temperature on adsorption of copper (II) on PGSP

IV. CONCLUSION

Adsorption process is rapid at the starting and a becomes slow at the standard stage. It dependent on initial concentration of adsorbate and also time for adsorption. This adsorption is good agreement with Freundlich adsorption isotherm and also for Langmuir adsorption isotherm Adsorption process is good at pH 5. The cost of adsorbent is very low & is easily available

V. ACKNOWLEDGMENT

The author are grateful to the Principal of S.M.B.S.T.College, Sangamner, Ahmednagar.

VI. REFERENCES

[1]. Jiaping P.C.; CRC Press print ISBN : 2012,978,1,4398-1667.
[2]. Jarup L.; British Medical Bulletin, 2003, 68,167-182.
[3]. Ayhan.D ;Journal of Hazardous Materials,2008, 157, 220-229.
[4]. Mckay.G,: Ho.Y.S,: Wase.D.A.;Forster.C.F.; Adsorption Science and Technology, 2000,
[5]. 18(7), 637-650.
[6]. Mohsen A.; Hashem Y.S.; Journal of Physical Sciences, 2007, 2 (7), 178-184.
[7]. BarakatM.A.; Arabian Journal Chemistry,2011, 4, 361-377.
[8]. Dhakal R.P.; Ghimire K.N.; Inoue K.; Hydrometallurgy,2005,79, 182-190.
[9]. DemirbasA;.Journal of Hazardous Materials,2008,157, 220-229.
[10]. Mohammed A;,Abdullah P.G.; Devi P.A.; Journal of Chemical Engineering research, 2009,1 51-62.
[11]. Prasad A; Satya S.P.; International Journalof Chemical Engineering research, 2010, 2, 253-258.
[12]. Ferda G.; SelenS.D.; African Journal of Biotechnology, 2012,11,1250-1258.
[13]. Vijayaraghavan.K.; PalaniveluK.; VelanM.; Journal of Hazardous Materials,2005, B119, 251-254.
[14]. AzouaouN,;SadaouiZ,; DjaafriA.; MokaddemH.; Journal of Hazardous Materials, 2010,184, 26-134.
[15]. Gopal M,;Pakshirajan S.; SwaminathanT.; Applied Biochemistry and Biotechnology, 2002,102,227-236.
[16]. Lee C.K.; Low K.S.; Journal of Environmental Technology, 1989, 10, 395-404.
[17]. Can C.; Jianlong W.;Journal of HazardousMaterials, 2007, 151, 65-70.
[18]. Sudha B.R., Emilia A., Bioresourse Technology,2003,87,17-26.
[19]. DumitruB.; Laura B.; Journal of BioresourcTechnology,2002,103, 489-493.
[20]. Gupta V.K.; RastogiA.; Journal ofHazardous Materials, 2007,152, 407-414.
[21]. Mohammad M.M.;ParisaR,; AtefehA.; Ali R.K.; Journal of Hazardous Materials,2011, 185, 401-407.
[22]. FerdaG.; SelenS.D.; African Journal of Biotechnology, 2012, 11,1250-1258.
[23]. DemirbasA.; Journal of Hazardous Materials, 2008,157, 220-229.
[24]. Larous S.; MeniaiA.H.; LehocineM.B.;Desalination, 2005, 185,483-490.
[25]. UysalM.; ArI.; Journal of Hazardous Materials, 2007, 149, 482-491.
[26]. Qi B.C.; Aldrich C.; Journal of Bioresource Technology, 2008, 99, 5595-5601.
[27]. AtalayE,;GodeF.; Sharma Y.C.; Practice periodical of hazardous toxic and radioactive waste management,2010,14, 132-138.
[28]. Ogunsuyi H.O.; Ipinmoroti K.O.; Amoo I.A.; Ajayi O.O.; JournalTechnoscience, 2001,5, 75-83.
[29]. BanumS.J.; Introduction to Organic and Biological Chemistry, 3rd ed., Macmillan Publishing Co., New York, NY. 1982,541

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Bioactivity study of Thiazolyl Chromones against Pseudomonas Flurescence

S D Mhaske[1], Salve S P[3], Sadavarte P S[3], B K Karale[2] , S J Takate[3*]

[1]Department of Chemistry, Dada Patil Rajale Arts & Science College, Adinathnagar, Ahmedabad, Gujarat, India
[2]Department of Chemistry, Radhabai Kale Mahila Mahavidyalya, Ahmednagar, Gujarat, India
[3]Department of Chemistry, New Arts, Commerce and Science College, Ahmednagar, Gujarat, India

ABSTRACT

Heterocyclic compounds are widely known for their bioactivities. Owing to potential bioactivities of thiazole and chromones, these are attractive lead compounds for drug development. In present investigation thiazolyl chromones were studied for their bioactivity against Pseudomonas flurescence using ampicillin as reference drug.

Keywords : Pseudomonas Flurescence, Thiazolyl Chromones, Chromones, Heterocycles

I. INTRODUCTION

Microbial infections are one of the major reasons for human diseases. Numerous microorganisms have been recognized as human pathogens. For treatment of these infectious diseases, there is an increasing need of potential medicinal agents.

As a part of drug development various heterocycles have been extensively studied for their therapeutic properties. Especially sulphur, oxygen and nitrogen containing heterocycles are dominating the field of synthetic as well as medicinal chemistry.

Owing to versatile spectrum of biological activities, thiazole has been proved as an important lead compound for drug development. Thiazole possesses broad spectrum of bioactivities including antimicrobial, antifungal, anticancer and antitubercular[1-6].

Chromones belong to group of natural compounds widely known for its potential bioactivities[7-10]. As a part of our previous studies we have synthesized thiazolyl chromones via Baker-Venkatraman transformation and studied them for their antimicrobial activities[11]. In continuation to that in present study some of the thiazolyl chromones were studied against *Pseudomonas flurescence*.

II. RESULTS AND DISCUSSION

In present study chromones **5b-d** and **5f** were evaluated against *Pseudomonas flurescence*. The results showed that all the compounds **5b-d** are weakly active against test organism while compound **5f** is moderately active at higher concentration against test organism.

Scheme:

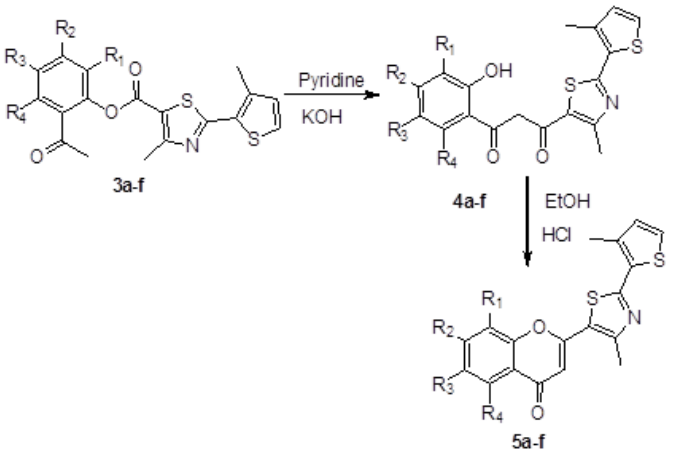


Table 1. Characterization data

Compd	R_1	R_2	R_3	R_4	M.P. (°C)	Yield (%)
5b	Cl	H	Cl	H	182	77
5c	H	H	Br	H	242	72
5d	H	CH_3	Cl	H	210	75
5e	CH_3	H	CH_3	H	216-218	70
5f	H	H	Cl	H	182-184	74

Biological Activity:

Table 2. Antimicrobial Activity (% Inhibition)

Compd	100 µg/ml	30 µg/ml	10 µg/ml
5b	42.12	16.60	9.86
5c	28.63	20.18	10.11
5d	72.11	31.87	6.46
5e	NT	NT	NT
5f	83.17	66.50	40.53
Ampicillin	97.0	95.2	92.2

Experimental:

6,8-Dichloro-2-[4-methyl-2-(3-methylthiophen-2-yl)-1,3-thiazol-5-yl]-4*H*-chromen-4-one, **5b**

Compound **4b** was dissolved in 10 mL ethanol and to this 1mL HCl was added. Reaction mixture was heated under reflux for 1 hr. After completion of heating, the reaction mixture was cooled and poured over crushed ice. The resulting product was separated by filtration and purified by recrystallization from ethanol to yield **5b**. Compounds **5c-f** were obtained using the same procedure.

5b: IR (KBr): 3076, 3026, 1654, 1598, 1560 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.55 (s, 3H), 2.81 (s, 3H), 6.69 (s, 1H), 7.08 (d, 1H, J = 5.0 Hz), 7.64 (d, 1H, J = 5.0 Hz), 7.95 (d, 1H, J = 2.52 Hz), 8.06 (d, 1H, J = 2.52 Hz); MS: *m/z*: (M+1) 408.

5c: IR (KBr): 3086, 3062, 1595, 1232, 1157, 1001, 839, 773, 721 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.73 (s, 3H), 2.95 (s, 3H), 7.19 (s, 1H), 7.27 (d, 1H, J = 5 Hz), 7.65 (d, 1H, J = 8.96 Hz), 7.99 (d, 1H, J = 4.92 Hz), 8.08 (dd, 1H, J = 8.9 and 2.24 Hz), 8.47 (d, 1H, J = 2.24 Hz).

5d: IR (KBr): 3113, 3047, 1593, 1546, 1236, 1150, 840, 792 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H), 2.55 (s, 3H), 2.75 (s, 3H), 6.58 (s, 1H),7.08 (d, 1H, J = 5 Hz),7.48 (s, 1H), 7.64 (d, 1H, J = 5 Hz), 7.68 (s, 1H

5e: IR (KBr): 3061, 2968, 1635, 1614, 1265, 825cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H), 2.48 (s, 3H), 2.52(s, 3H), 2.75 (s, 3H), 6.72 (s, 1H), 7.02 (d, 1H, J = 5.0 Hz), 7.25(bs, 1H), 7.62 (d, 1H, J = 5 Hz), 7.8 (s, 1H).

5f: IR (KBr): 3074, 3007, 1602, 1566, 1255, 1238, 1157,1035, 1006, 837, 767, 725 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.55 (s, 3H), 2.75 (s, 3H), 6.64 (s, 1H), 7.08 (d, 1H, J = 5.04 Hz), 7.49 (d, 1H, J = 8.9 Hz), 7.64 (d, 1H, J = 5.03 Hz), 7.72 (dd, 1H, J = 2.6 and 8.9 Hz), 7.80 (d, 1H, J = 2.59 Hz).

III. REFERENCES

[1]. Mhaske P C, Shelke S H, Raundal H N & Jadhav R P, J Korean Chem Soc, 58(1), 2014, 62.

[2]. Ramirez J, Svetaz L, Quiroga J, Abonia R, Raimondi M, Zacchino S & Insuasty B, Eur J Med Chem, 92, 2015, 866.

[3]. Rouf A & Tanyeli C, Eur J Med Chem, 2014, 1.

[4]. Khloya P, Kumar S, Kaushik P, Surain P, Kaushik D & Sharma P K, Bioorg Med Chem Lett, 25(6), 2015, 1177.

[5]. Patil A, Jadhav R, Raundal H, Patil S, Bobade V, Sharma L & Badgujar R, Indian J Chem, 54(B), 2015, 918.

[6]. He H, Wang X, Shi L, Yin W, Yang Z, He H & Liang Y, Bioorg Med Chem Lett, 26(14), 2016, 3263.

[7]. Gaspar A, Matos M J, Garrido J, Uriarte E & Borges F, Chem Rev, 114, 2014, 4960.

[8]. Keri R S, Budagumpi S, Pai R K & Balakrishana R G, Eur J Med Chem, 78, 2014, 340.

[9]. Singh P, Anand A & Kumar V, Eur J Med Chem, 85, 2014, 758.

[10]. 1Gomes A, Freitas M, Fernandes E & Lima J L F C, Mini Rev Med Chem, 10, 2010, 1.

[11]. Karale B K, Takate S J, Salve S P, Zaware B H & Jadhav S S, Indian J Chem, 54B, 2015, 798-804.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Scope of Using Carbon Nanobeads Prepared from Castor Seeds as Biosensors

Swapna Gijare[1], Suma Jebin[1], Umesh Mhapankar[2]

[1]Assistant Professor, Department of Humanities and Basic Sciences, Fr. C. Rodrigues Institute of Technology, Vashi, Navi Mumbai, India
[2]Assistant Professor, Department of Industrial Electronics, Fr. Agnel Polytechnic, Vashi, Navi Mumbai, India

ABSTRACT

This paper presents the possibility of development of biosensors from Carbon Nano beads (CNBs) prepared from castor seeds as a precursor by CVD method. The semiconducting nature of prepared nanobeads is confirmed by photoluminescence (PL) technique. The required study for determining the hormone content of the urine sample in the presence of CNBs as a sensing material has been carried out using a capacitance and resistor circuit. The variations found in the voltage across the capacitor give the scope for developing the biosensors from CNBs. This can be further calibrated and modified to measure the percentage of concentration of hormones by a non-invasive and simple method.

Keywords: Carbon Nano beads, photoluminescence technique, band gap, Biosensors.

I. INTRODUCTION

Carbon nanomaterials are getting popularized because of their special properties and possibility of their unique applications. Diverse nature of carbon bonding in organic molecules allows carbon to form some of the interesting nanostructures, particularly carbon nanotubes.. It's so called extraordinary properties like manifold stronger than steel, harder than diamond, electrical conductivity higher than copper etc. are turning it into a material of great demand in the near future. So there is a need to develop the process of synthesis using precursors which are not derived from sources like petroleum products so that in the event of the sources getting depleted the production of carbon nanomaterials is not adversely affected. Therefore plant based precursors are preferred over petroleum based precursors. The idea is, these precursors could be harvested as and when needed and even in the event of scarcity of petroleum derived precursors, the production of CNMs would not be affected.Therfore, seeds of Castor plant (*Ricinus communis*) was used as a precursor. Carbon nanobeads were prepared by Chemical vapour deposition method using the above mentioned precursor and Iron **(**Fe) as a nanocatalyst at a pyrolysis temperature of 900^0C with Argon as a carrier gas.

The term photoluminescence describes any process in which light is absorbed, generating an excited state, and then light of lower energy is re-emitted upon relaxation to a ground state. Photoluminescence absorption spectra of synthesized CNBs have been obtained in order to study their semiconducting nature. The absorption peak was found to occur at a particular wavelength (at around 600nm.). The analysis of this peak and the calculation of band gap show that CNBs prepared by above mentioned method are semiconductor in nature.

Endocrine system consists of various glands located throughout the body, hormones produced by the glands, receptors in various organs and tissues that recognize and respond to hormones. Nanomaterials are used in designing novel sensing systems and enhancing their performance. Use of nanotechnology in sensors and sensor hardware is resulted in development of a number of miniaturized, ultrasensitive and inexpensive methods. The surface chemistry and electronic properties make the use of carbon nanomaterials ideal for chemical and biochemical sensing. Carbon nanomaterials have the ability to enhance the binding of biomolecules and increase the electrocatalytic activities. With carbon Nanomaterials, the detection of several analytes is possible at low applied potential. No need to use electronic mediators and hence interferences are reduced.

After confirming the semiconductor nature of CNBs, a circuit is designed by using capacitor, resistor and 9 volt

battery for determining the hormone content of the urine sample in the presence of CNBs which act as a sensing device.The variation in the voltage with change in the concentration of hormone gives the scope for the development of biosensors.

II. MATERIALS AND METHODS

2.1. Preparationof Carbon Nano beads

2.1.1. Precursor: Seeds of Castor (*Ricinus communis*) was collected from the wild plants growing in Navi Mumbai (Vashi, Nerul). These seeds are rich in oil and carbohydrates, thus a good source of hydrocarbons. Oil and carbohydrates are stored in seeds in storage cell having a specific anatomy of their own. Inherent morphology of plant materials have been found to give rise to very complicated porous carbon nanomaterials which would be extremely difficult to synthesize in the laboratory and might be having very useful applications (Sharon & Sharon 2013). Keeping these facts in mind castor seed was selected as the precursor. An initial elemental analysis of seeds was done to get an idea about the Carbon content. Castor seeds were found to contain 55.728%carbon.Castor seeds contain 12 – 15% water, to remove the water from seeds they were kept in a hot air oven at 100^0 C for one hour, and then crushed to form powder.

2.1.2. Catalyst Preparation: Iron **(**Fe) was used as catalyst for Carbon Nano beads (CNBs) synthesis because of their high solubility in carbon at high temperature and high carbon diffusion rate. Nano sized catalyst can be prepared using nitrates of these metals, because oxides of nitrogen escape leaving behind metal oxides. Known weight of nitrate salt of iron metal was mixed with urea in the ratio 1:5 and was ground thoroughly till it became liquid while grinding. Liquid mixture was heated in the muffle furnace by gradually increasing the temperature from 100^0C to 600^0C until the entire liquid was charred and turned into metal oxide. Reduction of oxide was carried out by keeping the oxide in a quartz boat at 900^0C for 1hr in a split furnace, flushed with Argon (Ar) gas. Then Hydrogen gas was passed to convert entire oxide into pure metal.

2.1.3. CVD set-up used for pyrolysis: A Horizontal CVD furnace was used for synthesis of CNMs from castor seeds. One gram dried crushed seeds mixed with catalyst to be tested were kept in quartz boat and inserted in quartz tube which was then kept in CVD furnace. Ar gas was flushed inside for the quartz tube for 10 minutes to create inert atmosphere. After that the flow of Ar gas was maintained at 50 cc/minute throughout the experiment. Furnace was then heated to 900^0 C and kept at this temperature for 2 hours. The furnace was then cooled to room temperature and the carbon was collected from the quartz boat.

2.1.4. Purification of CNM: Pyrolysed product, along with carbon also contains remnants of metal catalyst, some amorphous carbon, possibly same as and other impurities. To get pure CNM, the product obtained from pyrolysis was suspended in 1N HCl and kept on stirrer overnight. Then it was filtered through Buckner funnel with the help of Whatman filter paper and finally dried in a muffle furnace at 60^oC for 30 min.Purified CNMs were weighed.

2.1.5. Characterization of CNM: The morphological observations of as-synthesized and purified CNM were carried out by Scanning Electronic Microscope (SEM). SEM was conducted using a Hitachi (S-4700) SEM by placing the as-prepared samples on conductive carbon tape. SEM results showed that CNM prepared by above method is chain of Carbon Nano beads. The SEM images of CNB are shown in Fig.1 and Fig.2.

2.2. Photoluminescence study of Synthesized CNBs:-

The detection of band gap of nanobeads prepared by CVD method is carried out by photoluminescence (PL) technique. Photoluminescence is a highly sensitive spectroscopic method to investigate optical transition energies of semiconductors.CNBs are luminescent The ISS PC-1Spectrofluorometer has been used for the photoluminescence study of CNBs, since this Spectrofluorometer covers a large range of excitation and emission energies.

The spectra of three samples prepared in three experiments keeping all the parameters constant have been studied. All the three samples showed the absorption peak at same wavelength at around 600nm.

The band gap energy of the CNBs was found to be 2 eVolts.It is calculated by using formula: - Band gap

Energy (E) = hc/λ. A Sample PL spectra are shown in Figure 3.

The analysis of these peaks and the calculation of band gap showed that CNBs prepared by above mentioned method are semiconductor in nature.

2.3. Experimental study of CNB as a biosensor:

Hormones are the substances produced by the body that have chemical effect on the other parts of the body. Women produce hormones like estradiol (estrogen), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and progesterone. Certain hormones rise at the various times of menstrual cycle and during pregnancy. Estrogen is the predominant female hormone.Estrodiol is the major form of estrogen produced in ovary. On the first day of Menstrual cycle, estrogen and progesterone levels are low which signal the pituitary gland to produce FSH.FSH begins the process of maturing a follicle. Follicle produces more estrogen to prepare uterus for preganancy.At ovulation, usually around 12^{th} to 14^{th} day ,increased estrogen level triggers a sharp rise in LH from pituitary gland causing release of egg from the follicle. Ruptured egg secretes progesterone and estrogen to continue to prepare uterus for pregnancy. If egg is not fertilized, estrogen and progesterone levels drop on 28^{th} day and menses begin.The Female hormone cycle showing the estrogen and progesterone level is shown in Figure 4.

In order to test the sensing ability of CNBs towards female hormones, urine sample of a healthy woman at different stages of menstrual cycle is used. Testing is carried out by designing a simple circuit containing capacitor, resistor and 9V battery which shown in Fig.5. The voltage across the capacitor with CNBs and without CNBs for various conditions of the subject has been measured and tabulated in Table.1. Observed variation of voltage in the presence of CNBs ensures the contribution of CNBs in sensing hormones in urine. Thorough analysis and calibration of this result in comparison with a normal menstrual cycle can be done in order to calculate the exact percentage of the hormone content in the urine.

III. RESULT AND DISCUSSION

From the above observations, the voltage in the absence of CNBs is same in all the cases whereas the voltage varies in the presence of CNBs and at the different stages of hormonal cycle. It is a well known fact that the female reproductive hormone levels are not constant throughout the menstrual cycle. Therefore, the observed voltage decreases with increase in the hormone content. This relation can be understood from Table.1.and Fig.4. This result gives the scope for developing biosensors from CNBs for measuring the quantity of female hormones by a non-invasive and a simple method. These voltage values can be further calibrated and modified so that it can be used for diagnosis of hormonal imbalance and related disorders.

IV. REFERENCES

[1]. Kumar M et al.in "Nano forms of Carbon and its application" Ed. by Maheshwar Sharon &Madhuri Sharon. Monad Nanotech Publisher, Mumbai, India, 2007

[2]. Sharon Maheshwar&Madhuri Sharon. Carbon Nanoforms –Application.McGraw Hill, USA, 2010.

[3]. Chales P Poole Jr., Frank J. Owens, Introduction to Nanotechnology, Willey India Edition (2012).DOI: 10.5339/connect.2013.45 (published online on 08 Dec 2013

[4]. Swapna Gijare et al, Advanced science letters vol.22.,No.4,(April 2016),pp.715-1092)

[5]. Ruquan Ye Andrew R.Barron, Photoluminescence and applications.

[6]. Mohammed Al Araimi et.al,'A dioxoborine cyanine dye as a photoluminescence for sensing carbon nanotubes',Beilstein Journal of Nanotechnology,pp1991-1999.

[7]. B.Hatting, Photoliminescence of single walled carbon nano tubes,(2009).

[8]. Achintya N Bezbaruah and Harjyotikalita Chapter 3. Sensors and Biosensors for endocrine disrupting chemicals: state-of-the-art and future trends.

[9]. M.A.Lopez-Gordo et al, Dry EEG Electrodes, sensors2014,ISSN 1424-8220, pp:12847-12870.

V. TABLES AND FIGURES

Table 1. Variation of voltage across the capacitor with and without CNB for various condition of the subject.

Sr.No	Materials	Voltage (v)	Condition of the subject
1	Without CNB	2.80	Menses
2	With thin layer of CNB	3.34	
3	With thick layer of CNB	4.9	
4	Without CNB	2.80	7^{th} to 9^{th} day of menstrual cycle
5	With thin layer of CNB	3.25	
6	With thick layer of CNB	4	
7	Without CNB	2.82	12^{th} to 14^{th} day of menstrual cycle
8	With thin layer of CNB	1.2	
9	With thick layer of CNB	1.7	

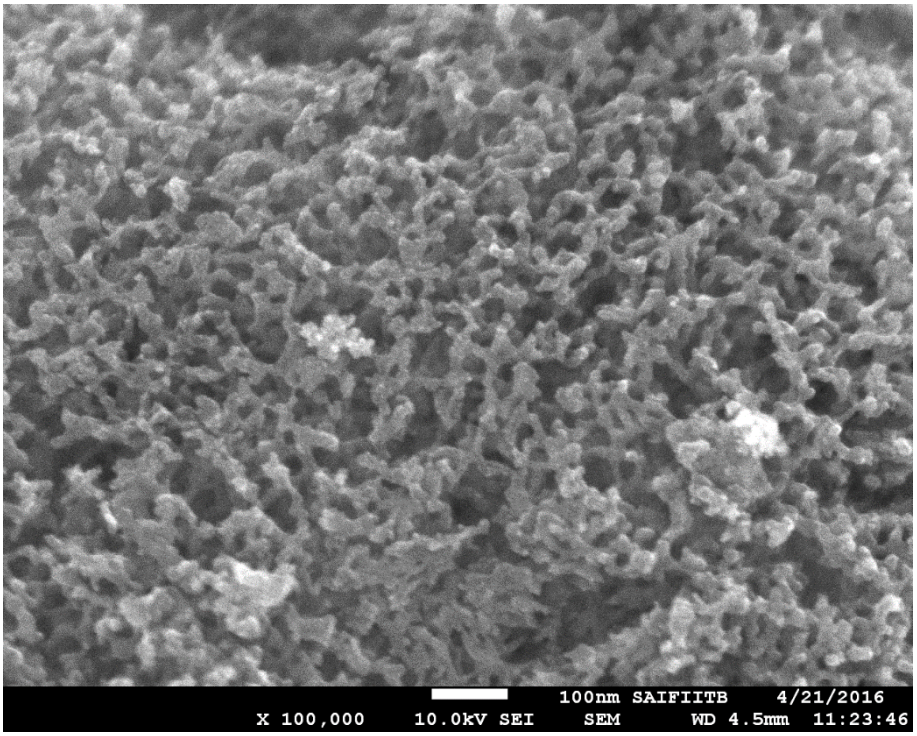


Figure 1. SEM image (1) of CNB

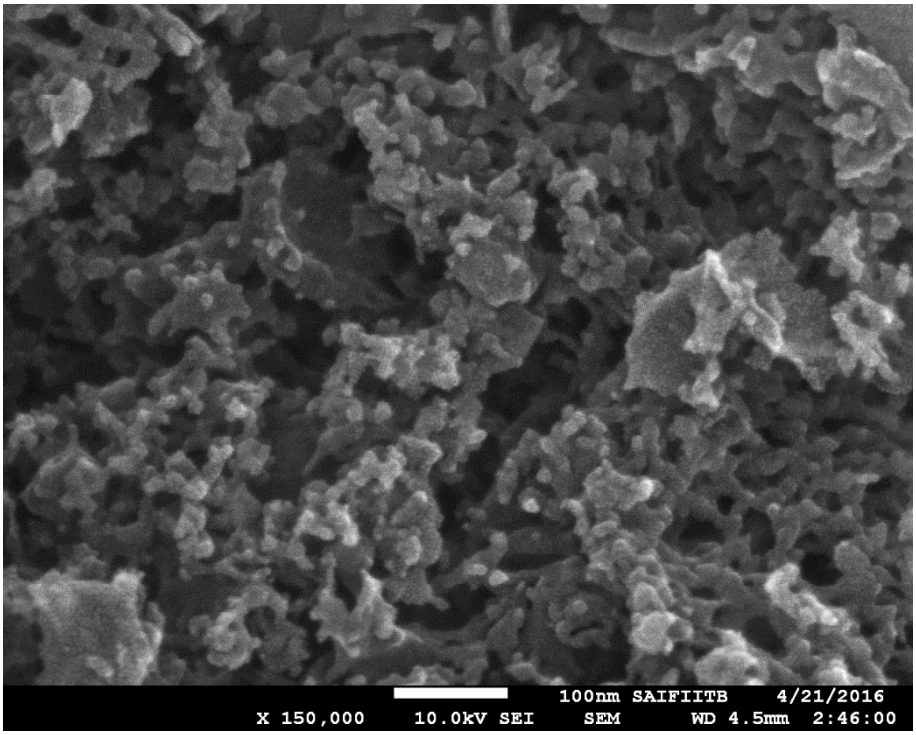


Figure 2. SEM image (2) of CNB

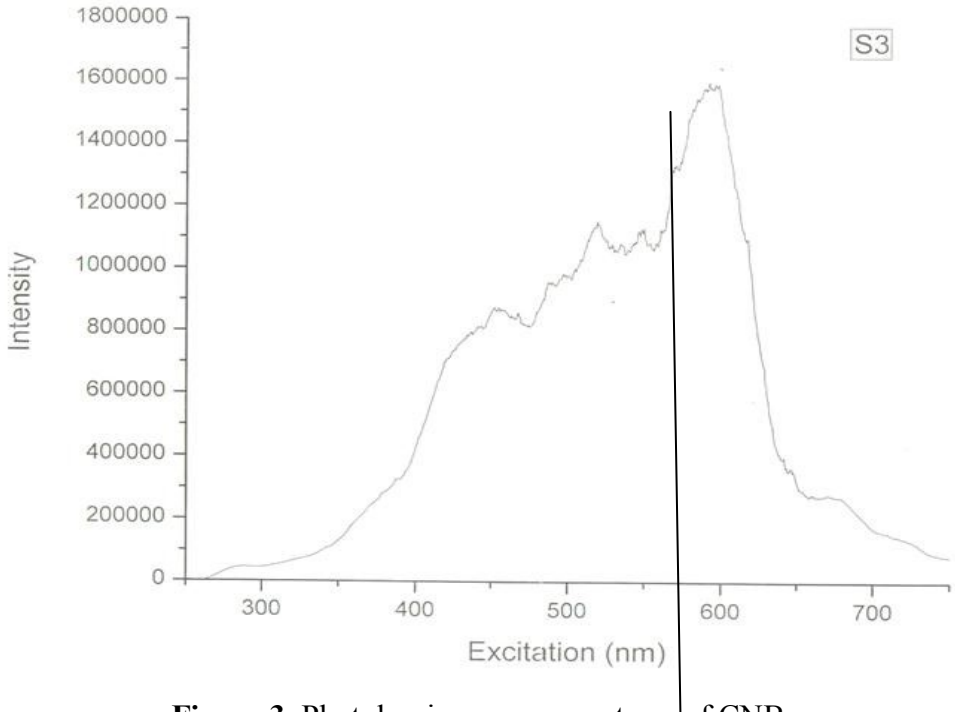


Figure 3. Photoluminescence spectrum of CNB

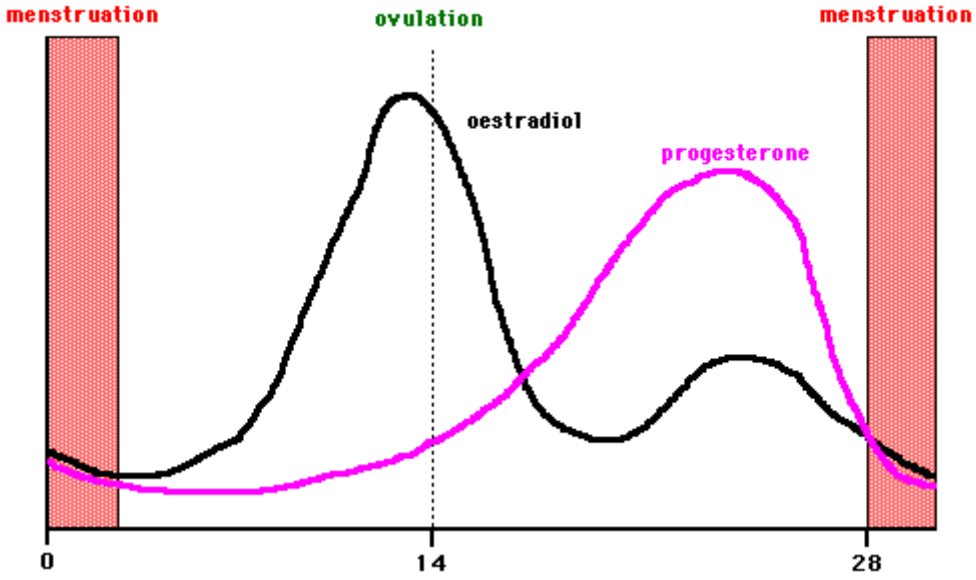


Figure 4. Estradiol and progesterone in the menstrual cycle.

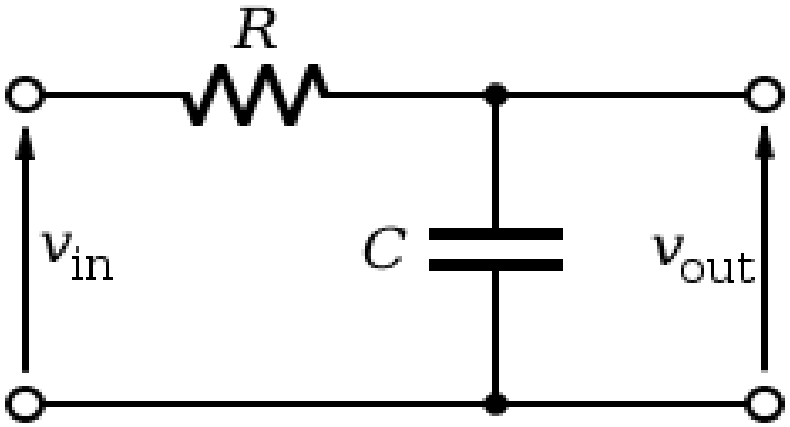


Figure 5. Circuit Diagram to test the sensing ability of CNBs towards female hormones

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Simple Procedure for the One Pot Synthesis of Ascorbic Acid As Efficiency And Recyclable Media

Vishal Gholap, Vijay Talekar, Mangesh Gholap, Anil Kanawade, Pradip Phapale
Email : talekarvijay16@gmail.com, vishalgholap123@gmail.com

ABSTRACT

A method for producing L-ascorbic acid which compromise forming substantially anhydrous slurry of 2-keto-l-gulonic acid, and reacting said slurry with a substantially hydrous acid catalyst to convert said 2-keto-l-gulonic acid to l-ascorbic acid. L-ascorbic acid is produced in a high yield and no efficiency by converting 2-keto-l-gulonic acid to L-ascorbic acid by acid catalyst as anhydrous condition.

Keywords : L-Ascorbic Acid, HCl, Isopropanol

I. INTRODUCTION

Ascorbic Acid is a naturally occurring organic compound. Ascorbic acid one form of Vitamin C. Vitamin C is an essential micronutrient with several important biological functions. It reduces the symptoms of Cold & Flu, accelerating the recovery process. Conventional method for organic synthesis usually needs reacting said slurry with sub surfactant anhydrous, Hydrogen Chloride gas as an acid catalyst. Which result in the excessive use HCl Gas generation in environmental pollution. In these new process no gas generation, and not environmental pollution. If want to preparation of ascorbic Acid from 2-keto-hexnoic acid using alkaline earth silicate catalyst. But in these process used acidic media. The process carried out in water, alcohol or in a variety of polar or moderately polar solvent or solvent mixture & proved for simple workup purification of ascorbic acid The new process were developed by considering important parameter and minimum by-products formation leading to maximum yield of the pure product with desired quality.

The invention relates to a process for the preparation of L-ascorbic acid, in which a melt of C3—C1O-alkyl Z-keto -L gulonate is lactonization under acidic conditions. In the past, a large number of process variants for the preparation of L-ascorbic acid have been published. Are view is found, inter alia, in Crawford et al., Adv. Carbohydrate Chem. 37, 79 (1980) and in Ullmann's Encyclopaedia of Industrial Chemistry, Vol. A27, 551—557 (1996).A number of processes for the preparation of ascorbic acid by 15 reaction of 2-keto-L-gulonic acid With an acid are known. Thus, US. Pat. No. 2,185,383 describes the reaction of 2-keto-L-gulonic acid with concentrated hydrochloric acid and acetic acid as a solvent.

US. Pat. No. 5,391,770 describes the esterification of 2-keto-L-gulonic acid with subsequent base-catalyzed lactonization of the esters formed to give salts of L-ascorbic acid and liberation of the ascorbic acid by the addition of a strong acid.

Japanese published patent specification 22113/75 describes the esterification of 2-keto-L-gulonic acid with butanol and the subsequent acid-catalyzed lactonization in benzene as a solvent. EP-A-0 671 405 discloses a process for the preparation of methyl or ethyl 2-keto-L-gulonate by esterification of 2-keto-L-gulonic acid with methanol or ethanol in the presence of an acidic ion exchanger.

The above mentioned embodiments of the acid-catalyzed, single-stage process variant exhibit serious Weaknesses. Thus, as a rule the use of an inert solvent is unavoidable in order to suppress the secondary reactions of the ascorbic acid with aqueous hydrochloric acid. At the same time, the 2-keto-L-gulonic acid is always present undissolved and the form of a suspension at the start and in the course of their action and reaction only takes place on the crystal surface. The addition of surface-active substances alters the course of the reaction only slightly. What is more, this auxiliary can only be removed from the crude product with difficulty

and means additional Working-up steps in order to obtain the desired purity of the L-ascorbic acid.

It was therefore the object to make available a process for the preparation of L-ascorbic acid which does not have the above mentioned disadvantages.

This object was achieved by a process for the preparation of L-ascorbic acid which comprises lactonizing a melt of C3—C1O-alkyl 2-keto-L-gulonate under acidic conditions. In a preferred embodiment, the process according to the invention further more comprises

a) esterifying 2-keto-L-gulonic acid or 2,3:4,6-di-Oisopropylidene-2-keto-L-gulonic acid in the presence of an acidic catalyst using a C3—C10-alcohol,
b) Distilling off the excess C3—C1O-alcohol together With the Water of reaction formed and
c) Then lactonizing the C3—C1O-alkyl 2-keto-L-gulonate formed in the form of an anhydrous melt under acidic conditions.

In the course of the process according to the invention,2-keto-L-gulonic acid or 2,3:4,6-di-O-isopropylidene-2 keto-L-gulonic acid is first reacted to give the alkyl ester in a single-stage esterification step in the presence of an acidic catalyst. The esterification is carried out in a temperature range from —10 to 160° C., preferably from 20 to 100° C., particularly preferably in a temperature range from 40 to 106° C.

As a rule, the monohydrate of 2-keto-L-gulonic acid is obtained an crystallization from Water or Water-containing, solvents. By centrifuging off the crystal magma, organic moist monohydrate is accessible. This can be employed directly in the subsequent esterification reaction as a centrifuge-moist product or dried under mild conditions .It is also possible to employ a concentrated aqueous solution of the 2-keto-L-gulonic acid directly in the esterification reaction. The excess solvent is removed before or during the esterification reaction, Ex. by extraction and phase separation or azeotropic distillation.

Advantageously, higher alkyl esters of saturated, branched or unbranched alkyl alcohols having a hydrocarbon number of greater than or equal to 3, preferably having an alkyl radical of 3 to 10 carbon atoms, are suitable for the esterification, such as, for example, n- propanol , isopropanol,1butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2 propanol, 1-pentanol, 2-pentanol, 3-pentanol, 1-hexanol,2-hexanol, 1-heptanol,2heptanol, 1-octanol, 2-octanol,3-octanol, 1-nonanol, 2-nonanol, 1-decanol, 2-decanol,4-decanol.Those alcohols in Which L-ascorbic acid is poorly soluble are preferably employed for the esterification. Those particularly preferably suitable are C4—C8 alcohols, selected from the group consisting of n-propanol, isopropanol,1-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol,1-pentanol, 1-hexanol and 1-octanol and 1-butanol and1-pentanol.

2-Keto-L-gulonic acid is preferably employed for the synthesis as a starting material. The acid monohydrate can be employed here either in crystalline form, for example as a dried or centrifuge-moist or as an anhydrous compound or as an aqueous solution, for example as a concentrated fermentation solution.

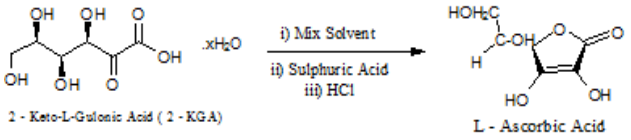
As a rule, the monohydrate of 2-keto-L-gulonic acid is obtained an crystallization from Water or Water-containing, organic solvents. By centrifuging off the crystal magma, moist monohydrate is accessible. This can be employed directly in the subsequent esterification reaction as a centrifuge-moist product or dried under mild conditions.

It is also possible to employ a concentrated aqueous solution of the 2-keto-L-gulonic acid directly in the esterification reaction. The excess solvent is removed before or during the esterification reaction, eg by extraction and phase separation or azeotropic distillation. This procedure is particularly suitable for a ketogulonic acid solution from a fermentative preparation process. After removal of the biomass by standard processes known per se, the fermentation solution, Which is usually colored, can preferably be employed directly Without further purification after liquid liquid extraction. The excess solvent is then removed, asdescribed above, before or during the esterification reaction, e.g. by phase separation or azeotropic distillation.

The drying or dehydration of the monohydrate of 2-ketoL-gulonic acid can advantageously be dispensed

With in the process according to the invention, as in the subsequent activation reaction according to the invention an azeotropic dehydration is carried out any Way.

SCHEME:



II. EXPERIMENTAL SCHEME

212g (1 mol) of hydrous 2-keto-L-gulonic acid Were suspended in 400 ml mix solvent n-butanol : toluene (37.5:62.5) and, after addition dehydrate reaction mass at 85 to88° C ,for quantity of water 18 ml .add 2 g of concentrated sulphuric acid, the mixture Was dehydrate to 410-435 mbar. After heating to 85° C. for 5-6 hr, for quantity of water 18 ml .

The viscous, brown coloured mass was cool up to 70-75 for 17 hr after addition of 22 ml conc. hydrochloric acid. After reaction completion dehydrated reaction mass at 70-75 at 460-475 mbar, for quantity of water 22 ml. Then cool reaction mass at room temp. stir well .

The precipitated L-ascorbic acid Was filtered off With suction, Washed With mix solvent n- butanol: toluene (37.5:62.5) and dried invacuo. 180 g (90%) of a pale gray crude product having a purity of 98.5% Were obtained. The crude product dissolved in Water. After clarifying filtration, a colourless solution was obtained from which it was possible to isolate L-ascorbic acid in a manner known from the literature. The residue (0.9%) consisted mainly of carbon. Purification of L- ascorbic acid by using n-butanol as solvent.

Reaction monitor by TLC (Acetonitrile : MeOH : Butanol : Acetic Acid) assay by HPLC(99%), SOR,

III. RESULT

The L-Ascorbic acid is a white crystalline powder, becoming freely soluble in water and sparingly soluble ethanol (95%). PH between 2.2 -2.5, specific optical rotation between + 20 – +21.5. The absorbance at 244 nm is about 0.56. Assay by titration 99.5% -100.5%.
IR (cm^{-1}): 3412 (s), 3317 (s), 3221 (s) OH , 3032 CH , 1753 C=O , 1668 C=C , 1500 CH, 1273 C-O-C, 1220 and 1197 C-C(=O)-O, 1026 C-O-H, 985 (s) C-H & O-H.

IV. DISCUTION

In previous patent describe the process preparation of ascorbic acid by dry HCl thus leading to charring of ascorbic acid. In previous patent describe the process preparation of ascorbic acid by dry HCl thus leading to poor yield and purity. Due to draw back associated with the process known in the literature for the preparation of ascorbic acid by using conc. HCl as catalyst. The present literatures have developed industrially advantages for the preparation of ascorbic acid of high purity and better yield. Which circumvent the drawback associated with process known in the prior art .After removal of desire quantity of water in first part dehydration do not heat more time at reflux. If we addition of Conc. HCl at 70-75°C, because the high temperature addition for Conc. HCl the charring the material. Give proper washing to Ascorbic acid by mixture of solvent dry under vacuum.

[1]. REFERENCES

[2].

[3]. Safety (MSDS) data for ascorbic acid https://web.archive.org/web/20070209221915/http://physchem.ox.ac.uk/MSDS/AS/ascorbic_acid.html). Oxford University

[4]. Lachapelle, M. Y.; Drouin, G. (2010). "Inactivation dates of the human and guinea pig vitamin C genesG". enetica.139 (2): 199–207. doi:10.1007/s10709-010-9537-x (https://doi.org/10.1007%2Fs10709-010-9537-x.)PMID 21140195 (https://www.ncbi.nlm.nih.gov/pubmed/21140195).

[5]. Story of Vitamin C's chemical discovery (https://profiles.nlm.nih.gov/WG/Views/Exhibit/narrative/szeged.html).Profiles.nlm.nih.gov. Retrieved on 2012-12-04. The outdated, but historically important industrial synthesis of ascorbic acid from glucose via the Reichstein process.

[6]. Davies, Michael B.; Austin, John; Partridge, David A. (1991)V. itamin C: Its Chemistry and Biochemistry. The Royal
[7]. Society of Chemistry. p. 48. ISBN 0-85186-333-7. Svirbelf, Joseph Louis; Szent-Györgyi, Albert (April 25, 1932), The Chemical Nature Of Vitamin C (https://profiles.nlm.nih.gov/WG/B/B/G/W/_/wgbbgw.pdf) (PDF). Part of the National Library of Medicine collection. Accessed January 2007
[8]. Jacobs, Carmel; Hutton, Brian; Ng, Terry; Shorr, Risa; Clemons, Mark (2015). "Is There a Role for Oral or Intravenous Ascorbate (Vitamin C) in Treating Patients With Cancer? A Systematic Review" (http://theoncologist.alphamedpress.org/content/20/2/210). The Oncologist. 20 (2): 210–223. doi:10.1634/theoncologist.2014-0381 (https://doi.org/10.1634%2Ftheoncologist.2014-0381.) PMC 4319640 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4319640) . PMID 25601965 (https://www.ncbi.nlm.nih.gov/pubmed/25601965). "Conclusion. There is no high-quality evidence to suggest that ascorbate supplementation in cancer patients either enhances the antitumorf ecf ts of chemotherapy or reduces its toxicity."
[9]. "Vitamin C Fact Sheet for Health Professionals" (http://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/). National Institutes of Health. Retrieved 2 June 2015. ""At this time, the evidence is inconsistent on whether dietary vitamin C intake affects cancer risk. Results from most clinical trials suggest that modest vitamin C supplementation alone or with other nutrients offers no benefit in the prevention of cancer... Some researchers support reassessment of the use of high-dose IV vitamin C as a drug to treat cance..r. It is uncertain whether supplemental vitamin C and other antioxidants might interact with chemotherapy and/or radiation" .
[10]. R, Caspi (Aug 19, 2009)," MetaCyc Compound: monodehydroascorbate radical"(http://www.biocyc.org/META/NEW-IMAGE?type=COMPOUND&object=CPD-318,) MetaCyc, retrieved 2014-12-08
[11]. https://books.google.com/books?id=AJW37Xc3uiAC&pg=PA311&dq=decomposition+of+ascorbic+acid
[12]. Weiss, Rick (May 20, 2007), "Tainted Chinese Imports Common" (https://www.washingtonpost.com/wp-dyn/content/article/2007/05/19/AR2007051901273.html,) Washington Post, retrieved 2010-04-25
[13]. UK Food Standards Agency:" Current EU approved additives and their E Numbers "(http://www.food.gov.uk/safereating/chemsafe/additivesbranch/enumberlist.) Retrieved 2011-10-27.
[14]. US Food and Drug Administration: "Listing of Food Additives Status Part I" (https://web.archive.org/web/20120117060614/http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm.) Archived from the original (http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm) on 2012-01-17. Retrieved 2011-10-27.
[15]. Australia New Zealand Food Standards Code"Standard 1.2.4-Labelling of ingredients" (http://www.comlaw

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Proximate Analysis of Terminalia Chebula Leaves

Shirish S. Pingale[1], Rajeshwari S. Oza[2]
[1]P.G. Department of Chemistry, Arts, Com. & Sci. College Narayangaon, Pune, Maharashtra, India
[2]Department of Chemistry, SN Arts, DJM Commerce & BNS Science College, Sangamner, Ahmednagar, Gujarat , India.

ABSTRACT

Natural products have traditionally provided many of the drugs in use. Despite the achievement of synthetic chemistry and advances towards rational drug design, natural products continue to be essential in providing medicinal compounds and as starting points for development of synthetic analogous. With the increasing power of screening programme and increasing interests in the reservoir of untested natural products, many future drug developments will be based on natural products. The Terminalia Chebula leaves were tested for proximate analysis and it shows presence of 0.99% foreign organic matter, 8.09% ethanol soluble extractives, 18.34% water soluble extractives, 8.52% total ash, 7.23% Acid insoluble ash, 11.92% loss on drying and 5.88% moisture content.
Keywords: Phytochemicals, Proximate Analysis, Terminalia Chebula Leaves

I. INTRODUCTION

Most of the crude drugs (Plant materials) are usually put in quarantine store and they remain there for long time. During storage proper ventilation, humidity controls, suitable temperature and light conditions should be ensured to maintain their original pharmacological action. However, it is observed that, crude plant materials, before being taken for processing, are not analyzed which can lead to changes in original characteristics. To avoid this, the crude drugs should be tested for the following tests as per the USP and Indian Herbal Pharmacopoeia (IHP). The Study includes foreign organic matter, Ethanol soluble extractives, Water soluble extractives, Total ash contents, Acid insoluble ash, Water soluble ash, Loss on drying and Percentage moisture content.

Medicinal plant materials should be entirely free from visible signs of contamination, i.e. moulds, insects and other animal contamination, including animal excreta, fungus and dust. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. Any soil, stone, sand, dust and other foreign organic matter must be removed before medicinal plant materials are cut or ground for testing. Macroscopic examination can conveniently be employed for determination of foreign matter in whole or specific plant material.

II. MATERIALS AND METHODS

Sampling

Terminalia Chebula leaves were collected from various places in Bhimashankar, Khed Taluka in bulk, cleaned to remove the dust particles on the surface of the plant material. Leaves were allowed to dry by spreading them on filter papers in shade.

Extractable Matter

This method determines the amount of phytoconstituents extracted with solvents from a given amount of medicinal plant material in the form of powder. Here according to Indian Herbal Pharmacopoeia ethanol and water were used as common solvents to determine the extractable matter.

Procedure

Accurately weighed five grams of leaves powder was placed in glass-stoppered conical flask. To it 100 cm^3 of water was added. The flask was shaken frequently for six hours, and then allowed to stand for eighteen hours. The contents were filtered rapidly to avoid loss of solvent. The filtrate was transferred to a previously weighed clean beaker and evaporated to dryness on a water-bath. After evaporation the extract was dried at

105°C for six hours and kept in desiccators for cooling. The beaker was weighed and percent extractable matter in water was calculated. The above procedure was repeated thrice for determination of water-soluble extractable matter.

Ethanol soluble extractable matter was determined by following the above procedure except ethanol was used instead of water, as extracting solvent. The experiment was repeated for three times.

Ash Content

The ash remaining following ignition of medicinal plant materials is determined by three different methods, which measures

The Total Ash method is designed to measure the total amount of material remaining after ignition. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter (e g sand and soil) adhering to the plant surface.

Acid-Insoluble Ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present as sand and siliceous earth.

Water-Soluble Ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Total Ash

The total ash was obtained by taking Accurately weighed 2 g of the dried plant material was taken in a tarred Silica dish and was ignited with a flame of Bunsen burner for about one hour. The ignition was completed by keeping it in a muffle furnace at 550°C ± 20°C till grey ash was formed. It was then cooled in desiccators and weighed. The process was repeated (ignition, cooling and weighing) till the difference in the weight between two successive weighing was less than 1 mg.

Acid Insoluble Ash

Acid Insoluble Ash was obtained by following method.

Procedure

Accurately weighed 2gm of the dried plant material was taken in a porcelain/silica dish and was ignited with a bunsen burner for about one hour. The porcelain dish was kept in a muffle furnace at 550°C ± 20°C till grey ash was obtained. The ash was moistened with concentrated HCI and evaporated to dryness after which it was kept in an electric air oven maintained at 135°C ± 2°C for 3 hr. After cooling, 25 cc. of dilute HCI was added, and was kept covered with watch glass and heated on a water bath for 10 minutes. It was then allowed to cool, and was filtered through Whatmann filter paper No. 41. The residue was then washed with hot water till washings were free from chloride (as tested with $AgNO_3$ solution). The filter paper and the residue were put in a dish and ignited in a muffle furnace at 550°C ± 20°C for one hour. The process of cooling in a desiccators and weighing was repeated till the difference between two successive weights was found to be less than one mg.

Water-Soluble Ash

Water soluble ash was obtained by following method.

Procedure

Twenty five cm3 of distilled water was added in a silica dish containing the total ash and boiled for ten minutes. The insoluble matter was collected on an ash-less filter paper. The residue was washed with hot water and ignited in a crucible for fifteen minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of the total ash and the water-soluble ash was calculated.

Loss on Drying

The percentage of loss on drying was obtained by following method.

Procedure

Five grams of powdered plant sample was weighed in wide mouthed stoppered weighing bottle. The bottle was then placed with lid open in an air oven maintained at 100°C ± 2°C. The sample was kept in an oven for 2 hours. The bottle was then removed, covered and placed in desiccators. The bottle was weighed after cooling to room temperature and weighed.

The bottle was again kept in the oven for 2 hrs. and the above procedure was repeated (heating. cooling and weighing) till the difference in the weight between two successive weighing was less than 1 mg. Three readings for each sample were recorded.

Moisture Content

The moisture of plant powder were obtained by Karl-Fischer Titrimetric Method

Procedure

Reaction vessel was rinsed thoroughly with methanol magnetic stirring rotor was inserted in the vessel and placed in proper position. The large rubber cork was removed and some K/F grade methanol was added using funnel, to the reaction vessel just enough to submerge the metal wires of sensors in the reaction vessel. The cork was replaced immediately. The K/F reagent and methanol bottles were placed in position. Then the instrument was turned on and the speed of magnetic stirrer was adjusted. Methanol was neutralized and the titer factor was determined by calibrating the K/F reagent. This was done by adding 10 µl of distilled water with the help of a µL syringe in the reaction vessel and completing the titration. The calibration of the reagent was done in triplicate. The readings were noted and the titer factor was calculated. The data for determination of titer factor is given in following table QC 8 and it was calculated using the following formula.

III. RESULTS AND DISCUSSION

The results of proximate analysis of leaves of ***Terminalia Chebula*** plant were tested for proximate analysis and it shows presence **0.99%** foreign organic matter, **8.09%** ethanol soluble extractives, **18.34%** water soluble extractives, **8.52%** total ash, **7.23%** Acid insoluble ash, **11.92**% loss on drying and **5.88%** moisture content.

IV. REFERENCES

[1]. Handbook of medicinal plants by S.K. Bhattacharjee IIIrd edn.

[2]. Reviews on Indian Medicinal Plants. Vol III

[3]. Wealth of India 1985

[4]. Plants that Heal by J.C. Krishnan Vol. IPower Companies A Practical Example (Part I) . *Database Systems Journal, IV*(1), 37-45.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Synthesis of 5-Cyano Uracil Derivatives and Study of their Deamination Reaction

P. B. Aware[1], R. B. Toche[2]
[1]CMCS College,Gangapur Road, Nashik, Maharashtra, India
[2]Dadasaheb Bidkar Arts ,Science and Commerce College, Peth, Nashik, Maharashtra, India

ABSTRACT

Facile synthesis of ureidopropenenitrileobtained by reaction of malenonitrile, orthoesterand substituted ureasat 100°C in good yields. The ureidopropenenitrile was cyclized to targeted 5-cyano Cytosine derivatives in presence of sodium ethoxide in ethanol with 60 -65% yields. The deamination of amino compound was studied to obtained uracil derivatives using ispentylnitrite.
Keywords: Ureidopropenenitrile, Substituted Urea, 5-Cyano Cytosine, One Pot Three Component Reaction.

I. INTRODUCTION

The development of efficient and mild method for synthesis pyrimidine heterocyclic compounds represents a broad area of organic chemistry [1-2]. Structures containing such as moiety often play an important role due to their biological activities these derivatives also used in cancer therapy and anti-viral research [3-6].Among these heterocyclic, pyrimidinesderivatives showed in important class of pharmaceutical discovery [7-8]. Some of these compounds are analgesics [9],antihypertensive [10], antipyretics [11] and anti-inflammatory drugs [12].Pyrimidine derivatives used in some pesticides [13], herbicides and plant growth regulators [14].Consequently synthetic methodologies for synthesis of novel pyrimidine or fused pyrimidine compounds are of particular interests in the medicine and agro chemistry[15].

The development of alternate and efficient strategies is of considerable interest in literature and various methods were reported towards pyrimidine synthesis [16-19].
The development of new chemotherapeutic agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to discover newer molecules, with higher specificity and reduced toxicity than existing ones. In addition, various types of new resistant microorganisms that are being discovered now days are the great challenge for scientists. Uracils occupy a distinct and unique place in medicine.
The cytocines and uracils derivativesare very important compounds. Various workers had been carried out the synthesis of these compounds.
We have reported the synthesis of these compounds by conventional method. The product obtained by this method was identical, confirmed by scanning the IR, NMR, MP, mixedMP and TLC method.

II. EXPERIMENTAL WORK

2.1 Materials and methods

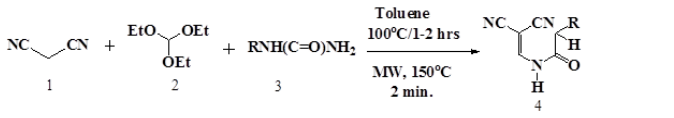
Melting points were determined on a Gallenkamp melting point apparatus. The 1H (300 MHz) and 13C (75MHz) NMR spectra were recorded on a Varian XL-300 Spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR-408 spectrophotometer. Mass spectra were recorded on a Shimadzu LC-MS:EI QP 2010A mass spectrometer with an ionization potential of 70eV. Elemental analyses were performed on Quest flash 1112 Series EA Analyzer. Reactions were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F254 Merck plates using UV light (254 and 366 nm) for detection and for column chromatography 5-20µm (Merck, 60-120 mesh) silica gel is used. Column dimension is 39× 2 cm and elution volume is 200-400 mL. Common reagent-grade chemicals are either commercially

available and were used without further purification or were prepared by standard literature procedures.

III. RESULTS AND DISCUSSION

The synthesis of cytosine derivatives was carried out in two steps. All the new compounds were well characterized by IR, NMR and elemental analysis given in experimental section.

3.1 Synthesis of ureidopropenenitrile, 4a-c



3a,R=Ph ;3b,R=CH_2 Ph;3c,R=CH_3

Theureidopropenenitrile**4**was prepared by using malononitrile**1,** triethylorthoformate**2**and substituted urea **3.** The reaction mixture was stirred in toluene at 100^o Cfor 1 hr. The completion of the reaction was checked by TLC, run in mixture ofHexane:Ethyl acetate (8:2). The solid precipitated on cooling was filtered and washed with ether to get required intermediate product **4**in60-65 % yield.

To prepared the same product in microwave, the above reaction mixture in round bottom flask attached with condenser was irradiated for 2 min. After cooling the flask, the obtained solid was stirred in 5 ml ether and then filtered to furnish **4** in 80-85 %

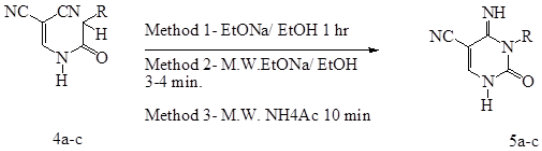
Table 3.1. The following table showing the detail for the synthesis of 3-cyano ureidopropenenitrile**4a, c**

1	Parameters	4a	4b	4c
2	Reaction Time in hrs.	1	1	1
3	Amount of solvent (ml)	20	25	20
4	% Yield	60	68	65
5	M.P. ^oC	265	258	245

The structure of the ureidopropenenitrile4,was also confirmed by analytical and spectroscopic studies.

e.g. The I.R. of **4c** showed the peaks at 3300 cm^{-1}, 2202 and 1667 was due to –NH_2, CN, and amide carbonyl respectively.The ^{1}HNMR($CDCl_3$) showed singlet at δ 2.73 for three protons of methyl group. The broad singlet at δ 7.15 and 10.73 corresponds to two NH protons and the sharp singlet at δ 8.38 is for =CH. The elemental analysis of compound **4a** is also in agreement with the molecular formula $C_6H_6N_4O$. Based on the above spectral and analytical data we have assigned the structure **4a**1-(2, 2-dicyanovinyl)-3-phenylurea for this product. The spectral and physical data for all compounds are explain in experimental part

3.2 Synthesis of 5-Cyano Cytosine derivatives, 5(a-c)



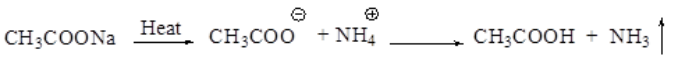
4a,R=Ph ;4b,R=CH_2 Ph;4c,R=CH_3

Method 1:

The ureidopropenenitrile**4a-c**, obtained in above method was cyclized to the required cytosine i.e. 5-cyano -4-aminopyrimidone. Compound **4** was refluxed in sodium ethoxideinethanol for 1 -2 hrs. Completion of the reaction was confirmed by TLC. The solvent was removed under vacuum. The residue was dissolved in cold water; then solid product was neutralization with 2N HCl, was filtered, and washed with water and purified. Yield obtained were **60-65%.**

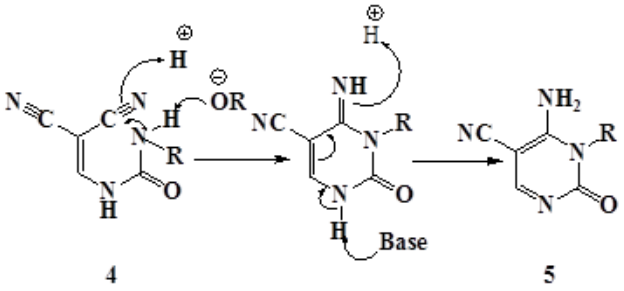
Method 2:

The same reaction also carried out in microwave using CH_3ONa/ CH_3OH(strongbase) for 3 minutes and furnished 60-67 % yield. ORThe above reaction mixture was heates in at 100^{o}Cfor NH_4OAC. The molten NH_4OAC acts as solventfor the reaction and also create weak basic medium due to evolution of $NH_3$by its decomposition. The use of NH_4OAC is novel eco friendly methods use for cyclization of ureidopropenenitrile



The ammonium acetate is non-toxic,non-poisonous, eco –friendly reagent. The products **5a-c** wascharacterized by analytical and spectral data.

Mechanism for cyclization reaction:



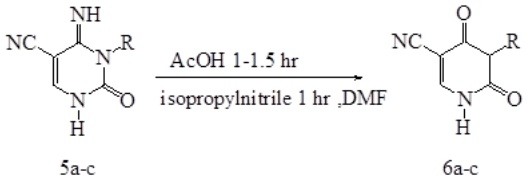
4 5

e.g.The I.R. spectrum **5a** showed the presence of NH_2 frequency at 3288 $cm^{-1}$and 3400 $cm^{-1}$and 2219 cm^{-1} corresponds to CN,the amide C=O observed at 1667 cm^{-1}.The ^{1}HNMR($CDCl_3$) showed multipletat δ 7.20-7.60 for aromatic protons. The sharp singlet for =C-H (olefin protons) is obtained at δ 8.40. Broad singlets at δ 7.15 and 10.73 correspond to two NH_2 protons. The elemental analysis this compound correspond molecular formula $C_{11}H_8N_4O$.

Table 3.2. The following table shows detail for the synthesis of compounds **5a,**

Parameters	5a	5b	5c
Reaction Time in hrs	1	1	1
Amount of solvent ml	20	2	20
% Yield	50	55	45
M.P. °C	282	270	251

3.3 Synthesis of Uracil Derivatives, 6a-cDeamination:



5a-c 6a-c

6a,R=Ph ;6b,R=CH_2 Ph;6a,R=CH_3

The removal of NH_2 group from the compound is deamination. The deamination of 4-amino -5-cyanopyrimidine 5a-c yield valuable product 5-cyano uracil **6a-c** by refluxing on glacial acetic acid.

Method 1:

The removal of NH_2 group from the compound is deamination. The deamination of 4- amino-5- cyano pyrimidine 5a-c yield valuable product 5-cyano uracil **6a-c**on using isopentylnitrile in DMF.

The identity of the products by both methods was proved by MP, TLC, IR and ^{1}HNMR methods.

e.g.The I.R. spectrum **6a** showed the presence of two carbonyls frequency at1746 $cm^{-1}$and the peak at 1668 $cm^{-1}$and CN at 2226 cm^{-1}.But no peakwas observed for NH_2 in the IR indicates the primary aminegroup of compound 5awhich was observed at 3388 cm^{-1} has been converted to carbonyl group.Similarly, the ^{1}HNMR showed 5 aromatic protonsappeared as amultiplet at δ 7.27-7.50. Broad singlet at δ 10.73correspond to NHprotons.The sharp singlet for =C-H (olefin protons) isobtained at δ 8.58.Theelemental analysis this compound correspond molecular formula $C_{11}H_7N_3O_2$.

Table 3.3. The following table shows detail for the synthesis of compounds **6a, c**

Parameters	5a	5b	5c
Reaction Time (hrs)	1	1.5	1
Solvent (ml)	20	25	20
% Yield	58	52	46
M.P.°C	264	247	233

IV. CONCLUSION

We have explored a facile and efficient protocol for the synthesis of 5-Cyano Cytosine derivatives with 60-89% yields. Particularly valuable features of present method include broad substrate scope, short reaction time, straight forward procedure and easy aqueous work up that facilitated 80-85% recovery of pure product and use of inexpensive chemicals and reagents.

V. AKNOWLEDGEMENT

Pankaj B. Aware thanks to the CIF, SPPU, Pune for spectral analysis; Principal, K.R.T. Arts, B.H. Commerce & A.M. Science College, Nashik 422 002 and Principal, CMCS College, Nashik 422 013(MS), India for facilities

VI. REFERENCES

[1]. Keay, J. G.; Toomey J.E; SuschitzkyH,;Scriven, E.F.V., Pergamum: Progress in Heterocyclic ChemistryOxford,1993;Vol.5,p 202

[2]. Ciufolini ,M.A.Advances in Heterocyclic Natural Product Synthesis;Pearson W.H.;Ed.; JAI : London,1996 ;

[3]. Wnuk,S.F.;Lewandowska,E.;Companioni ,D.R. ;Garcia's,P.I.,Jr.Secrist,Org.Biomol.Chem.2004,2, 120
[4]. Cushman, M.; Sambaiah, T.; Jin,G.; Illarionov, B.; FischerM.;Bacher,A. J.Org.Chem.2004,69 ,601
[5]. De Clercq , E. Anticancer Res.1986 ,6, 549
[6]. De Clercq , E.; Bernaerts,R.J.Biol.Chem.1987 , 263 , 14905.
[7]. Shishoo,C.J.;Shirsath V.S. ;Rathod.I.S.; Yande,Eur.J.Med,Chem.2000,35,351.
[8]. Chambhare, R. V.; Khadse,B.G.;Bobde , A.S.;Bahekar ,R.H. Eur.J.Med.Chem.38,89
[9]. Hassan , N.A.; Molecules 2000,5,827
[10]. Pemmsin, M,; Lbu-due, C;Hoguet ,F.; Gaultier ,C; Narcisse ,J.Eur.J.Chem.1988,23, 543.
[11]. Cannito,A.;Pernmsin ,M.;Lnu-Due.C;Hoguet , F., Gaultier, C ;Narcisse, J Eur.J. Chem.1990, 25,635.
[12]. Smith , P.A.S.; Kan ,R.O. / .Org. Chem. 1964 , 29 , 2261 .
[13]. Nega , s.; Aionso , J.;Diazj , A.; Junquere ,F.J.Heterocycl.Chem.1990,27 ,269
[14]. Shishoo ,C.J.; K.S. J.Heterocycl.Chem.1992 , 29, 883.
[15]. Peters J.U.;Weber,S.;Kritter,S.;Weiss P.; Wallier, A.; Zimmerli,D.;Boehringer,M.;Steger ,M.;Loeffer,B.M.; Bioorg. Med. Chem. Lett2004 ,14,3579.
[16]. Spivey , A.C.;Srikaran, R.;Diaper ,C.M.;Turner ,D..Org.Biomol.Chem.2003,1,1638
[17]. .Katrizky , A. R.; Soloducho , J.;Belyakov ,S. ARKIVOC 2000 ,(l),37
[18]. Bratusek ,U.;Meden ,A.;Svete ,J.;Stanovnik ,B. . ARKIVOC 2003,(v) ,77
[19]. Bowman , M. D. ; Jeske ,R.C.; Blackwell , H.E. Org.lett.2004 ,6,2019
[20]. R. B. Toche ,M.N. Jachak, N.S.Badgujar,A.B. Avhale and D.B.Kendre , OPPI, 37,405 (2005)

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Simultaneous Determination of Paracetamol and Mefenamic acid in Tablet Dosage Form by High Performance Thin-Layer Chromatography

Dr. Madhukar A. Badgujar
Department of Chemistry, Sheth J.N. Paliwala College Pali, Raigad, Maharashtra, India

ABSTRACT

A normal-phase simple, rapid and precise high-performance thin-layer chromatographic (HPTLC) method has been developed for simultaneous quantitative determination of Paracetamol and Mefenamic acid in a pharmaceutical formulation. The analysis was performed on silica gel 60F254 on aluminum plates with Acetonitrile-Toluene 7 : 3 (v/v), as mobile phase. Detection and quantitation were performed densitometrically at λ = 275 nm.. The developed method was validated for linearity, accuracy, precision and robustness parameters. Responses of Paracetamol standard and Mefenamic acid standard were linear functions of concentration in the ranges 36-180 µg/mL and 40-200 µg/mL, respectively.. The correlation coefficient of Paracetamol and Mefenamic acid were observed 0.9997 and 0.9992 respectively. Accuracy was checked by conducting recovery studies; average recovery from the pharmaceutical preparation was 99.88 ± 1.25% for Paracetamol and 99.92 ± 1.62% for Mefenamic acid. The Proposed HPTLC method has potential applications for simultaneous determination of Paracetamol and Mefenamic acid in combined tablet dosage form.

Keywords : Paracetamol, Mefenamic acid, HPTLC

I. INTRODUCTION

Paracetamol (PC) is chemically N - (4-hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent. It has a narrow therapeutic index – the therapeutic dose is close to the toxic dose. Mefenamic acid (MA) is 2-[(2, 3-dimethylphenyl) amino] benzoic acid Mefenamic acid, an anthranilic acid derivative, is a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs).. Literature survey revealed that various methods reported for the analysis of Paracetamol and Mefenamic acid in pharmaceuticals viz, UV spectrophotometry, reverse phase HPLC stability indicating, visible spectrophotometry. Aim of present work was to develop simple, economical, rapid, precise and accurate method for simultaneous determination of Paracetamol and Mefenamic acid. The key advantage of developed HPTLC method is that several samples can run using a small quantity of mobile phase. The present study describes HPTLC method for the determination of paracetamol and mefenamic acid from tablet dosage form.

II. EXPERIMENTAL

Chemicals and reagents:
Reference standards of Paracetamol and Mefenamic acid were procured from Blue Cross India Limited Nashik. AR grade methanol and acetonitrile were purchased from Baker (Mumbai, India).

Instrumentation and Chromatographic condition:
The samples were spotted in the form of bands of width 5mm with a desaga 100 µL sample syringe on silica gel precoated aluminum plate 60 F_{254}, with 200 µm thickness. These bands were applied with the help of Desaga AS 30 - sample applicator at a distance of 10mm from X axis and 15mm from Y axis at the edge of the HPTLC plate with the speed of 150nl/sec for methanol. The plates were pre-washed by methanol and activated at 110 °C for 5 min prior to chromatography. The space between two bands was kept at 10 mm. The slit dimension was kept at 4 x 3 mm and 4.0 mm/s scanning speed was employed. The monochromator bandwidth was set at 10 nm, each track was scanned thrice and baseline correction was used. The mobile phase consisted of Acetonitrile : Toluene in the volume ratio 7 : 3 v/v.

Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase.

The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C±2) at relative humidity of 55% ± 5. Subsequent to the development; TLC plates were dried in current of air with the help of air dryer. Detection and quantification was performed in the absorbance mode using Degasa TLC scanner with Pro-Quant software. During the method development the spots on the TLC plate were visualized in a UV chamber equipped with a UV lamp (λ =254nm). The developed TLC plate was scanned between 200 and 400nm wavelength using CD-60 Densitometer/scanner. The wavelength chosen for further quantification was 275nm.

Preparation of standard solutions:
45mg of Paracetamol and 50 mg of Mefenamic acid was accurately weighed and transferred to a 50cm^3 volumetric flask. It was dissolved in a minimum quantity of methanol and then diluted up to the mark with methanol. The concentration of the solution obtained was 900 µg/mL for Paracetamol and 1000 µg/mL for Mefenamic acid **(Solution A).** 5cm^3 of this **solution A** was diluted to 50 cm^3 in a volumetric flask with mobile phase. The concentration of the solution obtained was 90 µg/mL & 100 µg/mL for Paracetamol and Mefenamic acid respectively **Preparation of**

Sample solutions:
Twenty tablets (MFTEL FORTY, BLUE CROSS LABORATORIES LTD) were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to one tablet containing 450mg of Paracetamol and 500 mg of Mefenamic acid was taken in a 100mL dilution flask. Then about 50mL of diluent was added to it and sonicated for 20-25 mins at an ambient temperature with intermittent swirling, cooled to room temperature and diluted upto the mark with diluent. Then solution from the flask was filtered through syringe filter.

Validation of the Method:
The method was validated for linearity, precision (inter-day, intra-day and intermediate precision), accuracy, specificity. Standard plots were constructed for both paracetamol and Mefenamic acid in the range of 36-180 µg/mL and 40-200 µg/mL, respectively L. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively. The intermediate precision of the method was determined by repeating the experiment on two different instruments. Accuracy was determined by recovery studies. It was carried out by spiking 10%, 20% and 30% of the standard drugs to the pre-analysed marketed sample of Paracetamol and Aceclofenac. Three determinations were performed at each level. Further, specificity of the method was assessed by study of the resolution factor of the drug peaks from nearest resolving peaks. Robustness of the method was carried out by small changes in the mobile phase composition (± 0.1 mL for each component) were made and the effects on the results were examined. Time from spotting to chromatography and from chromatography to scanning was varied by ± 15 min.

Analysis of marketed formulation:
The developed method can be applied in determination of paracetamol and mefenamic acid in tablet MFTEL FORTY which is marketed oral solid dosage formulation. To determine the contents of paracetamol and mefenamic acid (label claim: 450mg paracetamol and 500 mg mefenamic acid per tablet), the contents of tablet were emptied and weighed. The drug from the powder was extracted with 10 ml methanol. To ensure complete extraction of the drug, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the supernatant was suitably diluted to give desired concentration. Ten microlitres of the solution was applied on TLC plate followed by development, visualization and scanned. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

III. RESULTS AND DISCUSSION

Optimization of the chromatographic conditions:
In order to develop an normal phase HPTLC method for the determination of paracetamol and mefenamic acid in combined dosage form the chromatographic conditions were optimized. For better separation and resolution the mixture of different solvents of varying polarity were tried. The different compositions of mobile phase were changed for getting better separation of analytes. Initially, chloroform-ethyl acetate 4: 6 (v/v) and acetonitrile, toluene 5:5 (v/v) were used. The best

results were obtained by the use of acetonitrile, toluene in the ratio of (7: 3 v/v). This mobile phase showed good resolution of paracetamol and mefenamic acid peak from other formulation components or excipients tested. Densitometric scanning of all the tracks showed compound with *Rf* value 0.51for mefenamic acid and 0.62 for paracetamol The present method uses acetonitrile –toluene (7:3 v/v) as the mobile phase for development. The present method is quicker as the time needed for development of plate is reduced considerably to less than half an hour for chamber saturation and development of plate as compared to the previously reported method.

Table 1. Optimized chromatographic conditions

Parameters	**Chromatographic conditions**
Development chamber	Twin trough chamber
Stationary phase	Silica gel
Mobile Phase	Acetonitrile : Toluene (7 : 3 v/v)
Chamber saturation	15 min
Sample applicator	AS 30 - SAMPLE APPLICATOR
Band	8mm
Space	12mm
Scanning speed	20mm/sec
Development distance	8 cm
Drying of plate	Room temperature
Densitometric scanner	CD 60 - DENSITOMETER / SCANNER
Lamp	Deuterium
Wavelength	275 nm
Volume	10µl

Method Validation:

Linearity and range

Linearity was observed over the concentration range of 36-180 µg/mL for paracetamol and 40-200 µg/mL, for mefenamic acid (see table 2). The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r^2 = 0.9997 for paracetamol and 0.9992 for aceclofeanc).

Table 2. Linear regression data

Drug	**Linearity range**	**Correlation coefficient (r^2)**	**Slope**	**Intercept**
Paracetamol	36-180 µg/mL	0.9997	3.430	-6.383
Mefenamic acid	40-200 µg/mL	0.9992	7.946	-13.73

Precision:

The developed method was validated for system precision and method precision.
The precision study of the proposed method gave the results in the prescribed limits of relative standard deviation. This is less than 2 % for both analytes. The low value of RSD showed that the proposed method was reliable and reproducible.

Table 3. Precision study for Paracetamol and Mefenamic acid

Obs No	Paracetamol		Mefenamic acid	
	Peak Area	% Assay	Peak Area	% Assay
1	2521	101.61	2665	101.89
2	2512	101.25	2689	99.82
3	2507	101.05	2710	98.24
4	2499	100.73	2708	98.34
5	2442	98.43	2650	97.65
6	2474	99.72	2743	99.53
	Mean	100.01	Mean	99.31
	S.D	0.899	S.D	1.307
	%R.S.D	0.8990	%R.S.D	1.310

Specificity:
An investigation specificity was conducted during the validation of identification tests, the determination of impurities and the assay. Demonstration of specificity requires that there should not be any interference of impurities and excipients. In practice this was done by taking the chromatogram of sample solution and the assay result was unaffected by the extraneous material. It has been found that there was no interference of the diluents , placebo at the Rf value of the analytes.

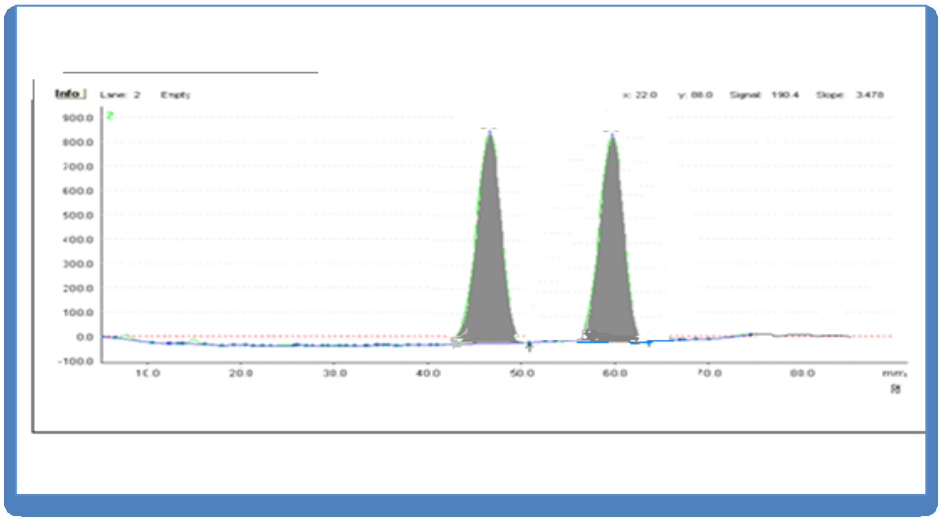


Figure 1. Typical HPTLC chromatogram 1) Mefenamic acid 2) Paracetamol

Accuracy (Recovery Experiment):

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10% , 20% and 30% of the standard drug of paracetamol and mefenamic acid were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for PC and

MA from the sample solution are shown in Table 4 and 5. The results are within the acceptance limit and hence the method is accurate.

Table 4. % Recovery of Paracetamol

Amount of Paracetamol in ppm								
Sr.No	**% Added**	**Original amount**	**Added amount**	**Total amount**	**Mean (n = 5)**	**% Recovery**	**S.D**	**% RSD**
1	10	450	45.40	495.40	495.45	99.97	0.3881	0.3786
2	20	450	90.87	540.87	540.02	99.50	0.4922	0.4896
3	30	450	135.66	685.66	685.66	99.10	0.645	0.681

Table 5. % Recovery of Mefenamic acid

Amount of Mefenamic acid in ppm								
Sr.No	**% Added**	**Original amount**	**Added amount**	**Total amount**	**Mean (n = 5)**	**% Recovery**	**S.D**	**% RSD**
1	10	500	50.36	550.36	550.12	100.15	0.9592	0.8582
2	20	500	100.38	600.38	600.33	99.49	0.4977	0.4902
3	30	500	150.16	650.16	650.87	99.82	0.8884	0.7953

IV. CONCLUSION

The HPTLC method for the determination of Paracetamol and Mefenamic acid from their tablet dosage form was found to be accurate , precise, specific and rapid. The results of the recovery studies show the high degree of accuracy of the proposed method. The advantage of the proposed method is that it require less time and cost effective method. Solvent consumption during the analysis is less. Therefore the proposed method can be applied successfully in routine analysis.

V. ACKNOWLEDGEMENT

The authors are thankful to Department of Chemistry, Mithibai College and for their support.

VI. REFERENCES

[1]. The United states of pharmacopeia NF 32

[2]. Rele R. V. Mali R.N. Sawant S.A. Analytical Chemistry: An Indian Journal. 2009, 8(2), 161-164

[3]. Badgujar M.A, Mangaonkar K.V. RJPBCS, 2014, 5(4), 110-117

[4]. Badgujar M.A; Mangaonkar KV, J.Chem.Pharm.Res, 2011, 3(4), 893-898

[5]. Godse V.P.; Deodhar M.N, Bhosale A.V. Sonawane R.A.; Sakpal P.S.; Borkar D.D.; Bafana Y.S. ; Assian Journal of Research in Chemistry.2009, 2(1) ,37- 40

[6]. Dongre Vaijanath G. Shah Sweta B, Bayes Gunaji S.; Phadke Manisha; Jadhav Vivek K. Chromatograpraphia, 2009, 69(9 -10), 1019- 1023

[7]. Vaidya, V. V.; Singh, G. R. Choukekar, M. P. Kekare, M. B.E-Journal of Chemistry. 2010, 7(1), 260-264.

[8]. Vidya V. DIghe, Ramesh T. Sane, Shashikumar N. Menon, Harsha N. Tambe JPC – Journal of planar chromatography – Modern TLC 2004, 17(5), 383-387
[9]. Vidya V. Dighe,; Ramesh T. Sane, ;Shashikumar N. Menon,; Harsha N. Tambe,; Sreedevi Pillai1,; Vijay N. Gokarn JPC - Journal of Planar Chromatography - Modern TLC.; 2006, 19(112), 443-448,
[10]. Harikrishan N., Gunssekaran V., Sathishbabu A., Rao G. Srinivasa, Roosewelt C. Assian Journal of Chemistry. 2007, 19(5), 3918 – 3922
[11]. Gandhi S.V, ;Barhate N.S,; Patel B.R, ;Panchal D.D and Bothara K.G, Acta Chromatogr., 2008, 20, 175 – 182.
[12]. Yadav, Alok; Singh, Raman M.; Mathur, Satish C.; Saini, Pawan K.; Singh, Gyanendra N.Journal of Planar Chromatography-Modern TLC. 2009, 22(6), 421-424.
[13]. Bhalerao, Santosh; Tambe, Santosh; Pareek, Vikas; Shinde, Rupali; Gupta, Lalit Kumar. Asian Journal of Pharmaceutical and Clinical Research. 2010, 3(1), 25-30.
[14]. Sethi P D, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations, 2nd Ed., CBS Publishers and distributors, New Delhi, India, 1996.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Isolation and Characterization of Herbal Surfactant from Selected Medicinal Plant

V. V. Chopade[*1], P. S. Uttekar[1], S. B. Takate[2]
[*1]P. E. Society's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India
[2]Agasti Arts, Comm. And Dadasaheb Rupwate Science College Akole, Ahmednagar, Gujarat, India.

ABSTRACT

The shade dried fruits of Sapindus mukorossiwas extracted with pet ether, chloroform and ethyl acetatesuccessively by soxhlation method, water by maceration method at room temperature and Preliminary phytochemicals screening of extracts of fruits of Sapindusmukorossiwith various solvents.Then we isolate the compound with high purity by flash chromatography technique. Mobile phase Benzene: ethyl acetate: water (3:4:3) and we got 11 phytoconstituents separated in different test tube and characterized fraction with parameter UV, IR, and Surfactant properties. The above findings lead to the conclusion that the saponin was sussefully isolated in fraction as surfactant of *S. mukorossi* fruits.

Keywords : Sapindus Mukorossiwas, S. Mukorossi Fruits, UV, IR, NMR, LC-MS

I. INTRODUCTION

Sapindus mukorossi (Fam: Sapindaceae), well known as soapnuts, are used medicinally as an expectorant, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, chlorosis, and migranes. Sapindus mukorossi is a popular ingredient in Ayurvedic shampoos and cleansers. Surfactants are term as surface-active agents also wetting agents,emulsifying agents or suspending agents depending on its properties and use. Surface-active agents are substances which, at low concentrations, adsorb onto the surfaces or interfaces of a system and alter the surface or interfacial free energy and the surface or interfacial tension.Surfactants are monomers, it has a characteristic structure possessing both hydrophobic groups / non-polar regions (their "tails") usually contain a C12–C18 hydrocarbon chain and hydrophilic groups / Polar Regions(their "heads").Therefore, they are soluble in both organic solvents and water, so they called amphiphilic present study carried out develop the method for extraction by various solvents, Identification of plant constituents by preliminary method, isolate plant constituents by using Flash Chromatography method, characterization of isolated plant constituents by UV, IR, NMR and LC-MS

II. MATERIALS AND METHODS

Collection and identification of plant material:
The plant material used in this study was collected during month of august in Nashik Dist, India and authentication was done from botanical survey of India pune A voucher specimen has been deposited.

Drying and grinding of plant materials:
Collected fruits of Sapindus mukorossi were dried under shade and pulverized to make coarse powder.

Preparation of the Extract:
The shade dried fruits of Sapindus mukorossi was extracted with pet ether, chloroform and ethyl acetate successively by soxhlation method, water by maceration method at room temperature, concentrated over water bath and evaporated under reduced pressure. The yields of extract were calculated

Preliminary phytochemicals screening:
The plants may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, tannins, saponins, flavonoids and sugars, etc. that exert physiological effects. These compounds are responsible for therapeutic effects, usually the secondary metabolites. All the extracts of the plant material were

subjected to preliminary phytochemicals screening for the detection of various plant constituents

Table: 1. Preliminary Phytochemicals Screening

Sr no	Plant constituents	Test /reagent	PTE	CHL	ETA	AQE
1	**Steroids**	**Salkovaski**	--	--	--	--
2	**Alkaloids**	**Dragendroff's test**	--	--	--	--
		Hager's test	++	++	++	++
		Mayer's test	++	++	++	++
		Wagner's test	++	++	++	++
3	**Saponins**	**Foam test**	--	--	++	++
		Haemolysis test	--	--	++	++
4	**Fats and oils**	**Filter paper test**	++	++	++	++
5	**Tannins and Phenolic**	**Ferric chloride test**	--	--	++	++
		Lead acetate test	--	++	++	++
		Pot. Dichromate	--	++	++	++
		Bromine water	--	--	++	++
6	**Flavonoids**	**Shinoda test**	--	++	++	++
		Lead acetate test	--	++	++	--
7	**Carbohydrates**	**Molisch test**	--	--	--	--
		Fehling's test	--	--	--	--
		Barfoed's test	--	--	--	--
8	**Proteins**	**Millon's test**	--	++	++	++
		Biuret test	--	++	++	++
9	**Amino acid test**	**Ninhydrine test**	--	++	++	++

+ ve -- present; -- ve absent

Isolation and Characterization

Isolation of constituents from ethyl acetate extract by flash chromatography

Flash chromatography is a fast and inexpensive separation technique for the purification of organic syntheses products e.g. in drug discovery or from natural

extracts. It is a popular alternative when other separation techniques cannot be used or are too difficult. Flash chromatography provides a rapid and inexpensive general method for the preparative separation of mixtures requiring only moderate resolution. It can be applied to normal-phase and reversed-phase separation. Flash chromatography can endure relatively high flow rate with low pressure, offering good separation in a short time under a proper chromatographic condition. In flash chromatography Columns are disposable plastic cartridges, advantage of cartridges are time save and reproducibility. Based on sample volume we may select different size of cartridges. Now a day's readily prepared cartridges are available based on particle size and stationary phase volume. Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we may isolate the compound with high purity. In the case of sample have more chemical constituents, without information of concentrations of that chemical constituents, preparative chromatography is preferable

Resulted Peak (ethyl acetate extract):

- ✓ Prepared a conc. of 2000µg/ml from above working standard solution (10,000 ppm).In 10 ml of volumetric flask pipette out 0.2 ml of working standard solution was mixed with 5 ml of 0.5N HCL and kept for 30 min for heating on water bath. After 30 min solution was diluted up to 10 ml with methanol.
- ✓ The prepared (2000 ppm) fractionsolution was adsorbed over silica gel (# 60 – 120) in the ratio 1:4 (drug to silica gel) and finally dried under vacuum below 60^0 C. A column of 5 litres capacity was first loaded with 1 to 2 g of silica gel (# 60-120) with chloroform as solvent (dry packing).

The adsorbed material (200 mg) was charged and eluted with chloroform: methanol gradient (100:0---90:10---80:20---70:30---60:40---50:50---40:60---30:70---20:80---0:100). Fractions of 100 ml were collected. The fractions collected were concentrated by distillation under vacuum using rota vapour and weighed

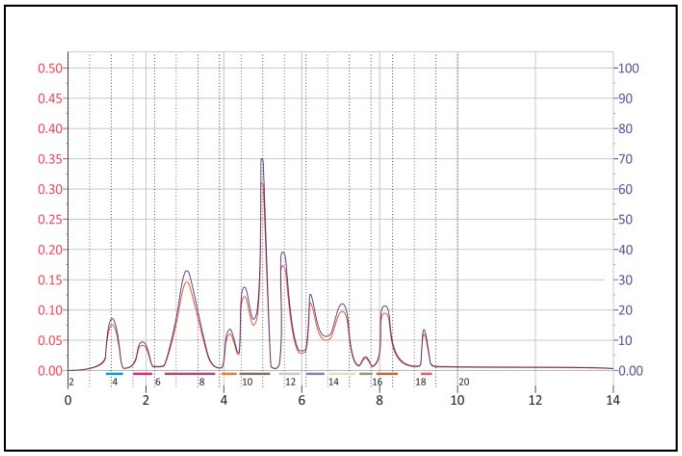


Figure 1. Flash Chromatogram of ethyl acetate extract at mobile phase benzene: ethyl acetate: water (3:4:3)

Characterization of fraction:

The structures of isolated fraction of degradation products were characterized by UV and functional groups were identified by IR spectra.

1) UV Spectra:

To analyze the collected fraction of ethyl acetate from flash chromatography samples was scanned under UV in the range of wavelength 200-400 nm. In followed UV spectra it shows change in wavelength that was at 242nm. From this result it was conclude that the fraction was isolated successfully.

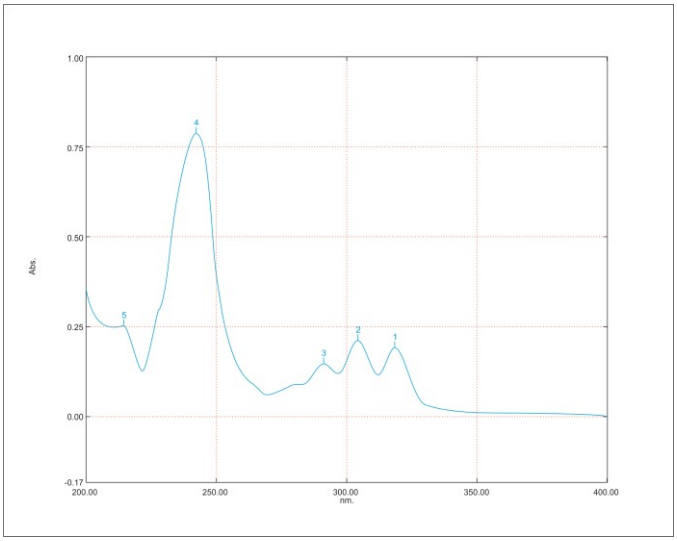


Figure 2. U V spectra of fraction

3) FT-IR:

Specification of FT-IR

Model - JASCO- M 4100 FT-IR

Preparation of sample for IR

The collected fraction adsorbed on sufficient Qty. of silica gel. This residue was then mixed with KBr in the ratio 1:300 and this sample was analyzed.

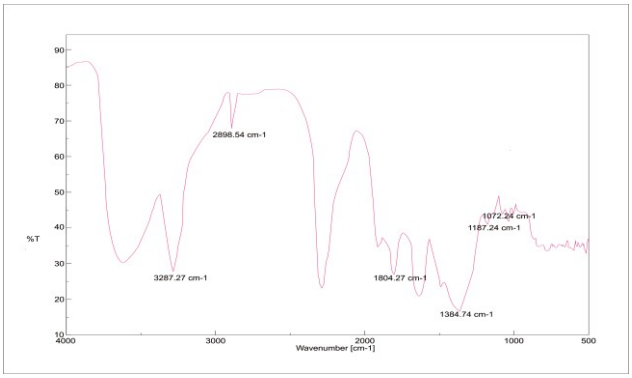


Figure 3. FT-IR Spectrum of fraction of extract

Surfactant Evaluation

Critical micelle concentration (CMC) measurement:

The CMC for fraction solution was estimated by employing Wilhelmy plate tensiometer (DCAT 11), from Data physics, Germany. Surface tension of double distilled water was measured using Wilhel my plate (platinum-iridium plate-PT 11). Then a controlled volume of fraction was added using calibrated micropipettes (20-200 µl, 200-1000 µl) into the double distilled water. This solution was stirred using Teflon needle for 60 sec duration and then allowed to equilibrate for 5 min before the surface tension measurement. The concentration of fraction solution was increased gradually by adding stock solution and surface tension was measured. The CMC was determined by noting the concentration above which the surface tension remained constant to the minimum value. The CMC using fraction solution as a mother solution was also determined.

Determination of emulsification activity with kerosene:

The fraction solution at CMC, were tested separately for the emulsification activity. This activity was checked by adding 1 ml of fraction solution in 4 ml of water and insoluble 6 ml of kerosene. Further, this mixture was vortexed vigorously for 2 min to obtain maximum emulsification. We also studied emulsification activity of aqueous SDS at CMC concentration (8.1 mM), henceforth we referred as SDS-c. After 48 h of settling down time, emulsification index was calculated by measuring percentage emulsion layer height i.e. ratio of height of emulsion layer to total height of liquid column. The results were obtained by averaging more than 3 realizations.

Emulsification assay with plant oils:

Emulsification activity to the various plant oils in water medium is also tested. In this case 3 ml of fraction solution was mixed with 0.5 ml of plant oils (coconut, mustard, soyabean, almond, castor, sunflower & olive) separately. It was vortexed vigorously for 2 min and incubated at room temperature for 1 h without disturbance for separation of aqueous and oil phase. Aqueous phase was removed carefully with the help of 1 ml micropipette and absorption was measured. Fraction solution without any oil was taken as a blank. Absorbance of aqueous phase was measured by using spectrophotometer. Emulsification activity per ml (EU/ml) was calculated by using the formula:

Emulsification unit = 0.01 × dilution factor. Such experiments were repeated more than thrice and mean value of EU was considered for activity.

The decrease in the surface tension of the aqueous solution as a function concentration is plotted It can be seen that the surface tension of aqueous surfactant solution decreases rapidly with increase in fraction concentration. For pure water it is 72.14 mN/m and saturates to minimum value of 41.21 mN/m when fraction concentration becomes 0.04 gm/cc. The CMC value for fraction is rather close to the reported value for chemically purified Sapindus saponin. Bio-surfactant fraction contains saponin which is responsible for its various functional It is clear that the crude ritha maintains CMC value, which is an essential functional property of a surfactant and could be used as an economical bio-surfactant.

Table 2. Variation of the surface tension of aqueous solution with fraction concentration

Fraction (gm/cc)	Surface tension (mN/m)
0	72.14
0.04	41.21
0.06	12.47
0.08	2.4

0.1	1.6
0.12	1.2
0.14	1.1
0.16	0.57
0.18	0.22

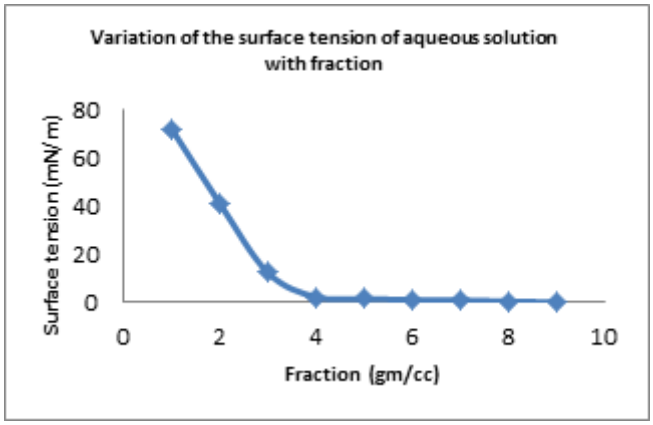


Figure 4. Variation of the surface tension of aqueous solution with fraction concentration

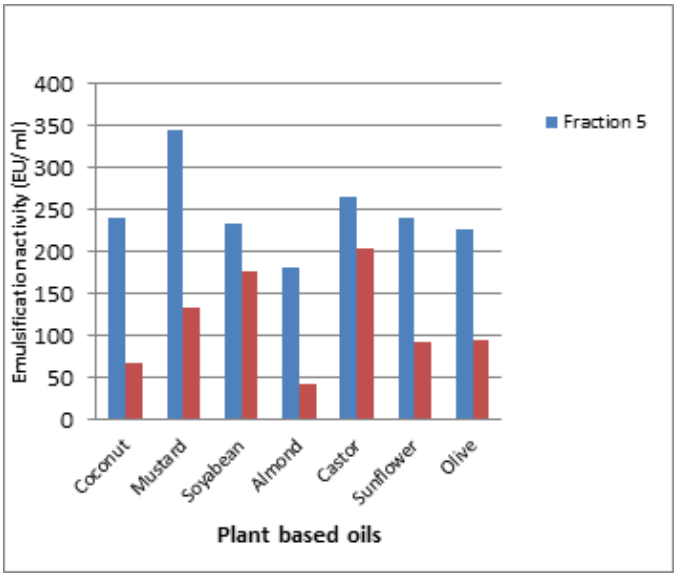


Figure 5. Emulsification activity of fraction and SDS with various plant oils.

III. DISCUSSION AND CONCLUSION

Emulsification activity is one of the important properties of a potent bio-surfactant. Due to amphiphilic nature of biosurfactant it can solubilize water insoluble substance/hydrocarbons.Therefore, we observed emulsification index for fraction. Emulsification index was tested for fraction and aqueous SDS-c solutions with kerosene. Emulsification activity for fraction solutions was approximately 72%. It is also seen that emulsification activity of fraction with kerosene is comparable to that of SDS-c solution, which is also 72%. Hence, fraction proves to be a good substitute for emulsification in comparison with synthetic surfactants. Fig. 2 shows a variation in the emulsification activity using fraction and SDS-c solutions for different plant oils. Excellent emulsification activity was shown by fraction solutions with the tested oils. These solutions show a highest activity for mustard oil followed by castor, soyabean and coconut oil. Fraction demonstrates superior emulsification activity. It is important to note that SDS-c solution exhibits least activity for coconut, almond, sunflower and olive oils. Relatively good activity was observed for castor and soyabean oils. This is the first report dealing with the emulsification activity of fraction with respect to the various plant oils.

The shade dried fruits of *Sapindus mukorossi* was extracted with pet ether, chloroform and ethyl acetate successively by soxhlation method, water by maceration method at room temperature Preliminary phytochemicals screening of extracts of fruits of *Sapindus mukorossi.* The plants may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, tannins, saponins, flavonoids and sugars, etc. that exert physiological effects. These compounds are responsible for therapeutic effects, usually the secondary metabolites. All the extracts of the plant material were subjected to preliminary phytochemicals screening for the detection of various plant constituents we got positive results with Steroids, Alkaloids, Saponins, Fats and oils, Tannins and Phenolic, Flavonoids, Carbohydrates, Proteins, Amino acid test, in different solvents we have concentrate on Saponins test was performed like Foam test, Haemolysis test and saponin was presents in ethyl acetate and water extract.

Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we isolate the compound with high purity. In the case of sample have more chemical constituents, without information of

concentrations of that chemical constituents, preparativev chromatography is preferable Mobile phase: Benzene: ethyl acetate: water (3:4:3) and we got 11 phytoconstituents separated in different test tube. To analyze the collected fraction of ethyl acetate from flash chromatography samples was scanned under UV in the range of wavelength 200-400 nm. In followed UV spectra it shows change in wavelength that was at 242nm. From this result it was conclude that the fraction was isolated successfully. The collected fraction of fraction adsorbed on sufficient Qty. of silica gel. This residue was then mixed with KBr in the ratio 1:300 and this sample was analyzed. The observed frequencies are Observed frequency in (cm-1), 1072.24, 1187.24, 1384.74, 1804.27, 2898.54.

The decrease in the surface tension of the aqueous solution as a function concentration is plotted in Fig. 3. It can be seen that the surface tension of aqueous surfactant solution decreases rapidly with increase in fraction concentration. For pure water it is 72.14 mN/m and saturates to minimum value of 41.21 mN/m when fraction concentration becomes 0.04 gm/cc. The CMC value for fraction is rather close to the reported value for chemically purified *Sapindus saponin*. Bio-surfactant fraction contains saponin which is responsible for its various functions. It is clear that the crude ritha contain CMC value, which is an essential functional property of a surfactant and could be used as an economical bio-surfactant. Above findings lead to the conclusion that the saponin was sussefully isolated in fraction as surfactant of *Sapindus mukorossi*

IV. REFERENCES

[1]. The Wealth of India. Raw Material. Publication and Information Directorate, CSIR, New Delhi, Vol. 9, 1972: 225

[2]. Kiritker KR and Basu BD: The Indian Medicinal Plants. Lalit Mohan Basu, Allahabad, Second Edition, Vol. 1, 1933:631.

[3]. Chopra R and Ghosh S: Poisonous plants of India. The manager of publishers, Delhi, 1946: 308.

[4]. USDA resource page. United states drug administration website. Available at http//www.plants.usda.gov/java/profile. Accessed –August 21,2008

[5]. Dev I, Guha SRD: Glyceride composition of Sapindus mukorossi (soapnut) oil. Indian Journal of Forestry 1979; 2(3):261-263.

[6]. Sengupta A and Basu SP: Chemical Investigations of the Sapindus mukorossi Seed Oil. Fette, Seifen, Anstrichmittel 2006; 84(10): 411 – 415.

[7]. Azhar I, Usmanghani K, Perveen S, Ali MS and AhmadVU: Chemical constituents of Sapindus mukorossi. (Sapindaceae). Pakistan Journal of Pharmaceutical Sciences 1994; 7(1): 33-41.

[8]. Zikova NI and Krivenchuk PE: Chemical study of flavonoids from the leaves of Sapindus mukorossi Gaerth. Farm. Zh 1970; 25:43-45. Article in Ukranian.

[9]. Chirva V, Kintya PK, Sosnovskii VA, Krivenchuk PE and Zykova NY: Triterpene glycosides of Sapindus mukorossi. II The structure of Sapindoside A& B. Chemistry of Natural Compounds 1970; 6(2): 213-215.

[10]. Chirva V, Kintya PK and Sosnovskii VA: Triterpene glycosides of Sapindus mukorossi. III. The structure of sapindoside C. Chemistry of Natural Compounds 1970; 6(3): 380.381.

[11]. Chirva V, Kintya PK, Sosnovskii VA and Zolotarev BM. Triterpene glycosides of Sapindus mukorossi. IV. The structure of sapindoside D. Chemistry of Natural Compounds. 1970; 6(3): 316-318.

[12]. Chirva V, Kintya PK and Sosnovskii VA. Triterpene glycosides of Sapindus mukorossi. V. The structure of sapindoside E. Chemistry of Natural Compounds. 1970; 6(4): 440-442.

[13]. Yao HK, Hui CH, Li-Ming YK, Ya-Wen H, Kuo-Hsiung L, Fang-Rong C and Yang-Chang W: New Dammarane-Type Saponins from the Galls of Sapindus mukorossi. Journal of Agriculture and Food Chemistry 2005; 53 (12): 4722 -4727.

[14]. Huang HC, Tsai WJ, Morris-Natschke SL, Tokuda H, Lee KH, Wu YC and Kuo YH: Sapinmu saponins F-J, bioactive tirucallane-type saponins from the galls of Sapindus mukorossi. Journal of Natural Products 2006; 69(5): 763-767.

[15]. Huang HC, Wu MD, Tsai WJ, Liao SC, Liaw CC, Hsu LC, Wu YC and Kuo YH: Triterpenoid saponins from the fruits and galls of Sapindus mukorossi. Phytochemistry2008; 69(7): 1609-1616.

[16]. Nakayama K, Fujino H, Kasai R, Mitoma Y, Yata N and Tanaka O: Solubilizing properties of saponins from Sapindu smukorossi Gaertn. Chemical and Pharmaceutical Bulletin 1986; 34(8): 3279-3283.
[17]. Hostettmann K, Marston A (1995) Saponins. Cambridge University Press, Cambridge
[18]. Yangxin YU, ZHAO Jin and Andrew E. Bayly. Development of Surfactants and Builders in Detergent Formulations. Chinese Journal of Chemical Engineering. 2008, 16(4), 517—527.
[19]. Menghao Du, Sumei Huang, Jinping Zhang, Jingwen Wang, Lisong Hu, Jingmin Jiang. Isolation of Total Saponins from Sapindus mukorossi Gaerth. Open Journal of Forestry, 2014. Vol.4, No.1, 24-27
[20]. Runner R.T. Majinda. Extraction and Isolation of Saponins. Natural Products Isolation, Methods in Molecular Biology. 2012. vol. 864, 415-427.
[21]. Yamunadevi M, Wesely E G, Johnson M. Chromatographic finger print studies on saponins of aervalanata(l.) Juss. Ex schultes by using HPTLC. International Journal of current Pharmaceutical Research. 2012 vol 4, issue 2, 52-57

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Green Chemistry - New Approach in Drug Synthesis

Patil Tejaswini D[*1], Harak Shilpa S.[1], Dr. Amrutkar Sunil V.[1]
[*1]GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik, Maharashtra, India

ABSTRACT

Green Chemistry approach is need of the hour in the field of chemistry. Considering the damage to environment, all Pharmaceutical and chemical industry develop interest in new synthetic processes using green chemistry. Most of the drugs are designed, developed and synthesized using various approaches of green chemistry. Microwave assisted synthesis, sonochemistry, solvent free reactions, reuse of catalyst are various approaches of green chemistry used in drug and chemical synthesis. These approaches will be promising in obtaining drug with good yield, less toxic to environment and cost effective.

Keywords: Green Chemistry, Microwave Assisted Synthesis, Solvent Free Reaction, Sonochemistry

I. INTRODUCTION

In early days, drug synthesis is based on the pollute-and-then-clean-up approach. Now a day's more ecofriendly approach of drug synthesis was adopted called Green chemistry. This concept was introduced in the early 1990s in a special program launched by the US Environmental Protection Agency (EPA). It was adopted by mass-media as the new approach of synthesis.[1, 3]

Green chemistry, also called **sustainable chemistry**, is an area of chemistry and chemical engineering focused on the designing of products and processes that minimize the use and generation of hazardous substances.[1,2]

Attention must be paid towards the issues related to safety, health and protection of the environment, due to reactants (starting materials, products and reagents), auxiliaries (mainly solvents) and waste; in order to evaluate the greenness of a particular process. Green Chemistry insists that our synthetic objectives are achieved while assuming additional considerations related to the unnecessary environmental burden created during operations.[4]

Pharmaceutical industry produce a higher ratio of waste per kilogram of product when compared to their peers, such as petrochemical, bulk, fine chemical, and polymer firms. The chemical industry uses two measures to quantify the waste generated by a process:

1) E-factor, which is defined as the unit of waste generated per unit of product (API);
2) PMI, which is defined as unit of raw material used per unit of product.

A lower value on both is desirable, and is the goal that the pharmaceutical industry is driving towards. Cost Savings, Consumer Awareness, Regulations, Development of innovative new products, Senior Management Commitment and increased R&D investment, are key factors for driving adoption of green chemistry by pharmaceutical industry. [5]

In some country to enhance the research and implementation of green chemistry various awards are also given.

- ✓ Australia's Green Chemistry Challenge Awards overseen by The Royal Australian Chemical Institute (RACI).
- ✓ The Canadian Green Chemistry Medal.
- ✓ In Italy, Green Chemistry activities center arrange an inter-university consortium known as INCA.
- ✓ In Japan, The Green & Sustainable Chemistry Network oversees the GSC awards program.
- ✓ In the United Kingdom, the Green Chemical Technology Awards are given by Crystal Faraday.
- ✓ In the US, the Presidential Green Chemistry Challenge Awards recognize individuals and businesses

12 Principles of Green Chemistry: [1, 2, 4,11]

1. **Pollution Prevention:** It is much better to prevent environment pollution rather than first pollute and then clean up.
2. **Atom Economy:** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Synthesis:** Wherever **it is possible**, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. **Designing Safer Chemicals**: The design of chemicals with minimal toxicity reduce the potential risk to human health and the environment; decrease the costs of production and site remediation; and increasing team commitment to workplace health and safety.
5. **Safer Solvents and Auxiliaries:** Reduction or elimination of solvents is often possible while in some cases where solvent is needed, less hazardous solvents should be employed.
6. **Design for Energy Efficiency:** Energy requirements of chemical reactions should be minimized for environmental or economic impacts. If possible, the synthetic methods should be conducted at the ambient temperature and pressure.
7. **Use of Renewable Feedstocks:** Whenever technically and economically practicable, raw material or feedstock should be renewable rather than depleting it.
8. **Reduce Derivatives:** Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis :** Catalytic reagents are superior to stiochiometric reagent. They enhance the selectivity of reaction and extent of conversion to products by reducing temperature.
10. **Design for Degradation :** Chemical products should be designed so that, at the end of their function they break down into degradation products and do not persist in the environment.
11. **Real-time analysis for Pollution Prevention :** It is always important to monitor the progress of the reaction to know when the reaction is complete or to detect the generation of unwanted byproducts. Methods and technologies should be developed so that the prevention or minimization of generation of hazardous waste is achieved.
12. **Inherently Safer Chemistry for Accident Prevention:** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions and fires.

Advantages of Green Chemistry: [7, 8]

Green chemistry has many advantages like

- Non toxic
- Environment Friendly
- Simple
- Sustainable
- Economical
- Safe
- Avoid Waste

Disadvantages Green Chemistry:

- High implementing costs.
- Lack of information
- Lack of awareness about alternative chemical or raw material inputs
- No known alternative process technology
- Uncertainty about performance impacts
- Lack of human resources and skills.

Green Chemistry Approaches:

1) Solvent Free Reactions: [6,7]

Reaction in absence of solvent is one of the method to reduce waste generation of chemicals from reagent. Hence designing of reaction with no use of hazardous and expensive solvents, e.g., "solvent-free" reactions, has gained special attention. Green Chemistry reaction should ideally, be conducted under solvent-free conditions with minimal or no side-product formation and with utmost atom-economy. A solvent-free or solid state reaction may be carried out using the reactants alone or incorporating them in clays, zeolites, silica, alumina or other matrices. Thermal process or irradiation with UV, microwave or ultrasound can be employed to bring about the reaction. Various heterocycles like jasminaldehyde, 4(3H)-quinazolinones, isobenzofuran, pyrazolones, N-substituted pyrroles, Sulphonamide, 6- amino uracil and pregabalin can be synthesized by using solid support. Various name reactions like Aldol condensation reaction, Pinacol-Pinacolone reaction, Pericyclic reaction, Bayer-Villiger Oxidation, Michael Addition are carried out in solvent free environment.

2) Microwave induced green synthesis: [3,9,12]

Microwave reactions involve selective absorption of electromagnetic waves by polar molecules, non-polar molecules being inert to microwaves. In microwave induced organic reactions, the reactions can be carried out in a solvent medium or on a solid support in which no solvent is used. It is one of the good green approach of synthesizing drugs. As it reduces time and also economic, this approach is widely used in synthesis. Only disadvantage is large scale production is not possible. Hence It is used in research, for enhancing the time required for synthesis.

Furans and benzofurans , Pyrroles, indoles , indolizines , Thiophenes , Imidazoles, pyrazoles and benzimidazoles, thiazoles, benzoxazoles , benzothiazoles , Triazoles , Pyridines, quinolines, isoquinolines, Benzopyrans and many more have been synthesized by using microwave. Over-the-counter analgesics such as aspirin, acetanilide, phenacetin, and acetaminophen are conveniently prepared in a microwave at 30% power for five minutes

3) Catalysis: [2,5]

A Green catalyst can play a very important role in chemical processes by replacing reagents, by enabling more efficient processes, by reducing the environmental impact of processes and by reducing the costs of the processes. Biocatalysis offers many attractive features such as mild temperatures, less solvents, biodegradable nature of the enzyme catalyst, high selectivity and functional group compatibilities, all of which favour green chemistry. One of these biocatalysts is Candida Antarctica Lipase, which catalyses alcoholysis, ammoniolysis, and perhydrolysis reactions. Clay-supported zinc chloride (clayzic), HMS-supported zinc triflate, Zeolites are popularly used catalyst.

4) Aqueous mediated synthesis:

Aqueous mediated reactions offer useful and more environmentally friendly alternatives to their harmful organic solvent versions and have received increasing interest in recent years. benzothiazoles/benzothiazolines, Thioesters, Fischer indole synthesis and Michael addition of nitroalkanes are few examples of Aqueous mediated reactions.

5) Ionic liquids: [2, 4]

A new process to separate problematic chemicals from ionic liquids was proposed by Dame et al. Ionic liquids occur in two main categories, namely simple salts (made of a single anion and cation) and binary ionic liquids (salts where equilibrium is involved). For example, [EtNH3][NO3] is a simple salt whereas mixtures of aluminum(III) chloride and 1,3-dialkylimidazolium chlorides (a binary ionic liquid system).

Synthesis of (S)-Naproxen in the ionic liquid BMIM-PF4 has been reported. The very common organic reaction, Friedel-Crafts alkylation proceeds smoothly and efficiently in chloroaluminate (III) ionic liquids.

6) Sonochemistry in Organic Synthesis, without Solvents

Sonochemistry is also considered a methodology of organic reactions without solvents. Their use has been described before and it is obvious that their applications in organic chemistry will be extended further. High yields, low energy requirements, low waste, no use of solvents are some of the fundamental advantages of these sonochemical techniques.

Some application of green Chemistry in Drug Synthesis:

Ibuprofen was synthesized by conventional method in 1960 and widely used. In 1990 new green chemistry

approach is used. It is synthesized in three steps using Nickel (Raney nickel) as catalyst which can be recycled and reused.

Adipic acid is a very important starting material for Nylon-6,6 and catehole (which is used in the pharmaceutical and pesticide industries). Other scientists promoted the biocatalytic method of synthetic adipic acid from D-glucose. It is achieved with genetically transgenic bacteria *Klebsiella pneumoniae*, a non-toxic strain of *Escherichia coli, (Enterobactiriaceae)*.[13]
Methods for synthesis of Aspirin with microwave irradiation using catalysts such as $AlCl_3$, H_2SO_4, H_3PO_4, $MgBr_2.OEt_2$, $CaCO_3$, NaOAc, Et_3N and solvent-free approach have been designed.[14]

Anastas et al has described synthesis of Naproxen with chiral metal catalyst containing BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthyl) ligand with good yields.[15]

The green synthesis for a key intermediate of atorvastatin has been developed in two steps. First step involves the biocatalytic reduction of ethyl-4-chloroacetoacetate using a ketoreductase in combination with glucose and a NADP-dependent glucose dehydrogenase (GDH) for cofactor regeneration. The (S)ethyl-4-chloro-3-hydroxybutyrate product is obtained in very good yield. In the second step, a halohydrindehalogenase (HHDH) is employed to catalyze the replacement of the chloro substituent with cyano, by reaction with HCN at neutral pH and ambient temperatures. These natural enzymes were highly selective for the reactions. [16]

Tetrahydropyrimidinones was synthesized by Biginelli reaction using conventional heating. A. k. Bose et al has used modified version of Toda"s method of grinding solids together for solvent-free chemical reactions called Grindstone Chemistry to synthesize tetrahydopyridinones using p-toluenesulfonic acid as an acid catalyst. [3]

II. ACKNOWLEDGEMENTS

We are grateful to Gokhale Education Society and its members for their encouragement and support.

III. REFERENCES

[1]. Rajiv Dua,et al, 'Green Chemistry and Environmentally Friendly Technologies: A Review', Middle-East Journal of Scientific Research, 11 (7), 2012, 846-855.
[2]. Smita Talaviya and Falguni Majmudar, 'Green chemistry: A tool in Pharmaceutical Chemistry', NHL Journal of Medical Sciences, 1 (1), 2012, 1-13.
[3]. Ajay K. Bose, 'A simplified green chemistry approach to the Biginelli reaction using Grindstone Chemistry', Tetrahedron Letters, 45, 2004, 8351–8353.
[4]. Santosh V. Padghan et al, 'The Use of Green Chemistry Approach in Organic Synthesis: Focus and Review', www.epitomejournals.com, I(VII), 2015, 1-9.
[5]. Vivek Sharma, 'Applicability of Green Chemistry in Pharmaceutical Processes', Pharma Bio World (Features), 2015, 34-36.
[6]. Gopalpur Nagendrappa, 'Organic Synthesis under Solvent-free Condition. An Environmentally Benign Procedure – I', Resonance, 2002, 59-68.
[7]. M. Himaja et al 'Green Technique- solvent free synthesis and its advantages', International journal of research in Ayurveda and Pharmacy, 2(4), 2011, 1079-1086.
[8]. Monu Bhardwaj and Neelam, 'The Advantages and Disadvantages of Green Technology', Journal of Basic and Applied Engineering Research, 2(22), 2015, 1957-1960.
[9]. Veena Yadav, Nishant Yadav, 'Microwave assisted green chemistry approach to the synthesis of some novel N1-amino-methyl-substituted 1,4-Benzodiazepine derivatives', Archives of Applied Science Research, 3 (1), 2011, 139-144.
[10]. Sonali R. Sharma, 'Green Chemistry, Green Solvents and Alternative Techniques in Organic Synthesis', International Journal of Chemical and Physical Sciences, 4, 2015, 516-520.
[11]. W. Wardencki, 'Green Chemistry- Current and Future Issues', Polish Journal of Environmental Studies, 14 (4), 2005, 389-395.
[12]. Savita Belwal, 'Green revolution in chemistry by microwave assisted synthesis: A review', Modern Chemistry, 1(3), 2013, 22-25.

[13]. Draths CM, Frost JW. 'Microbial catalysis. Synthesis of adipic acid from D-glucose', ACS Symposium Series, 577, Chapter 3, 1994, 32-45.
[14]. Anastas PT, Lauren BB, Mary MK, Tracy CW., 'The Role of Catalysis in the design, development, and implementation of Green Chemistry' Catalysis Today, 55(1), 2000, 11-22
[15]. Ingrid M, David S, Marilyn G, Joaudimir C and Johanna F., 'A Greener Approach to Aspirin Synthesis using Microwave Irradiation', J Chem Edu, 83(4), 2006, 628
[16]. Steven KM, John G, Chris D, Lisa N, David G, Alica W et al., 'A green-by-design biocatalytic process for atorvastatin intermediate', Green Chem, 12, 2010, 81-86

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Phytochemical Study of White Variety Seed of Abrus Precatorius. Linn (Leguminosae) an Unexplored Medicinal Plant of India

Jyoti Randhawane*, Shinde, Dr. Shobha Borhade
Department of Chemistry, Akole College, Akole, Ahmednagar Maharashtra, India

ABSTRACT

Plant medicines are great importance in the primary healthcare in many developing countries. According to World Health Organization (WHO) still about 80% of the world population rely mainly on plant-based drugs. In Ayurveda, Siddha, and Unani, utilizing a large number of medicinal plants were used for the treatment of human diseases [1].The medicinal plants occupied a unique place in human life. It provides more information about the use of plants or plant parts as medicine[2]. Medicinal plants are one of the most sensitive commodity areas of research in the world today. The medicinal plants have been used by humans from the pre-historical times.Medicinal plants play a vital role in drug discovery . About 50 drugs have been discovered from ethnobotanical leads by translating folk knowledge into new pharmaceuticals [3]. Plant-based drugs used in the traditional medicine have paid great attention because it is easily available, less expensive and also have no side effects [4].Plants have the ability to synthesize a wide verity of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailments for mankind. The evaluation of these drugs is primarily based on phytochemical, pharmacological and allied approaches including various instrumental techniques such chromatography, microscopy and others. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential[5]. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties [6].Plants have a great potential for producing new drugs and used in traditional medicine to treat chronic and even infectious diseases [7]. India has a unique position in the world, where a number of recognized indigenous systems of medicine are available for the health care of people. No doubts that the herbal drugs are popular among rural and urban community of India. The demands for plant based medicines are increasing very fast in India. Among the traditional system of medicine Abrus precatorius L is one of the important herb commonly known as Indian licorice Fabaceae.

Keywords : Medicinal Plants, World Health Organization, Abrus Seeds, Ulcerogenic

I. INTRODUCTION

The seeds of Abrus precatorius have been used through history in a variety of roles. The Abrus seeds have also been used for medicinal purposes, including the treatment of chronic eye disease. Arabic culture has purportedly used the seed as an aphrodisiac known as coq's eye. The toxicity of theAbrus seed was associated with its use as a fish poison as well as a homicidal agent.The poisoning by the seeds of Abrus precatorius has been reviewed and reported often in literature. Death has been reported with twenty seeds bended with water. The symptoms included vomiting of blood, severe pain in the eyesand burning of ears. Death ensued in two days [8].

The word "Phyto" is the Greed word for plant. Phytochemicals, which not only that they are nonnutritive. Phyto chemicals that have protective or disease preventive properties but also protect human from a host of diseases [9]. From thousands of years, plants have been utilized as medicines. Major constituents of more than 50% of all the drugs in clinical use are natural products and their derivatives [10]. Medicinal herbs constitute effective sources of antimicrobial and antioxidant natural products[11]. Medicinal herbs are an important source for the therapeutic remedies of various ailments [12]. Plant was

used asanthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were reported for more research scholars and scientist. There has been an growing interest in the study of medicinal plants as natural products in diverse parts of the world[13]. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases [14]

This article aims to provide a comprehensive review on the phytochemical study of white variety seed of aspects of *Abrus Precatorius L*

Taxonomical classification

Kingdom: Plantae
Division: Magnoliophyta
Order: Fabales
Family: Facaceae
Subfamily: Facoideae
Tribe: Abreae
Genus: Abrus
Species: Abrus precatorius

Common names in India

Sanskrit	Gunja
Hindi	Rati,Gaungchi,Gunchi, Gunja
Bengali	Kunch,Koonch,Chunhali
Gujarati	Gumchi,Chanothi
Kannada	Gurugunji
Kashmiri	Shangir
Malayalam	Kunni,Gundumani
Persian	Gunchi,Chashami-Khurosa
Punjabi	Mulati
Tamil	Gundumani, Kunthamani
Telugu	Guruginia

Common name according to different countries

Egypt	Rosary pea
Nepal	Crab's eye
Philippines	Jequerity
USA	Precatory bean
Indonesia	Saga
Pakistan	Gunchi

Figure.

II. MATERIALS AND METHODS

Collection of seed sample

The white seeds were collected from the garden cleaned and chopped into small pieces, shade dried and coarsely powdered and stored in a sealed vessel wrapped with a polyethylene bag at 4^0c.

Preparation of extracts

After cleaning and removal of the sand and foreign materials, the dried and powdered material were ground to a fine powder using a grind. The seed powder sample was extracted with methanol and n-hexane (1:4 w/v) by continuous extraction in a soxlet apparatus for 48 h at 40-60^0c. The extracted was separated from solvent by rotavapour at 40^0c. After extraction and purification the sample were filtered and stored.

Preliminary Phytochemical screening

Test for alkaloids:

Extract was dissolved individually in dilute Hydrochloric acid and Solution was clarified by filtration.

a. Mayer's Test:

Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b. Wagner's Test:

Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide)Formation of brown/reddish precipitate indicates the presence of alkaloids.

c. Dragondroffs Test:

Filtrate was treated with Dragondroffs reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for Flavonoids

a. Alkaline Reagent Test:

The Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute HCl acid, indicates the presence of flavonoids.

b. Lead acetate Test:

The Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for Carbohydrate

a.Molisch's Test:

To 1 ml of extract, 2 drops of Molisch's regent was added in a test tube and 2 ml of concentrate H2SO4 was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of carbohydrate.

b.Benedict's Test :

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brownprecipitate to show a positive result for the presence of carbohydrate.

c. Fehling's Test:

Extracts were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A
& B solutions. Formation of red precipitate indicates the presence of carbohydrate.

Test for phenols

a. Ferric Chloride Test:

The aqueous solution of extract was treated with three drops of freshly prepared 1% ferric chloride and potassium ferrocyanide. Formation of bluish-green colour was taken as positive. The methanol extract was dissolved in water. Few crystals of ferric sulphate were added to the mixture. Formation of dark violet colour indicated the presence of phenolic compounds.

Test for Saponins

a. Froth Test:

Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Test for Proteins

a.Biuret Test:

Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Phytosterols

a. Salkowski's test:

The extract was dissolved in 2 ml chloroform in a test tube. Conc. Sulphuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface
indicated the presence of a steroid ring i.e., glycoside.

b. Liebermann Burchard's test:

The Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for Oil & Fats

a.Filter paper test:

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicates the presence of fixed oils.

Test for Glycosides

a.Keller Killiani Test:

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

b.Borntrager's Test:

To the 3ml of aqueous extract, dil. H2SO4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Test for Tannins

a. Ferric chloride test

The extract was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

b. Lead acetate test.

The extract was dissolved in water and to that 10% Lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Coumarins

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescenceindicated the
presence of coumarins

Test for Anthraquinones

About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath, filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrates. Few drops of 10% ammonia was added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthroquinone.

Test for Anthocyanins

The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract with 2 mL of 2 N HCl. The appearance of apink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins

Detection of Diterpenes

Copper acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

Table 1. Results of phytochemical analyses of the seed (white variety) of Abrus precatorius. L

Sr.No.	Variable	Methanol extract	n-hexane extract
1	Alkaloids	+	+
2	Flavonoids:	++	+
3	Carbohydrate:	-	-
4	Phenols:	+	+
5	Saponins	+	+
6	Proteins	+	+
7	Phytosterols	-	-
8	Oil	-	+
9	Fats	-	-
10	Glycosides:	+	+
11	Tannins	-	-
12	Coumarins	-	-
13	Anthraquinones	-	-
14	Anthocyanins	+	+
15	Diterpenes	-	-

(+) = Presence, (-) = Absence

III. REFERENCES

[1]. Valsaraj R, Pushpangadan P, Smitt UW, Adersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol1997; 58: 75-83.

[2]. SaikiaB. Ethanomedical plants from Gohpur of Sonitpur district,Assam. Ind j Traditional knowledge 2006; 5(4): 529-530.

[3]. Cox, PA. 1994. The ethnobotanical approach to drug discovery: Strengths and limitations.25-41

[4]. Cathrine L, Prabavathi N. Preliminary Phytochemical Analysis and Antibacterial Activityof leaf extracts of Vitex leucoxlonL.F. IntJ Curr PharmRes2011;l3: 71-73.

[5]. R. K. Gupta, Medicinal & Aromatic plants, CBS publishers & distributors, 1st edition, 2010, 116-117

[6]. Mahesh B,Sathish S. Antimicrobial Activity of some important medicinal plant against plant and HumanPathogens. World J AgriSci 2008; 4(suppl):839-843.

[7]. Panda SK, Thatoi HN, Dutta SK. Antibacterial activity and phytochemical screening of leaf and bark extracts of Vitex negundofrom Similipal biosphere reserve Orissa. J Med Plant Res2009;3(4):294-300

[8]. Buchanan E., Grove man dies after eating rosary beans. Miami Hearld1976; Miami FI, USA.

[9]. Suleiman M. N, Der Pharmacia Sinica, 2011, 2 (4):108-111.

[10]. Satwinderjeet kaur, Subodh kumar, American Journal of Biomedical Sciences, 2010, 2(2):164-177.
[11]. Calixto, B.J, Brazilian journal of Medicinal and Biological Research, 2000, 33(2): 179 – 189.
[12]. Moses A.G, Maobe, European journal of Applied Science,2013, 5 (1): 01-06.
[13]. Rajeswari G, Murugan M and Mohan VR, Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012, 3(4): 301-308.
[14]. Rajeev Nema, International journal ofPharm.Phytochemical.Res, 2012,1(5): 283-286.
[15]. Wagner KH, Elmadfa I. Biological relevance of terpenoids: Overview focusing on mono-di and tetraterpenes. AnnNutr Metab 2003; 47:95-106.
[16]. Dasgupta S, Parmar A, Patel H. Preliminary phytochemical studies of Kalanchoe Gastonis-bonnieri.Int J Pharm Bio Sci 2013; 4:550-557.
[17]. Kharat SS, Kumkar PB, Siddhesh RR, Sonawane KS. Qualitative phytochemical screening of
[18]. Gnidia glauca(Fresen) Gilg. Plant extract. Int J Pharm Bio Sci 2013; 4:144-148. Kavitha R, Premalakshmi V. Phytochemical analysis of ethanolic extract of leaves of Clitoria ternatea L. Int J Pharm Bio Sci 2013; 4:236-242.
[19]. Raphael E. Phytochemical constituents of some leaves extract of Aloe veraand Azadirachta indicaplant species. Glo Adv Res J Environ Sci Toxicol 2012; 1:14-17.
[20]. Kumari SPK, Sridevi V, Lakshmi MVVC. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. J Chem Bio Phy Sci 2012; 2:1326-1332.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Validated HPTLC Method for the Quantification of B-Sitosterol In Leaves, Bark of Putranjiva Roxburghii Wall

Kalyani Abhimanyu Kedar[*1,2], Sanjay R. Chaudhari[3], Avanapu S. Rao[4]

[1]P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India
[2]Jawaharlal Nehru Technological University (JNTU), Hyderabad, Andra Pradesh, India
[3]Rasiklal M. Dhariwal Institute of Pharmaceutical Education and Research, Pune, Maharashtra, India.
[4]Bhaskar Pharmacy College, Yeknapally, Moinabad (Mandal), R.R. (Dt), Hyderabad, Telangana, India

ABSTRACT

Objective: A simple and sensitive high-performance thin-layer chromatography method was developed and validated for the determination of β-sitosterol in Putranjiva roxburghii Wall leaf and bark
Methods: Analysis of samples was performed on TLC aluminium precoated plate (60F 254) by using mobile phase toluene: ethyl acetate: formic acid (9:1:0.1v/v/v). TLC plate derivatized with vanillin sulphuric acid reagent. The method was validated using International Council for Harmonization (ICH) guidelines, including linearity, precision, accuracy, and robustness.
Results: A good linearity relationship was found to be with correlation coefficient (r2) value of 0.9951 for β-sitosterol, from calibration curve it shows presence of 0.16%w/w for β-sitosterol in leaf extract, 0.07% w/w in bark extract of Putranjiva roxburghii Wall (Family:Euphorbiaceae). Limit of detection and limit of quantitation was found to be 0.04, 0.13 ng spot-1 respectively for β-sitosterol. The interday and intraday precision was found to be 1.33%, 1.99% (%RSD). Accuracy of the method was performed by recovery studies at three different concentration levels and the average percentage recovery was found to be 98.05% for β-sitosterol.
Conclusion: The proposed method for the quantitation of β-sitosterol was found to be simple, specific, accurate and robust in Putranjiva roxburghii Wall.
Keywords: Putranjiva roxburghii Wall; Euphorbiaceae; β-sitosterol; HPTLC; Method validation.

I. INTRODUCTION

Euphorbiaceae family having 220 genera and 4,000 plant species found in various tropical regions of India [1-2]. Following genera of Euphorbiaceae are reported as medicinal plants: *Acalypha, Aleurites. Bridelia, Jatropha, phyllantus, Putranjiva, Ricinus* [2-3,4]. The species commonly seen in India is *Putranjiva roxburghii* Wall which is known as child's amulet tree or child-life tree [5]. *Putranjiva roxburghii* is evergreen tree with drooping branches with corky bark coriaceous leaves, dioeciously flowers [6].

Most frequently recorded folk remedy claims of *Putranjiva roxburghii* Wall mentioned that the plant leaf, bark, seed, nuts are medicinally useful. Paste of seeds of *Putranjiva roxburghii* applied on forehead to check pain. The seeds of this plant species are given daily for one month to women for conception [6]. The bark and the seeds are usefull in antidotal treatment of snake-bite. Its leaves and fruits, stones of this plant have been traditionally used for the treatment of fever, muscle twisting, aphrodisiac, arthralgia and rheumatism [7-9]. It is also used as antinociceptive, antipyretic, anti-inflammatory, antioxidant [10]. This plant has reported various phytoconstituents such as putranjivanonol, putranjic acid, friedelin, putranjivadione, friedelanol and roxburgholone from the trunk bark of *Putranjiva roxburghii* [11-13]. Roxburghonic acid, putraflavone were isolated from the alcoholic extract of *Putranjiva roxburghii* leaves [14].

β-Sitosterol is a dietary supplements, found in a variety of plants and plant oils. Phytosterols are similar in structure to cholesterol except some minor structural differences [15]. β-Sitosterol was estimated by HPLC in

Ampelocissus latifolia (Roxb.) species [16] Based on literature β-Sitosterol has promised antidiabetic activity and it shows antioxidant effects and specifically useful for hypercholesterolemia and radioprotective acivity [17-20]. The was also studied Some reports are available on plant sterols and plant stanols use full in the management of hyperlipidaemia [21]. β-sitosterol isolated from *Syzygium cumin* (L.) inhibits the activity of α-amylase enzyme and thus slows down the glucose release in the blood stream. Previous analytical work includes that amentoflavone, β-amyrin and stigmasterol determined from *Putranjiva roxburghii* by HPTLC method [22, 23]. So an attempt has been made to carry out chromatographic analysis of leaves and bark of *Putranjiva roxburghii* Wall.

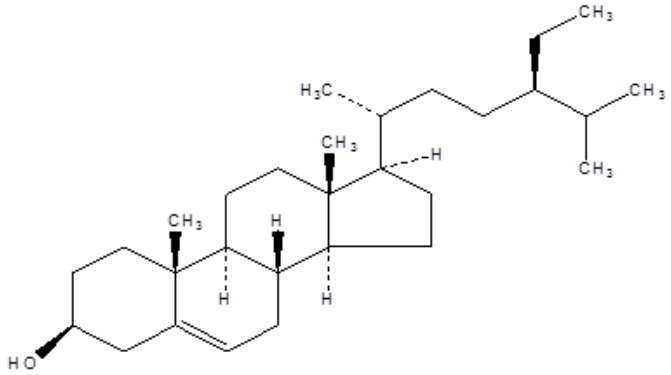


Figure 1. Chemical structure of β-sitosterol

II. MATERIALS AND METHODS

Plant material

The leaves and trunk bark material of fully grow tree of *Putranjiva roxburghii* Wall was collected from Khadaki region of Maharashtra, India in June 2014. The taxon is authenticated from Botanical Survey of India, Pune dated 18/08/2014 with Voucher number BSI/WRC/Cert./2014 and collection no.KKA 01. The herbarium specimen is deposited in the Modern college of pharmacy, Nigdi, Pune.

Chemicals and reagents

β-sitosterol was purchased from Sigma-Adrich (USA). All other solvents, reagents and Silica gel 60 F_{254} precoated HPTLC Plates (20 × 20 cm) were purchased from Merck (Germany).

HPTLC instrumentation and experimental conditions

Method development parameters includes sample and test solution preparation, HPTLC instrumentation condition, preparation of developing chamber, derivatization reagents were carried out as per guideline mention in USP (Ch.203). According to this chromatographic analysis was done on 10×10 cm HPTLC Silica gel F_{254} plates. Samples of extracts, formulations and standards were applied as band length 8 mm wide and 8mm apart by Camag Linomat 5 sample applicator. The application rate of sample on plate was $150nl^{-1}$. The plate was developed in previously saturated 10×10 cm twin-trough glass chamber at room temperature. Initially different mobile phases were use for chromatogram development from this best resolution was observed in the composition of toluene: ethyl acetate: formic acid (9:1:0.1v/v/v) for β-sitosterol. Dry TLC plate derivatized with vanillin sulphuric acid reagent, heat plate at 105^{o}C and observed separation of bands it helps in analysis of β-sitosterol in leaf and bark Extracts [24]. Analysis was done at 540nm in absorbance remission mode by win CATS Chromatography software.

Preparation of standard solution

A stock solution of β-sitosterol (100µg/ml) was prepared by dissolving 10mg of accurately weighed β-sitosterol in 100ml chloroform. For calibration 0.2-1.2µl standard solution was applied to HPTLC plate in the range 20-120 ng per band.

Preparation of sample solution

Extract of leaves and bark were prepared by weighing 50gm of dried powdered drug of leaves and bark of *Putranjiva roxburghii* and extraction was carried out by soxhlet extraction assembly for 6hrs. Solution was filtered, concentrated and use for HPTLC analysis. From this weigh 10mg leaf and bark extract and transferred to a 10ml volumetric flask. Chloroform was added to volumetric flask to make final concentration (1000µg/ml)

Method validation

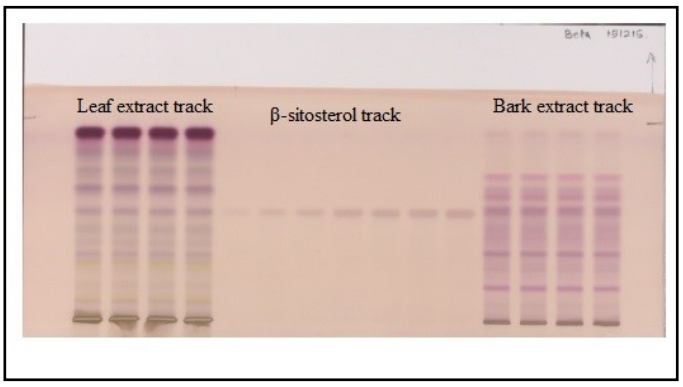
The analytical method was validated for linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantitation (LOQ) according to ICH guidelines (ICH, 2005). The linearity was carried out by

applying different concentration of standard β-sitosterol. Quantitation of marker in sample was carried out by calibration curve. LOD, LOQ were carried out according to formula {LOD= 3.3(SD/S) and LOQ= 10 (SD/S)}. Precision studies include, repeatability and system precision. Accuracy by recovery studies were carried out by spiking known concentration of standard to pre-analyzed samples. The robustness was carried out by making small variation in optimized method parameters such as variation in composition of mobile phase, chamber saturation time etc. The specificity of the method was determined by comparing R_f values.

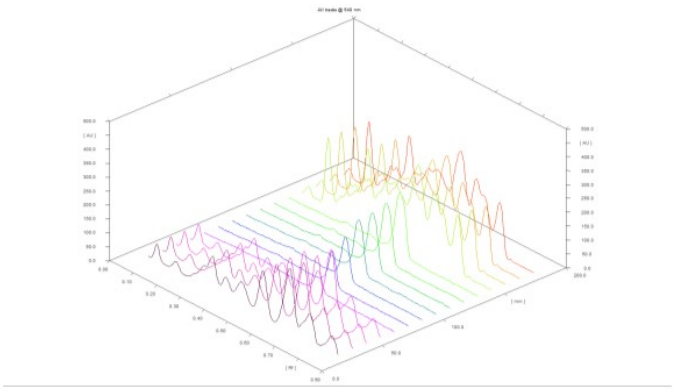
III. RESULTS AND DISCUSSION

Solvent system optimization

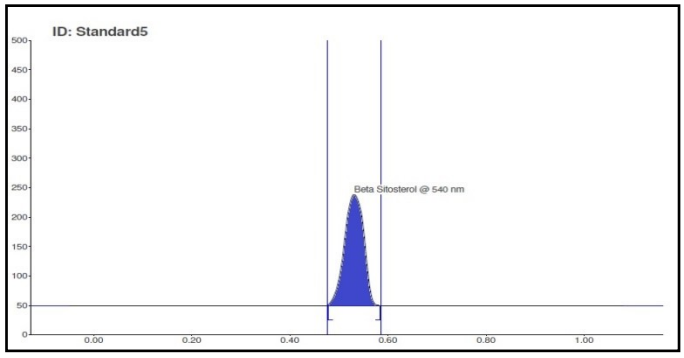
For optimization of solvent system various compositions of mobile phases were use. When mobile phase consisting toluene: ethyl acetate (10:1) component in samples not get resolved. In ordered to improve resolution in between peaks mobile phase in composition of toluene: ethyl acetate: formic acid (9:1:0.1v/v/v), gives compact peak of standard and standard in samples. Observation shows the same R_f value (0.5) (Fig. 3) for β-sitosterol in standard and samples.



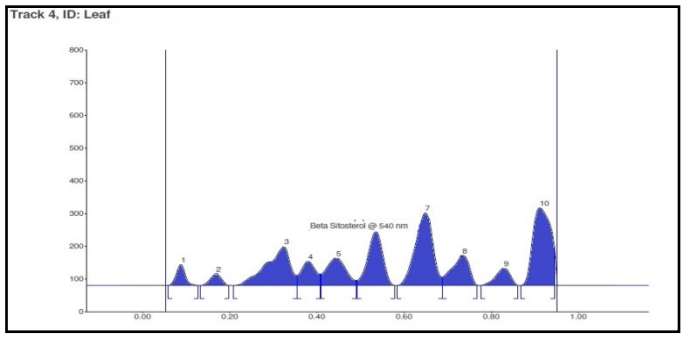
(a)



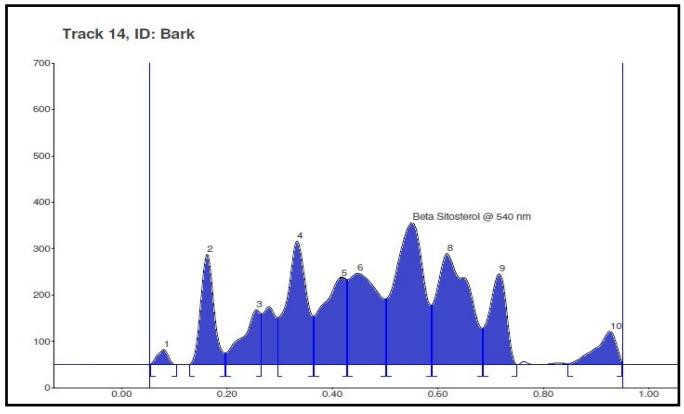
(b)



(c)



(d)



(e)

Figure 3. HPTLC Chromatogram of leaf, bark with β-sitosterol (a), 3D display of leaf, bark with β-sitosterol chromatogram (b), HPTLC Chromatogram of standard β-sitosterol (c), leaf extract (d), Bark extract (e) at 540nm

IV. METHOD VALIDATION

Linearity

For determining the linearity range of standard β-sitosterol, a series of spots of different volumes (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 µl) were applied so as to get 20-120 ng quantity of standard per band, respectively. Linearity was evaluated in triplicate. The plate was scanned at 540nm and curve was prepared with respect to area *vs.* amount per spot (Figure 2). A good linearity relationship was found to be with correlation coefficient (r^2) value of 0.9951 for β-sitosterol (Table 1 and Figure 2)

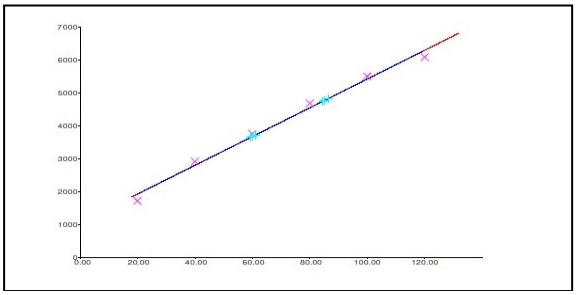


Figure 2. Calibration curve of Standard β-sitosterol at 540nm

Quantification of β-sitosterol

0.6µl of the plant leaf, bark extract were applied to HPTLC plate in triplicate and the amount of β-sitosterol in samples were determined by using calibration curve of standard.

This method shows the presence of 0.16%w/w for β-sitosterol in leaf extract, 0.07% w/w in bark extract.

Limit of Detection and quantitation

In order to determine limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on standard deviation (SD) and slope (S) of the calibration curve at levels approaching to the LOD according to formula { LOD= 3.3(SD/S) and LOQ= 10 (SD/S)}. LOD and LOQ calculated and found to be 0.04, 0.13 ng $spot^{-1}$ for β-sitosterol (Table 1).

Table 1. Method validation parameters for the quantitation of β-sitosterol by HPTLC.

Parameters	Results
Range of linearity (ng $spot^{-1}$)	20-120
Regression of equation	Y = 1048 + 43.54 * X
Slope	43.54
Correlation coefficient (r^2)	0.9951 ±0.003
LOD	0.043 ng
LOQ	0.13 ng

Precision

Precision studies were carried out to show the reproducibility of the proposed developed method. Intraday precision study was carried out by applying six times 60ng per band of same concentration. It can be analyzing at three different times in a day for intraday precision and the same procedure was followed for three different days to determine interday precision. The results were reported as SD (%RSD) (Table 2). The % RSD was found to be 1.33 %, 1.99% for interday and intraday precision. The low %RSD indicated the method is precise for the analysis (Table 2).

Table 2. Interday and intraday precision for quantitation of β-sitosterol by HPTLC densitometric method.

Concentration (ng $spot^{-1}$)	β-sitosterol			
	Interday precision		Intraday precision	
	Mean peak area ± SD*	%RSD**	Mean peak area ± SD	%RSD
60	3775.182±50.49	1.33	2781.45±55.41	1.99

* SD-standard deviation, ** RSD-relative standard deviation.

Specificity

The specificity of the method was determined by analysing standard drug and sample. The presence of β-sitosterol in leaf, bark were confirmed by comparing R_f of sample with standard.

Recovery studies (Accuracy)

Accuracy of method was studied by performing recovery studies at 3 levels of β-sitosterol. The pre-analyzed samples were spiked with 80%, 100% and 120% of the standard β-sitosterol and analyzed by the proposed HPTLC method. The experiment was conducted six times the percentage recovery at three different levels of β-sitosterol was found to be 98.14, 99.01, 97% respectively (Table 3).

Table 3. Accuracy (recovery study) determined for the TLC-densitometric method.

% of standard spiked to the sample	Theoretical content (µg $spot^{-1}$)	Experimental content (µg $spot^{-1}$±SD*)	Recovery (%)	RSD* (%)
80	0.108	0.106	98.14	0.94
100	0.12	0.1188	99.01	0.43
120	0.13	0.1261	97.00	1.36

* SD-standard deviation, ** RSD-relative standard deviation.

Robustness

Robustness was studied in triplicate at 60ng $band^{-1}$ by making small variation in optimised method parameters

such as variation in composition of mobile phase, chamber saturation time. The results were examined in terms of relative standard deviation (%RSD) and standard error of peak area (Table 4). Mobile phase prepared by solvent system such as Toluene: Ethyl acetate: Formic acid in composition (9: 1:0.1 v/v/v), (9.1:0.5: 0.1 v/v/v), (8.9:1.1:0.1 v/v/v) etc. Duration of saturation time change during chromatograph development (15, 20 and 25 min) respectively. The plate was activated at 110^0C for 20 min and analysed at 540nm. By introducing small changes into TLC method % RSD was obtained less than 2% proved the robustness of proposed method.

Table 4. Robustness study

Parameters				
Mobile phase (Toluene: Chloroform)				
Actual (v/v)	Used(v/v)	Level	Mean peak area ± SD*	%RSD**
Toluene: Ethyl acetate: Formic acid (9:1:0.1v/v/v)	9.1:0.5:0.1	+1	3291.34±36.34	1.11
	9:1:0.1	0	3387.7±55.04	1.62
	8.9:1.1:0.1	-1	3281.94±47.94	1.46
Saturation Time (Minutes)				
Actual				
20	15	-5	3358.73±54.22	1.61
	20	0	3331.34±46.63	1.39
	25	+5	3267.89±1.43	1.43

* SD-standard deviation, ** RSD-relative standard deviation.

V. CONCLUSION

In present study HPTLC method was developed and validated for the determination of β-sitosterol in *Putranjiva roxburghii* Wall leaf and bark extracts, which shows 0.16, 0.07% w/w respectively. The developed method found to be simple, accurate, specific and robust for the analysis of β-sitosterol in crude drug sample. Based on these results leaves of this plant contains higher β-sitosterol may be use full for analysis of various biological activity. So this proposed method may be useful in analysis of β-sitosterol containing plant species and polyherbal formulations.

VI. ACKNOWLEDGMENTS

Authors are thankful to Botanical Survey of India (BSI), Pune, Anchrom Pvt. Ltd. Mumbai for instrumental facility.

VII. CONFLICTS OF INTEREST

There are no conflicts of interest.

VIII. REFERENCES

[1]. Anonymous, The wealth of India: raw materials, New Delhi Publications and Information Directorate, C.S.I.R; 2003.p.134-5

[2]. Kirtikar KR, Basu BD. Indian Med. plants, 2nd edition. International Book distributors Dehradun; 1999.p.2190.

[3]. Ambasta SP. The useful plants of India. C.S.I.R. New Delhi; 1986.

[4]. Nadkarni KM. Indian Materia Medica. 3rd ed. Bombay Prakashan; 2002.p.1036

[5]. Vaidya U, Nishteswar K. Review Article PUTRANJIVA- A HERB FOR PUMSAVAN (MALE PROGENY FACILITATOR)?. Inter J of Ayu and Pharma Research 2015; 3: 11-6.

[6]. Kumar S. Unexplored Ethnobotanical Uses of Some Plants of Family Euphorbiaceae. Journal on New Biological Reports 2012; 1: 67-69.

[7]. Baslas RK. Phytochemical studies of the plants of the genera Euphorbia part II. Herba Hungerica 1982; 21: 115-26.

[8]. Boonyaprapat N, Chokechaicharoenporn A. Samunprai Maipuenban, Prachachon, Bangkok; 1999.p.262-73

[9]. Chand J. Phytogeographical Analysis of Medicinal Plants in Renuka Forest Division, Research Reformer 2014; 26:21-36.

[10]. Durre S, Muhammad AR, Afifa S, Madiha R, Faiza IC, Merva J, Sami U, Saif U. Antioxidant potential of the extracts of Putranjiva roxburghii, Conyza Bonariensis, Woodfordia fruiticosa and Senecio chrysanthemoids, African J of Biotech 2012; 11: 4288-95.

[11]. Garg HS, Mitra CR. Putranjiva roxburghii Wall.-II triterpenes of the trunk bark, Phytochemistry 1968;7: 2053-55.

[12]. Sengupta P, Mukherjee J. Terpenoids and related compounds XI the structure of roxburgholone, a new triterpenoid constituent of Putranjiva roxburghii, Tetrahedron 1968; 24: 6259-64.

[13]. Mukherjee T, Sarkar T, Paul P, Chakraborty AK, Jaisankar P, Mukhopadhyay SB. Putralone, a novel 10alpha-hydroxy-25-nor D: A friedo-

oleanane triterpenoid from Putranjiva roxburghi, Nat Prod Communi 2012; 7: 511-13.
[14]. Garg HS, Mitra CR. Putraflavone, a new biflavonoid from Putranjiva roxburghii. Phytochem 1971; 10: 2787-91.
[15]. Mark AM. Complementary and alternative medicine for prostate and urologic health. In: Complementary and alternative medicine interventions for BPH/LUTS, Springer Publisher; 2014:43.
[16]. Pednekar PA, Kulkarni V, Raman B. High performance liquid chromatographic method development and validation for the simultaneous determination of gallic acid and beta sitosterol in Ampelocissus latifolia (roxb.) Planch. Asian Journal of pharmaceutical and clinical Research 2014; 7(2):86-89.
[17]. Vinutha K, Vidya SM, Suchetha NK, Ganesh S, Nagendra HG, Pradeepa, Vaman CR. Radioprotective activity of Ficus racemosa ethanol extract against electron beam induced dna damage in vitro, in vivo and in silico. International Journal of pharmacy and Pharmaceutical Sciences 2015; 7(6); 110-119.
[18]. Alam MR, Rahman AB, Moniruzzaman M, Kadir MF, Haque MA, Alvi MR, Ratan M. Evaluation of antidiabetic phytochemicals in Syzygium cumini (L.) skeels (Family: Myrtaceae). J of App Pharma Sci 2012; 2: 94-98.
[19]. Gupta R, Sharma AK, Dobhal MP, Sharma MC, Gupta RS. Antidiabetic and antioxidant potential of ß-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes 2011; 3: 29-37.
[20]. Soodabeh S, Azadeh M, Ahmad RG, Mohammad A. The Story of Beta-sitosterol- A Review. Euro J of Med Plants 2014; 4: 590-609.
[21]. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegard L, Jessup W, Jones PJ, Lutjohann D, Maerz W, Masana L, Silbernagel G, Staels B, Borén J, Catapano AL, De Backer G, Deanfield J, Descamps OS, Kovanen PT, Riccardi G, Tokgözoglu L, Chapman MJ. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. European Atherosclerosis Society Consensus Panel on Phytosterols. Atherosclerosis 2014; 232: 346-360.
[22]. Ravishankara MN, Pillai AD, Padh H, Rajani M. A sensitive HPTLC method for estimation of amentoflavone, a bioactive principle from Biophytum sensitivum (Linn) and Putranjiva roxburghii Wall. J. Planar Chromatogr 2003; 16:201-5.
[23]. Badole M. β-amyrin and stigmasterol reported from Putranjiva roxburghii WALL by high performance thin layer chromatography. International Journal of Pharma and Bio Sciences 2011; 2: 346-52.
[24]. Wagner H, Bladt B. Plant Drug Analysis. 2nd edition., Appendix A, Springer Publications;1996.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Synthesis Characterization of Photoactive Complex and Study its Photochemical Reaction

Namrata Gadekar*, Nikita Gadekar
Department of Chemistry,Dr.D.Y.Patil ACS College pimpri , Pune, Maharashtra, India

ABSTRACT

The mixed ligand complex of potassium tris(oxalate)ferrate(III)trishydrates have been synthesized & characterized the resulting complex were characterized by Gravimetric analysis, volumetric analysis & spectrophometrically. (uv visible spectro) studied. Also study its photochemical reaction .from the analytical &spectral data the result is conclude that %purity of the complex is of 90-95% exept for the preparation in terms of oxalate ion &Its found that complex shows the colour change on irradiation therefore is Photoactive.

Keywords: Photoactivity, Gravimetric, Volumetric, Spectrophotimetric

I. INTRODUCTION

with multi functional properties due to their potential application in molecular magnet chiral materials super conductors , ferromagnetic metals & photophysics.in the area of photochemistry a photochemical reaction are valuable in organic & inorganic chemistry because they proceed differently than thermal reaction.these reaction are serious nuisance ex.degradation of PVC.

II. EXPERIMENTALS

Materials & Methods:- Under the world of green chemistry we synthesis of characterize the photoactive complex & studied its photochemical reaction all the chemicals & solvent used were of A.R. grade. the spectra were recorded on a spectrophotometer in the frequency range 700nm ferroferri-cynide complex against the non irradiated ferrioxalate solution .and the graph obtain by spectrophotometer rate constant were recorded.

Synthesis of ligand complex:

1) By Single stage method:

Prepartion of potassium trisoxalatoferrate (III) trihydrate

$$K_3[fe\ (C_2O_4)_3].H_2O$$

Weighed quantity of FeCl3 and potassium oxalate mixed. After adding methanol cooling done on ice bath. Crystal of $K_3[Fe(C_2O_4)_3]3\ H_2O$ will appear in solution dry the crystals under vaccum.

From these therotically & practically %Yield is calculated.

2) By Two Stage Method-

1) Preparation of ferrous Oxalate $[Fe(C_2O_4),2\ H_2O]$-

From FAS:

Dissolve Weighed Quantity of FAS in the acidified warm water .then freshly prepaired Oxalic acid solution is Added To the solution Of FAS .Heat The above solution Cautiously to avoid the bumping. Withdraw the heat. And allow the granuler yellow precipitate of iron (II) oxal ate dehydrate to settle. Decant the supernatant liquid. Wash the yellow solid with hot distilled water,and with a few ml of acetone.

2) Preparation of Potassium Trioxalatoferrate (III) trihydrate:

$K3[Fe\ (C_2O_4)_3].3H_2O$ From Ferrous Oxalate-

Weighed quantity of dried iron (II) Oxalate dehydrate prepared in first step .is dissolve in little quantity of distilled water . also prepare the solution of potassium oxalate hydrated $[K_2C_2O_4H_2O]$ in 30 ml distilled water and pour this solution into the above solution . resulting the formation of orange intermediate iron (II) Copmplex. Warm the solution.to it. Add 30% 10 ml H_2O_2 dropwise .at this stage brown precipitate of $Fe(OH)_3$ if

formed. Heat the mixture to boiling to it add 1M Oxalic acid solution .lime green solution should appear. This result formation of tris oxalate ferrate (III) ion $[Fe(C_2O_4)_3^{3-}]$. Cool the above solution add 95% ethanol . cool in ice bath .the product potassium trisoxalatoferrate (III) is Form . Which is Photosensitive. From this practical yield ,theoretical yield ,% Practical yield is calculated.

Characterization of a$K_3[Fe(C_2O_4)_3].3H_2O$:

1) % H2O (moisture) in $K_3[Fe(C_2O_4)_3].3H_2O$can be calculated by gravimetrically.(% Purity)

2) % Of Oxalate ion in $K_3[Fe(C_2O_4)_3].3H_2O$ can be calculated by volumetrically.(% Purity)

3) % Of iron in the $K_3[Fe(C_2O_4)_3].3H_2O$ can becalculated by Spectrophotometrically.

Gravimetric study:

Sr No.	Description	Two Stage Preparation method	Single Stage Preparation method
1	Wt. of the empty silica crucible+lid(w_1)	26.315gm	26.113gm
2	Silica crucible +lid+complex(w_2)	26.856gm	26.613gm
3	Weight of sample taken (W_2-W_1)	0.541gm	0.50gm
4	After heating at $110^0{}_c$ For 45 min	26.725gm	26.585gm
5	(after heating at 100^0c for 60 minW3) & onward	26.797gm	26.558gm
6	Weight of residue (W_2-W_3)	0.059gm	0.0545gm
7	% of moisture	10.9%	10.90%

Volumetric study:

Sr. No.	Description	Result for 2 stage	Direct method
1	Exact normality of KM nO_4	0.055N	0.05N
2	Theoretical %of $C_2O_4^{2-}$ ions	53.76%	53.76%
3	Practical % of $C_2O_4^{2-}$ ions	25.085%	53.33%
4	% purity of complx in terms of oxalate ion	46.85%	99.20%

Spectrophotometric study:

Sr. No	Description	Result forTwo Stage method	Result for single Stage Preparation method
1	Theoretical % Of Fe(III) ions	11.404%	11.404%
2	Practical % of Fe (III) ion sample –I	10%	9.5%
3	% Purity of complex in terms of Fe sample I	87.71%	83.33%

For this preparation of standered Fe(III) solution is done which is used as blank solution and by using blank solution obtained the absorbance at 530 nm then from graph determine the amount of Fe(III) solution Plotting of calibration curve for single stage & double stage method was carried out From this concentration of Fe in Sample is obtained.

Study the Photochemical reaction:

1) By Prepairing a stock solution of ferrioxalate by dissolving 0.06 gm of Fe $NO_3.9H_2O$ in 100 ml distilled water and add 0.04 gm of oxalic asid with A.R. grade is added in above soltion .and dilute it by 100ml distilled water. Store it in adark by wrapping black carbon paper around it.

2) In 8 large test tube take 10ml of above solution .kept one of the test tube in dark which is used as blank solution

3) The solution in other test is exposed to sunlight for the period of 18 min. at the interval of 3 min

4) Add 1ml of 0.05 M $K_3[Fe(CN)_6]$ solution to each of the irradiated solution. On irradiation The reference solution remains pale yellow while the irradiated solution developed th blue colour.

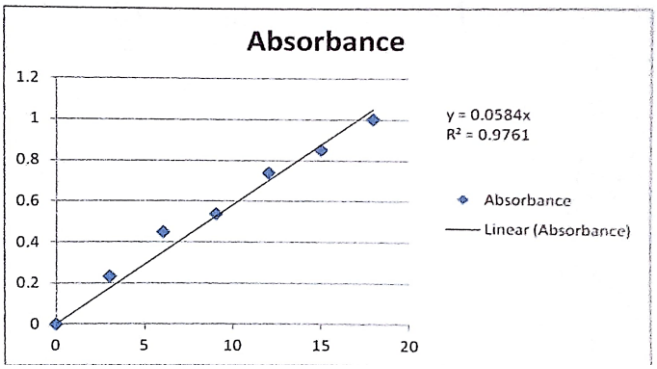
5) above solution is characterizes by spectrophotometer and calculate the rate constant from the first Order reaction kinetics. From the graph of log of absorbance v/s time in minute . rate constant can be calculated as

K= 2.303*slope

Observation Table 1. Irradiation of complex solution (with time Intervals 3 Minute)

Sr no	Time in minute	absorbance	Rate constant
1	0	0	0.133574
2	3	0.233	
3	6	0.449	
4	9	0.537	
5	12	0.737	
6	15	0.852	
7	18	1	

A graph of Absorbance Vs Time in min.



III. RESULT

Fe (II) ions formed by above method is proportional to no of photons absorbed .this in turns is proportional to the time of exposure.

IV. DISCUSSION

1) Photoactive Complex i.e.Potassium trisoxalatoferrate(III) trihydrate $K_3[Fe(C_2O_4)_3].3H_2O$ can be synthesized by both methods Viz. Single stage as well as double stage method.
2) By both method s green crystals of Pottasium trisoxalatoferrate(III)trihydrate complex are obtained out % practical yield by two stage method is more than single stage method
3) though the % practical yield by single stage method is less but % Purity of complex is about 99%
4) As irradiation time interval increases rate constant also increases .
5) Synthesised potassium trisoxalatoferrate(III) trihydrate complex is photoactive.

V. REFERENCES

[1]. Klan,J.Wirz Photochemistry Of organic Compound:from Concept to Practice. Wiley, chinchester,2009,ISBN978-1405190886
[2]. savelyev,G.G.(2003). The Photochemistry Of Pottassium trisoxalatoferrate(III)trihydrate in the solid state . Journal Of solid state Chemistry
[3]. Duncan,J.(2010) Experiment 1: Synthesisand analysis of an inorganic compound.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Preparation of Various Derivatives of Dibenzalpropanone by using Lithum Hydroxide as Green Approach

Pankaj Naikwadi*, Dr. S. B. Takate, S. K. Malujkar, Sandesh Kasar, Arun Wakchaure, N. D. Phatangare, Rupeshkumar Bhalerao, Rutuja K., Deshmukh, Asha B. Dhokare, Sonali Vaidya, Suvidha Papal

Department of Chemistry, Agasti Arts Commerce & Dadasaheb Rupwate Science College akole Sangamner College, Sangamner, Akole, Ahmednagar, India

ABSTRACT

The α,β-unsaturated ketones known as benzalacetones are an interesting class of compounds frequently used as key intermediates in organic synthesis. Due to their conjugated system, benzalacetone and derivatives have been described as radical scavengers with potential antioxidant properties. We report here a simple and direct method to prepare functionalized α,β-unsaturated ketones via a microwaves activated Aldol Condensation reaction. The experimental protocol developed selectively produces benzalacetones of self-condensation product in very short reaction times and good yields.

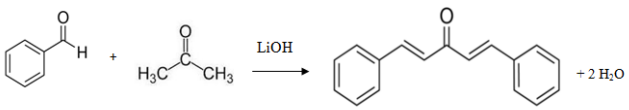
Keywords: Benzalacetones, microwave, Aryl Aldehyde.

I. INTRODUCTION

The synthesis of α,β-unsaturated ketones known as benzalacetones, which possess interesting properties for organic synthesis. Due to their conjugated system, benzalacetone and derivatives have been described as radical scavengers with potential antioxidant properties. Various methods of synthesis for this type of compounds have been described in the literature. The Aldo condensation reaction is one of the simplest condensation methods. This reaction is typically catalyzed by acids ($AlCl_3$ or HCl) and more often by bases with or without solvent at room temperature or under conventional heating . In order to increase the yield and to avoid the formation of by-products, several protocols relative to aldol condensation reaction have also been reported using different catalysts, sonochemical activation or microwaves irradiation. However, in all these conditions, side reactions start decreasing the yield of the desired product and entail further purification steps. Consequently, we were particularly interested in developing an efficient preparation of benzalacetones from acetone and aromatic substituted aldehydes in basic conditions under microwave-activation.

1) Preparation of (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one:

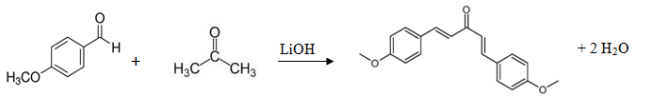
Place 0.9ml benzaldehyde in to conical flask containing acetone 0.3ml add 95% ethanol . Over a period of 15 min. Slowly added 2ml 40% LiOH solution to the mixture at room temp. For 10 min, pour the reaction mix. into the ice & stir until the precipitation occurs. The progress of the reaction was monitored by TLC. The separated solid was filtered & recrystallized from ethanol. Their physical constant. data give in table.



Benzaldehyde Acetone (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one

2) Preparation of (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one:

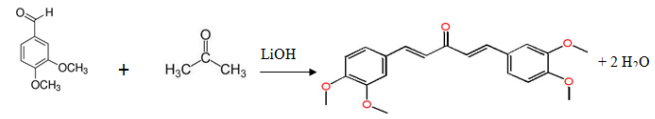
Place 0.9ml 4-Methoxybenzaldehyde in to conical flask containing acetone 0.3ml add 95% ethanol . Over a period of 15 min. Slowly added 2ml 40% LiOH solution to the mixture at room temp. For 10 min, pour the reaction mix. into the ice & stir until the precipitation occurs. The progress of the reaction was monitored by TLC. The separated solid was filtered & recrystallized from ethanol. Their physical constant. data give in table.



4-Methoxybenzaldehyde Acetone (1E,4E)-1,5-bis(4-methoxyphenyl)-penta-1,4-dien-3-one

3) Preparation of (1E,4E)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one

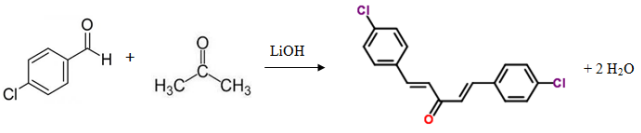
Place 0.9ml 3,4-Dimethoxybenzaldehyde in to conical flask containing acetone 0.3ml add 95% ethanol. Over a period of 15 min. Slowly added 2ml 40% LiOH solution to the mixture at room temp. For 10 min, pour the reaction mix. into the ice & stir until the precipitation occurs. The progress of the reaction was monitored by TLC. The separated solid was filtered & recrystallized from ethanol. Their physical constant. data give in table.



3,4-Dmethoxybenzaldehyde Acetone (1E,4E)-1,5-bis(3,4dimethoxyphenyl) penta-1,4-dien-3-one

4) Preparation of (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one :-

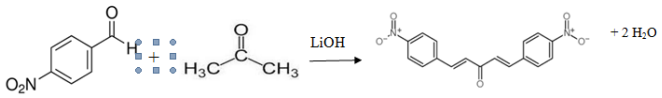
Place 0.9ml P-Chlorobenzaldehyde in to conical flask containing acetone 0.3ml add 95% ethanol. Over a period of 15 min. Slowly added 2ml 40% LiOH solution to the mixture at room temp. For 10 min, pour the reaction mix. into the ice & stir until the precipitation occurs. The progress of the reaction was monitored by TLC. The separated solid was filtered & recrystallized from ethanol. Their physical constant. data give in table.



P-Chlorobenzaldehyde Acetone (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one

5) Preparation of (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one:-

Place 0.9ml P-Nitrobenzaldehyde in to conical flask containing acetone 0.3ml add 95% ethanol. Over a period of 15 min. Slowly added 2ml 40% LiOH solution to the mixture at room temp. For 10 min, pour the reaction mix. into the ice & stir until the precipitation occurs. The progress of the reaction was monitored by TLC. The separated solid was filtered & recrystallized from ethanol. Their physical constant. data give in table.



P-Nitrobenzaldehyde Acetone (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one

II. RESULT AND DISCUSSION

- ✓ Microwave activation for the synthesis of benzalacetones has not been widely described in the literature. Kappe et *al* reported the aldol condensation of *p*-methoxybenzaldehyde with acetone using microwave activation.
- ✓ As a wide variety of aryl aldehydes is commercially available, microwave activation would provide a higher degree of flexibility with respects to functional groups which may be introduced in the benzalacetone skeleton. The details of the synthesis were previously described by our group.
- ✓ Following our interest in establishing an efficient, rapid and selective access to benzalacetones and considering our results previously obtained under conventional heating.

Sr. No.	Compound Name	Molecular formula	Molecular Weight	M.P. ^{0}C	Yield %
01	(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one	$C_{17}H_{14}O$	234.298	130°C	**85**
02	(1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one	$C_{19}H_{18}O_3$	294.35	175°C	65
03	(1E,4E)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one	$C_{21}H_{22}O_5$	354.396	142°C	40
04	(1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one	$C_{17}H_{12}Cl_2O$	303.182	172°C	70
05	(1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one	$C_{17}H_{12}NO_2O_5$	324.292	220°C	72

All the compound synthesized were adequately characterized by their spectral IR, ^{1}H-NMR & Mass Spectra.

***Spectral Study of (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one :-**

IR(KBr cm^{-1}):1725cm^{-1}(C=O),1570cm^{-1}(C=C), ^{1}H NMR (ppm) ($CDCl_3$):7.60(dt, 1.5Hz & 8 Hz, 2H); 7.40(dt 1.5 & 8Hz, 2H); 7.33(dt, 1.5 & 8Hz ,1H); 7.82(d,18Hz ,1H); 7.03(d,18Hz, 1H).

***Spectral Study of (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one**

IR(KBrcm^{-1}):1725cm^{-1}(C=O),1570cm^{-1}(C=C);^{1}HNMR(ppm) ($CDCl_3$):7.62(td, 0.5 & 8 Hz, 2H); 6.94(td, 0.5 & 8Hz, 2H); 3.83(s, 3H); 7.82(d, 18Hz,1H); 7.03(d, 8Hz, 1H)

***Spectral Study of (1E,4E)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one**

IR(KBrcm^{-1}):1725cm^{-1}(C=O),1570cm^{-1}(C=C);^{1}HNMR(ppm) ($CDCl_3$): 7.18(dd, 1.5 & 8Hz, 1H); 6.94(dd, 0.5 & 8Hz, 1H); 7.22(dd, 1.5&0.5Hz, 1H); 3.83(s, 6H); 7.82(d, 18Hz, 1H); 7.03(d, 18Hz, 1H)

***Spectral Study of (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one**

IR(KBrcm^{-1}):1725cm^{-1}(C=O),1570cm^{-1}(C=C);^{1}HNMR(ppm) ($CDCl_3$):7.44(td, 1.5 & 8Hz, 2H); 7.68(td, 1.5 & 8 Hz, 2H); 7.82(d, 18Hz, 1H); 7.03(d, 18Hz, 1H).

***Spectral Study of (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one**

IR(KBrcm^{-1}):1725cm^{-1}(C=O),1570cm^{-1}(C=C);^{1}HNMR(ppm) ($CDCl_3$):8.03(td, 0.5 & 8Hz, 2H); 8.21(td, 0.5 & 8Hz, 2H); 7.96(d, 18Hz, 1H); 7.32(d, 18Hz, 1H).

III. ACKNOWLEDGEMENTS

We are thankful to the department of chemistry (Agasti College Akole) for providing excellent facilities and we are grateful to my Principal Dr. S.B.Takate, Prof. S.K. Malunjkar,Dr.S.M.Sonawane,Prof.M.D.Banait,Prof.S.D. kasar,Prof.A.L.Wakchaure Lab.Asst.K.L.Sabale .

IV. REFERENCES

[1]. Conard, C. R.; Dolliver, M. A. (1943). "Dibenzalacetone". Org. Synth.; Coll. Vol., 2, p. 167

[2]. Rao, G. N.; Janardhana, C.; Ramanathan, V.; Rajesh, T.; Kumar, P. H. (November 2006). "Photochemical Dimerization of Dibenzylideneacetone. A Convenient Exercise in [2+2] Cycloaddition Using Chemical Ionization Mass Spectrometry". J. Chem. Educ. 83(11): 1667. doi:10.1021/ed083p1667.

[3]. Narender, T.; Papi Reddy, K. A simple and highly efficient method for the synthesis of chalcones by using borontrifluoride-etherate. Tetrahedron Lett. 2007, 48, 3177–3180.

[4]. Jayapal, M.R.; Prasad, K.S. and Sreedhar N.Y., Synthesis and characterization of 2,6-dihydroxy substituted chalcones using PEG-400 as a recyclable solvent. J. Pharm. Sci. Res. 2010, 2, 450-458.

[5]. Drake N.L.; Allen P. Benzalacetone. J.Org. Synth. 1923, 3:17.

[6]. Bogdal D.; Loupy A. Application of Microwave Irradiation to Phase-Transfer Catalyzed Reaction. Org. Process. Res. Dev. 2008, 12, 710–722.

[7]. Ordomsky, V.V., Sushkevich, V.L. and Ivanova, I.I. Journal ofMolecular Catalyst A: Chemical, 2010,333.

[8]. Kumar D.S.; Sandhu J.S. An efficient green protocol for the synthesis of chalcones by a Claisen – Schmidt reaction using bismuth(III)chloride as a catalyst under solvent-free condition. Green Chem. Lett. Rev. 2010, 3, 283-286.

[9]. Sashidhara K.V.; Rosaiah J.N.; Kumar A. Iodine catalyzed mild and efficient method for the synthesis of chalcones. Synth. Commun. 2009, 39, 2288–2296.

[10]. Blois M.S. Antioxidant determinations by the use of a stable free radical. Nature. 1958, 181, 1199–1200.

[11]. Rayar A.; Sylla-Iyarreta Veitía M.; Ferroud, C. An efficient and selective microwave-assisted Claisen-Schmidt reaction for the synthesis of functionalized benzalacetones. Springer Plus 2015, 4, 221-226.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

pH-Metric Study on Determination of Metal-Ligand Stability constants of some substituted Isoxazolines

P. J. Parmar
Department of Chemistry Nanasaheb Y. N. Chavan Arts, Science and Commerce College, Chalisgaon, Maharashtra, India

ABSTRACT
The interaction of Cu(II), Co(II), fe(III), Al(III) and Nd(III) with 3-(2"-hydroxy-3"-nitro-5" methylphenyl)-5-phenyl isoxazoline (Ligand-1), 3-(2"-hydroxy-3"-nitro-5"-methylphenyl)-5-(4'-methoxyphenyl)-isoxazoline(Ligand-II), 3-(2"-hydroxy-3"-nitro-5" methylphenyl)-5-(3'-nitrophenyl)-isoxazoline(Ligand-III) and 3-(2"-hydroxy-3"-nitro-5" methylphenyl)-5-(3', 4'-methylene dioxyphenyl)-isoxazoline (Ligand-IV) have been studied by spectrophotometric technique at 0.1 M ionic strenghth and (30±0.1° c) in 70 % dioxane-water mixture. The data obtained were used to estimate the values of metal-ligand stability constant of substitututed isoxazolines. Spectrophotometric investigation of Co(II), Cu(II), Fe(III), Al(III) and Nd(III) complexes with Ligand(I), Ligand (II), Ligand (III) and Ligand(IV) showed l:l and l:2 complex formation simultaneously. The information of complexes has been studied by Calvin-Bjerrum titration technique. The results obtained of stability constants are in good agreement.
Keywords: Stability Constants, Complex Formation, Ph-Metric Study, Cu(II), Co(II), Fe(III), Al(III), Nd(III) Metal-Ligand Stability Constants, Substituted Isoxazolines.

I. INTRODUCTION

2-Hydroxy substituted isoxazolines are good chelating agents due to the presence of electron donor nitrogen and oxygen atom and liberation og H^+ ion from –OH group[1,2].

In view of biological importance and analytical applications[3,4] of substituted isoxazolines, it was interesting to know the physics-chemical properties such as stabilities of complexes with Cu(II), Co(II), Fe(III), Al(III) and Nd(III) metal ions pH-metrically. Study of complexes under identical set of experimental conditions is still lacking.

All chemical such as $NaNO_3$, HNO_3 and NaOH used were of AR grade. The ligand (I-IV) were prepared by literature method[1-4] . These ligands were crystallized and their purity was checked by analytical and spectral study. 1:4 Dioxane was purified by the standard methods. The metal ions used were in the form of their nitrates, A-VSI-01 AT digital pH-meter was used for measurement of pH. Standard NaOH and 1M $NaNO_3$ solution were prepared in double distilled water.

The pH-metric titrations of (i)1×10^{-2} M HNO_3 (ii)1×10^{-2} M ligand and (iii)1×10^{-2} M HNO_3+2×10^{-3} M ligand+4×10^{-4} M metal ion solutions against carbonate free 0.1008 M NaOH were carried out by Calvin[6] – Bjerrum pH-metric titration technique in 70 % dioxane water mixture at 0.1 M ionic strength maintained by addition of appropriate quantity of 1 M $NaNO_3$ The total volume of each system was made upto 50 ml. so that the solution were 70 %(V/V) with respect to 1:4 dioxane. All titrations were carried out in the environment of oxygen free nitrogen gas. The readings were recorded for each addition of 0.2 ml. The pH-metric titration data of acid,(acid+ligand) and (acid+ligand+metal) systems were used to construct acid, ligand and metal curves between volume of NaOH added vs. pH of respective system.

***Determination of Proton ligand stability constant(pK)**
The ligand(I-IV) in the present investigation are monobasic acids having only one dissociable proton from hydroxyl group of the ligand. In general, ligands can be represented as HL and dissociated as

$$HL \rightleftharpoons H^+ + L^-$$

It is found that , the deviation of (acid+ligand) curves from acid curve started at about pH 4. This indicates that dissociation of hydroxyl group occurs which is present

in the ligand part of the complex structure. The proton ligand formation number (n A) were calculated by the Irving and Rossotti expression[7].

The values of pK(dissociation constant) were estimated by noting the pH at nA=0.5 which were calculated by half integral method and presented in Table-1. Most accurate values were calculated from pointwise calculations.

Table 1. DETERMINATION OF PROTON-LIGAND STABILITY CONSTANT (pK) OF SOME SUBSTITUTED ISOXAZOLINES AT 0.1 M IONIC STRNGTH AT (30±0.1)° c. TEMPARATURE.

Sr. No.	System	Constant pK	
		By half integral method	By pointwise calculation
1.	Ligand-1, 3-(2"-hydroxy-3"-nitro-5" methylphenyl))-5-phenyl isoxazoline	9.57	9.62±0.05
2.	Ligand-2, 3-(2"-hydroxy-3"-nitro-5"-methylphenyl)-5-(4'-methoxyphenyl)-isoxazoline	8.25	8.35±0.03
3.	Ligand-3, 3-(2"-hydroxy-3"-nitro-5" methylphenyl)-5-(3'-nitrophenyl)-isoxazoline	8.79	8.87±0.06
4.	Ligand-4,3 -(2"-hydroxy-3"-nitro-5" methylphenyl)-5-(3', 4'-methylene dioxyphenyl)-isoxazoline	8.97	9.05±0.07

It can seen from table-1 that value of ligand-1 is greater than ligand-2, ligand-3 and ligand-4. This may be due to the fact that presence of electron withdrawing nitro group near to the –OH group of respective ligand. The electron withdrawing group reduces the pK value of the ligand.

***Determination of metal-ligand Stability constants(log K)**

The deviation of (acid+ligand) curves from (acid+ligand+metal) curves were observed at about 2.7 pH in media of 70 % dioxane-water mixture. This indicates the commencement of complex formation from this pH. During titration process there is colour change of solution from pale yellow to orange. This indicates the formation of complex between ligand and metal ion. The value of metal-ligand formation number (n) were evaluated by the Irving-Rosotti's expression. The values are presented in Table 2.

Table 2. DETERMINATION OF METAL-LIGAND STABILITY CONSTANTS (Log K) OF METAL COMPLEXES WITH LIGAND(I), LIGAND(II), LIGAND(III), AND LIGAND(IV) AT 0.1 M IONIC STRENGTH AND AT (30±0.1)° C TEMPERATURE.

Sr. No.	System	Constant	Constant
		Log K1	Log K2
1.	Cu(II) Ligand (I)	6.35	5.95
	Co(II) Ligand(I)	9.78	9.37
	Fe(III) Ligand(I)	10.11	9.74
	Al(III) Ligand(I)	9.15	8.63
	Nd(III) Ligand(I)	8.65	7.90
2.	Cu(II) Ligand (II)	8.65	8.17
	Co(II) Ligand(II)	8.57	8.09
	Fe(III) Ligand(II)	8.03	7.37
	Al(III) Ligand(II)	8.51	8.07
	Nd(III) Ligand(II)	7.55	6.95
3.	Cu(II) Ligand (III)	7.55	6.67
	Co(II) Ligand(III)	8.67	7.71
	Fe(III) Ligand(III)	8.53	8.17
	Al(III) Ligand(III)	8.37	7.41
	Nd(III) Ligand(III)	7.69	6.57
4.	Cu(II) Ligand (IV)	7.55	6.85
	Co(II) Ligand(IV)	8.57	7.77
	Fe(III) Ligand(IV)	8.35	7.45

	Al(III) Ligand(IV)	8.75	7.87
	Nd(III) Ligand(IV)	7.73	5.65

It could be seen from table 2 that there is slight difference between the values of log K_1 and log K_2. This indicates the stepwise complex formation between ligand and metal ion. The order of stability constant of complexes is represented as,

i) For ligand(I)

Fe(III)>Co(II)>Al(III)>Nd(III)>Cu(II)

ii) For ligand(II)

Cu(II)>Co(II)>Al(III)>Nd(III)>Fe(III)

iii) For ligand(III)

Co(II)>Fe(III)>Al(III)>Cu(II)>Nd(III)

iv) For ligand (IV)

Al(III)>Co(II)>Fe(III)>Nd(III)>Cu(II)

II. ACKNOWLEDGEMENT

The author is thankful to Principal, Nanasaheb Y. N. Chavan Arts, Science and Commerce College, Chalisgaon for providing necessary facilities.

III. REFERENCES

[1]. P. B. Raghuwanshi, A. G. Doshi and M. L. Narwade, J. Indian Chem. Soc., 73, 21 (1996).
[2]. P. R Athappan, P. Shanthi and C. Natranjan, Indian J, Chem., 34, 648 (1995).
[3]. G. F. Duffin and J. D. Kendall, J. Chem. Soc., 405(1954).
[4]. Z. H. Khalil and A. S. Yanni, J. Indian Chem. Soc., 58, 168 (1981).
[5]. A. I. Vogel, A Text Book of Practical Organic Chemsitry, Longmans, London (1966).
[6]. M. Calving and N. C. Melchior. J. Chem. Soc., 3270 (1948).
[7]. H. M. Irving and H. S. Rosotti, J. Chem. Soc., 3397 (1953).
[8]. D. Moore and R. Reynolds, X-Ray Diffraction and the Identification and Analysis of Clay Minerals, Oxford University Press, Oxford (1997).
[9]. M. M. Mortland, S. Shaobai and S. A. Boyd, Clays Clay Miner., 34, 581 (1986).
[10]. J. Lee, M. M. Mortland, C. T. Chiou, D. E. Kilc and S. A. Boyd, Clays Clay Miner., 38, 113 (1990).
[11]. C. T. Cowan and D. White, The mechanism reactions occurring between sodium montmorillonite n-primaty apliphatic amine salts (1986) (Translated by D. R. GarKarini and L. L. Lion).
[12]. S. A. Boyd, M. M. Mortland and C. T. Chou, Soil. Sci. Soc. Am. J., 52, 652 (1988).
[13]. R. M. Barer, Zeolites and Clay Minerals as Sort Molecular Sieves, Academic Press. London pp. 407-409 (1978).
[14]. S. A. Boyd, S. S. Kai and M.M. Mortland, in: K. G. Theng (Ed.), The Chemistry of Clay-Organic Reactions, Wiley, New York (1974).
[15]. A. R. Khan, R. Atullah and A. Al-hadded, J. Colloid Interface Sci., 194, 154 (1997).
[16]. S. Haydar, M. A. F. Garcia, J. R. Ultrilla and J. P. Joly, Carbon, 41, 387 (2003).
[17]. H. J. Lee, D. J. Song nad Y. W. Jeon, Sep, Sci. Tech, 32, 1975 (1997).
[18]. A. Wolborka, Water Res., 23, 85 (1989).
[19]. M. A. M. Lawrence, R. K. Kukkadapa and S. A. Boyd, Appl. Clay Sci., 13, 13 (1998).
[20]. A. Weiss, Appl. Clay Sci., 4, 193 (1989).
[21]. K. Krause, Process for Immobilizing Organic and Inorganic Pollutants in a Contaminated Soil Material on a Remediation Site, US 5651831 (1997).
[22]. B. C. Pan, Y, Xion, Q. Su, A. M. Li, J. L. Chen and Q. X. Zhang, Chemosphere, 51, 953 (2003).
[23]. M. Akcay and G. Akcay, J. Hazard, Mater., 113B, 189 (2004).
[24]. R. C. Zielke and T. J. Pinnavaia, Clay Clay Miner., 36, 403 (1988).
[25]. M. B. McBride, T. J. Pinnavaia and M. M. Mortland, Air Water Environ., 8, 145 (1977).
[26]. L. P. Mcir, R. Nueesch and F. T. Medsen, J. Colloid Interface Sci., 238, 20 (2001).

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Design New Pyrano Quinoline Derivatives and Study of their Anti-Microbial Activity

Rahul Watpade, Raghunath B. Toche

Organic Chemistry Research Centre, Department of Chemistry, K. R. T. Arts, B. H.Commerce and A. M. Science College, Gangapur Road, Nashik, Maharashtra, India

ABSTRACT

4,5,7-Trichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6]naphthyridine 1 was selectively converted to 5-iminoether 5 by the reaction with sodium alkoxy in corresponding alcohol and to 2-methylbenzo[h][1,6]naphthyridin-5(6H)-one 2 by acetic acid reflux. The reaction selectively occurs at C5-position of the benzo[h][1,6]naphthyridine. Further, 2-methylbenzo[h][1,6] naphthayridin-5(6H)-one 2 furnish O and N alkylation products 3 and 4 with bromoethylacetate respectively. The reaction of 2 with bromoacetamide yield O-acetanilide i.e. 2-(4-chloro-12-methyl-16,17-dihydro-15-thia-6,11-diaza-cyclopenta[a]phenanthren-7-ylsulfanyl)-N-phenyl acetamide 6 in major amount.

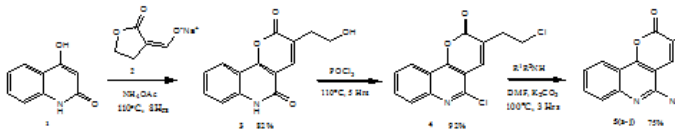
Keywords: Pyranoqunoline Reactions, benzo[h][1,6]Naphthyridines, Antimicrobial Activity

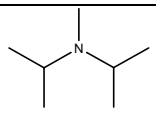
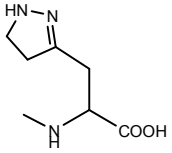
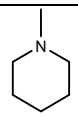
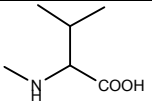
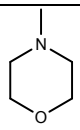
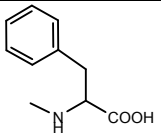
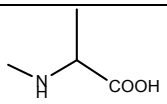
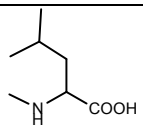
I. INTRODUCTION

Multifunctional benzo[*h*][1,6]naphthyridines showed broad spectrum of biological activities[1-3] including high affinity on 5-HT_4 receptors and high selectivity versus other receptors[4-7] and also promising antimalarial activity.[1] Here, we report the synthesis of benzo[*h*][1,6]naphthyridines derivatives linked with active C_5-iminoether and N_6- acetic acid ethyl ester. *sulfanyl)-N-(2,4-dichloro-phenyl)-acetamide* . iminotiaoether. at position of cyclopenta[*a*]phenanthren -7-ylsulfanyl)-*N*-phenyl acetamide. Further we report the novel synthesis of thiazolidinone derivatives on iminothioether linkers at C_7 position. 4-Thiazolidinone derivatives showed remarkable antimicrobial,[8-9] antibacterial,[10] antifungal,[11] anticonvulsant,[12] anticancer,[13] antituberculosis,[14] and anti-human immunodeficiency virus type 1 (HIV-1) activities.[15] We undertook the synthesis and investigated reactions of some new benzo[*h*][1,6]naphthyridine derivatives, which might good biological and medicinal applications.

In this paper, report the synthesis of derivatives having linkers of iminoether 2 position of benzo[*h*][1,6]naphthyridines In view of all these facts and as the continuation of our work on the synthesis of new heterocyclic derivatives by using α-acetyl γ-butyrolactone and heterocyclic amines.[16-18] We undertook the synthesis and investigated reactions of some new benzo[*h*][1,6]naphthyridine derivatives, which might have good biological and medicinal applications.

II. RESULTS AND DISCUSSION



Comp. 5	R^1/R^2	Comp. 5	R^1/R^2
a		**e**	
b		**f**	
c		**g**	
d		**h**	

The starting compound 4,5,7-trichloro-3-(2-chloroethyl)-2-methylbenzo[*h*][1,6]naphthyridine **1** was synthesized according to our previous reported procedure. The substitution of Cl at C_5 in compound **1** with alkoxide in corresponding alcohol was done refluxing compound **1** in sodium methoxide in corresponding ethanol yield pyranoqunoline derivative **5** in good yield. The C_5 position is more electron diffident than C_6 due to neighboring sp^2 nitrogen and also inductive effect of C_7-Cl, hence attack of nucleophile is preferred at C5, was proved with the help of X-ray crystallography of Compound **2**.

The synthetic strategy adopted to obtain the target compounds are depicted in Schemes 1-3. The iminechloride (-N=C-Cl) moiety in compound **1** was converted to lactum cabonyl[30] by refluxing in glacial acetic acid furnished 4,7-dichloro-3-(2chloroethyl)-2-methylbenzo[*h*][1,6] naphthyridin 5(*6H*)-one **2** in 93% yield. The structure of compound **2** was assigned by spectroscopic and analytical methods e.g. IR of compound **2** showed lactum carbonyl (C=O) stretching at 1676 cm^{-1} and NH at 3339 $cm.^{-1}$

Biological activity

The antimicrobial activities of all synthesized compounds were evaluated in vitro for three Gram-positive and Gram-negative organisms including Staphylococcus aureus, Bacillus subtilis, and Methicillin-resistant S. aureus and three Gram-negative organisms including Escherichia coli. The compounds 5a-h was tested against microorganism species at 1000 ppm concentration. The observed results of antibacterial screening reported in above table indicate that benzo[h][1,6]naphthyridine derivatives 5e, 5f and 5g are active against S. Aureus; compounds 5h and 5d are active against E. Coli; compound 5e active against P.Sedoaurious; compound 5e and 5f are active against streptococcus and compound 47d and 49a are active against B-megaterium. However, compounds 5b, 5c and 5i are less active against bacterial species while the other compounds showed mild activities against bacterial species.

Table 1. Antimicrobial screening of compounds 5a-t: Inhibition Zone in Diameter (mm) at 40 µg / mL

Compound No.	Conc. (µg/mL)	*E.coli*	*S.aureus*	*P.Sedoaurious*	*B. subtilis*
5a	40	15±0.8	16±0.9	18±1.1	17±0.9

5b	40	16±1.2	17±0.5	17±0.9	15±0.7
5c	40	18±0.8	18±0.6	17±0.6	18±0.6
5d	40	19±0.9	15±0.7	17±0.5	18±0.8
5e	40	19±1.3	18±0.8	**21±0.4**	**22±0.5**
5f	40	**20±0.3**	19±0.6	19±1.1	**20±0.8**
5g	40	18±0.6	18±0.7	17±0.7	**22±0.3**
5h	40	19±0.3	18±0.4	18±0.5	18±0.9
5i	40	15±0.8	14±1.3	16±1.1	18±0.9
Gentamycin	10	21±0.8	23±0.3	NT	NT
Flucouezole	20	NT	NT	24±0.2	22±0.5

III. EXPERIMENTAL

Common reagents grade chemicals are either commercially available and were used without further purification or prepared by standard literature procedures. The melting points were measured on Barnstead Electro Thermal melting point apparatus Mod. No. IA-9200 in open capillary tubes and were uncorrected. Elemental analyses were determined using Thermo Quest Model No. flash EA 1112-Elemental Analyzer. The IR spectra of compounds were recorded on Shimadzu IR-408, instrument in potassium bromide pellets. All mass spectra were recorded on Mat 112 Varian Mat Bremen mass spectrometer. Routine ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on VARIAN XL-300 instrument at 25 ^{o}C. The measurements were done using pronated solvents $CDCl_3$ and DMSO-d_6, with TMS as an internal reference standard. Coupling constants (*J*) are quoted to the nearest 0.1 Hz and chemical shift (δ-scale) are quoted in parts per million (ppm) using abbreviations s=singlet, d=doublet, t=triplet, q= quartet, m= multiplet, br =broad. Column chromatography was performed using silica gel with particle size (60-120 mesh, Merck). All reactions were monitored by TLC carried out 0.2 mm silica gel 60 F_{254} (Merck) plates using 254 and 366 nm UV light for detection.

3.1 4,7-Dichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6]naphthyridin-5(6H)-one **5a**. A mixture of 4,5,7-trichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6]naphthyridine **1** (3.60 g, 0.01 mol) in glacial acetic acid (25 mL) was refluxed for 15 min. After cooling down to room temperature, methanol (50 mL) was added, the crude product obtained was collected by suction filtration, dried and recrystallized from ethanol/DMF (9:1) to yield title compound **2** (3.17 g, 93%) as pink colored prisms; Rf (toluene/ethyl acetate 9:1) 0.51, mp 254 ^{o}C; IR (KBr): ν 3339 (NH), 3186, 3143, 1676 ($C=O_{lactum}$), 1249, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.91 (s, 3H, CH_3), 3.57 (t, J = 7.1 Hz, 2H, CH_2), 3.83 (t, J = 7.1 Hz, 2H, CH_2Cl), 7.59 (t, J = 7.5 Hz, 1H, C_9H), 7.77 (d, J = 7.5 Hz, 1H, C_8H), 8.1 (s, 1H, NH, D_2O exchangeable), 8.97 (d, J = 7.5 Hz, 1H, $C_{10}H$); ^{13}C NMR ($CDCl_3$): δ 23.26, 30.95, 42.65, 119.40, 121.72, 122.14, 126.75, 128.00, 128.20, 128.63, 128.74, 136.66, 142.23, 161.54, 170.61; MS: m/z (%): 347 (M+6, 10), 345 (M+4, 30), 343 (M+2, 50), 341 (M, 100), 274 (20), 198 (20), 99 (10); Anal. Calcd for $C_{15}H_{11}Cl_3N_2O$ (341.62): C, 52.74; H, 3.25; N, 8.20. Found: C, 52.47; H, 3.31; N, 8.29.

3.2 [4,7-Dichloro-3-(2-chloroethyl)-2-methyl-benzo[h][1,6]naphthyridin-5-yloxy]-acetic acid ethyl ester **5b**. Anhydrous potassium carbonate (0.136 g, 0.01 mmol) was added to the stirred solution of 4,7-dichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6] naphthyridine-5-(6H)-one **2** (0.341 g, 0.01 mmol) and 2-bromo-N-phenyl-acetamide (0.012 mmol) in DMF at 25^{o}C. The resulting reaction mixture was kept stirring for 2 h. The

progress of the reaction was monitored by TLC (toluene/ethyl acetate 8:2). After completion of reaction, the mixture was stirred in cold water (100 mL). The obtained solid was filtered washed with water, dried and purified by column chromatography eluting with gave title compound 6 was purified on silica column eluting with toluene. Yellow prisms; yield (0.328 g, 77%); Rf (toluene/ethyl acetate, 8:2) 0.53, mp 211 ^{o}C; IR (KBr): ν 2977, 2898, 1749 (C=O), 1589, 1562, 1375, 1213, 1060, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.92 (s, 3H, CH_3), 3.53 (t, J = 7.5 Hz, 2H, CH_2), 3.81 (t, J = 7.5 Hz, 2H, CH_2Cl), 4.28 (q, J = 7.2 Hz, 2H, OCH_2), 5.20 (s, 2H, CH_2), 7.44 (t, J = 7.8 Hz, 1H, C_9H), 7.76 (d, J = 7.8 Hz, 1H, C_8H), 8.88 (d, J = 7.8 Hz, 1H, $C_{10}H$); MS: m/z (%): 432 (M+6, 10), 430 (M+4, 15), 428 (M+2, 30), 426 (M+, 40) , 391 (30), 381 (50), 341 (60), 91 (40), 85 (100), 77 (30). Anal. Calcd for $C_{19}H_{17}Cl_3N_2O_3$ (427.72): C, 53.36; H, 4.01; N, 6.55. Found: C, 53.39; H, 4.07; N, 6.51.

3.3 [4,7-Dichloro-3-(2-chloro-ethyl)-2-methyl-benzo[h][1,6]naphthyridin-5-yloxy]-acetic acid ethyl ester, 5c

Yellow needles; yield (0.0426 g, 10%); R_f (toluene/ethyl acetate 8:2) 0.53, mp 237 ^{o}C. IR (KBr): ν 2993, 2960, 1731 (C=O), 1664 (C=O), 1533, 1394, 1253, 1126, 786 cm.$^{-1}$ ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 6.9 Hz, 3H, OCH_2CH_3), 2.89 (s, 3H, CH_3), 3.46 (t, J = 7.2 Hz, 2H, CH_2CH_2Cl), 3.78 (t, J = 7.2 Hz, 2H, CH_2CH_2Cl), 4.28 (q, J = 6.9 Hz, 2H, OCH_2CH_3), 5.27 (s, 2H, CH_2), 7.29 (t, J = 8.1 Hz, 1H, C_9H), 7.61 (d, J = 8.1 Hz, 1H, C_8H), 8.90 (d, J = 8.1 Hz, 1H, $C_{10}H$). MS: m/z (%): 432 (M+6, 10), 430 (M+4, 15), 428 (M+2, 30), 426 (M+, 100), 391 (30), 381 (50), 341 (60), 91 (50), 85 (90), 77 (40). Analysis Calculated for $C_{19}H_{17}Cl_3N_2O_3$ (427.72): Calcd: C, 53.36; H, 4.01; N, 6.55; Found: C, 53.39; H, 4.07; N, 6.51

3.4 5,7-Dichloro-3-(2-chloroethyl)-4-methoxy-2-methylbenzo[h][1,6]naphthyridine, 5d

4,5,7-trichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6]naphthyridine **1** (3.60 g, 0.01 mol) was refluxed in sodium methoxide in methanol for about 1 hour. The solvent was removed under reduced pressure. The solid obtained was stirred in cold methanol. The residue was filtered, dried and recrystallized from ethanol. White prisms, yield (0.298 g, 84%); Rf (Toluene) 0.81, mp 180 ^{o}C. IR (KBr): ν 2962, 2837, 1583, 1425, 1321, 1162, 1033, 776 cm.$^{-1}$; ^{1}H NMR ($CDCl_3$): δ 2.89 (s, 3H, CH_3), 3.44 (t, J = 7.6 Hz, 2H, CH_2CH_2Cl), 3.92 (t, J = 7.6 Hz, 2H, CH_2CH_2Cl), 4.19 (s, 3H, OCH_3), 7.54 (t, J = 7.5 Hz, 1H, C_9H), 7.94 (d, J = 7.5 Hz, 1H, C_8H), 8.83 (d, J = 7.5 Hz, 1H, $C_{10}H$).MS: m/s (%): 360 (M+6, 50), 358 (M+4, 60), 356 (M+2, 80), 354 (M, 100), 325 (100), 319 (60), 275 (70), 198 (20), 138 (50), 49 (70). Analysis Calculated for $C_{16}H_{13}Cl_3N_2O$ (355.65): Calcd: C, 54.03; H, 3.68; N, 7.88; Found: C, 55.22; H, 4.11; N, 7.53

3.5. 2-[4,7-Dichloro-3-(2-chloro-ethyl)-2-methyl-5-oxo-5H-benzo[h][1,6]naphthyridine-6-yl]-N-substituted phenyl acetamide 5e

Anhydrous potassium carbonate (0.136 g, 0.01 mmol) was added to the stirred solution of 4,7-dichloro-3-(2-chloroethyl)-2-methylbenzo[h][1,6] naphthyridine-5-(6H)-one **2** (0.341 g, 0.01 mmol) and 2-bromo-N-phenyl-acetamide (0.012 mmol) in DMF at 25^{o}C. The resulting reaction mixture was kept stirring for 2 h. The progress of the reaction was monitored by TLC (toluene/ethyl acetate 8:2). After completion of reaction, the mixture was stirred in cold water (100 mL). The obtained solid was filtered washed with water, dried and purified by column chromatography eluting with gave title compound 6 was purified on silica column eluting with toluene.

3.5.1 2-[4,7-Dichloro-3-(2-chloro-ethyl)-2-methyl-benzo[h][1,6]naphthyridin-5-yloxy]-N-p-tolyl-acetamide (**5f**). Yellow needles; yield (0.374 g, 77%); Rf (toluene/ethyl acetate 8:2) 0.51, mp 218 ^{o}C; IR (KBr): ν 3390 (NH), 2962, 2926, 2854, 1681 (C=O), 1589, 1537, 1300, 1184, 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.58 (t, J = 7.8 Hz, 2H, CH_2), 3.84 (t, J = 7.8 Hz, 2H, CH_2Cl), 5.35 (s, 2H, CH_2), 7.19 (d, J = 8.7 Hz, 2H, ArH), 7.49-7.52 (m, 3H, C_9H), 7.82 (d, J = 7.5 Hz, 1H, C_8H), 8.82 (s, 1H, NH, D_2O exchangeable), 8.91 (d, J = 7.5 Hz, 1H, $C_{10}H$); MS: m/z (%): 493 (M+6, 10), 491 (M+4, 10), 489 (M+2, 15), 487 (M+, 30), 452 (20), 381 (80), 341 (50), 325 (60), 147 (100), 106 (65), 91 (80), 77 (90). Anal. Calcd for $C_{24}H_{20}Cl_3N_3O_2$ (488.80): C, 58.97; H, 4.12; N, 8.60. Found: C, 59.02; H, 4.11; N, 8.64.

3.5.2 2-[4,7-Dichloro-3-(2-chloro-ethyl)-2-methyl-benzo[h][1,6]naphthyridin-5-yloxy]-N-(4-fluoro-phenyl)-acetamide (**5g**). Yellow needles; yield (0.369 g, 75%); Rf (toluene/ethyl acetate 8:2) 0.80, mp 121 ^{o}C; IR

(KBr): ν 3354 (NH), 3273, 2926, 1670 (C=O), 1595, 1590, 1317, 1224, 833 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.95 (s, 3H, CH_3), 3.57 (t, J = 7.2 Hz, 2H, CH_2l), 3.84 (t, J = 7.2 Hz, 2H, CH_2Cl), 5.35 (s, 2H, CH_2), 7.11 (d, J = 7.2 Hz, 2H, ArH), 7.49 (t, J = 7.2 Hz, 1H, C_9H), 7.61 (d, J = 7.2 Hz, 2H, ArH), 7.85 (d, J = 7.5 Hz, 1H, C_8H), 8.91 (d, J = 7,5 Hz, 1H, $C_{10}H$), 9.21 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 25.09, 29.66, 41.16, 66.78, 115.67, 115.96, 119.20, 121.66, 122.23, 123.52, 123.98, 125.72, 128.27, 128.80, 129.19, 131.39, 133.33, 143.12, 143.91, 155.14; MS: m/z (%): 497 (M+6, 15), 495 (M+4, 20), 493 (M+2, 30), 491 (M+, 40), 456 (20), 482 (30), 342 (40), 325 (50), 149 (100). 91 (40), 77 (40). Anal. Calcd for $C_{23}H_{17}Cl_3FN_3O_2$ (492.77): C, 56.06; H, 3.48; N, 8.53. Found: C, 56.12; H, 3.42; N, 8.56.

3.5.3 N-(4-Chloro-phenyl)-2-[4,7-dichloro-3-(2-chloro-ethyl)-2-methyl-benzo[h][1,6]naphtha yridin-5-yloxy]-acetamide (**5i**. Yellow needles; yield (0.371 g, 73%); Rf (toluene/ethyl acetate 8:2) 0.82, mp 231-232 ^oC; IR (KBr): ν 3355 (NH), 2926, 2840, 1683 (C=O), 1594, 1510, 1318, 1224, 850 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.72 (s, 3H, CH_3), 3.46 (t, J = 7.6 Hz, 2H, CH_2CH_2Cl), 3.57 (t, J = 7.6 Hz, 2H, CH_2CH_2Cl), 4.41 (s, 2H, CH_2), 7.35 (d, J = 8.3 Hz, 2H, ArH), 7.54 (t, J = 7.8 Hz, 1H, C_7H), 7.61 (d, J = 8.3 Hz, 2H, ArH), 7.83 (d, J = 7.8 Hz, 1H, C_8H), 8.85 (d, J = 7.8 Hz, 1H, C_6H), 9.81 (s, 1H, NH, D_2O exchangeable); MS: m/z (%): 515 (M+8, 10), 513 (M+6, 10), 511 (M+4, 15), 509 (M+2, 20), 507 (M+, 40), 472 (30), 381 (30), 341 (30), 325 (100), 166 (80). Anal. Calcd for $C_{23}H_{17}Cl_4N_3O_2$ (509.22): C, 54.25; H, 3,37; N, 8.25. Found: C, 54.30; H, 3.35; N, 8.27.

IV. ACKNOWLEDGEMENTS

The authors thank to University of Pune, India for financial support to this research project. Authors also thanks to Principal KTHM College Nashik, for spectral and analytical facilities.

V. REFERENCES

[1]. Roseman K. A.; Maribeth M. G.; Linfield, W. M. and Edwards Ben, E. J. Med. Chem.1969, 13, 230-233.

[2]. Hinschberger, A.; Butt, s.; Lelong, V.; Boulouard, M.; Dumuis, A.; Dauphin, F.;Bureau, R.; Pfeiffer, B.; Renard P.; Rault, S. J. Med. Chem. 2003, 46, 138-147.

[3]. Gorlitzer, K.; Bode, M.; Jones, P.G.; Jomaa, H.; Wiesner, J. Pharmazie, 2007, 62, 15-26.

[4]. Gorlitzer, K.; Bode, M.; Jones, P.G.; Jomaa, H. Pharmazie, 2005, 60, 172-174.

[5]. Chrzastek, L.; Mianowska, B.; Sliwa, W. Aus, J. Chem. 1994, 47, 2129-2133.

[6]. (a) Coatney, G. R. Survey of Antimalarial Agents. Public Health Service Monograph

[7]. No. 9; U. S. Government Printing Office: Washington DC. 1952; (b) Sliwa, W. Heterocycles

[8]. 1979, 12, 1207-1237; c) Matusiak, G.; Sliwa, W. Acta Chimica Hungarica. 1988, 125, 267-274.

[9]. Martindale, R. J. The exta parmacopoeia. 30th ed.; Royal Pharm Soc Great Britain,Pharmaceutical: London, 1993; p 460.

[10]. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.;Saxena, P. R.; Humphrey, P. P. Pharmacol. Rev. 1994, 46, 157-203.

[11]. (a) Eglen, R. M.; Wong, E. H. F.; Dumuis, A.; Bockaert, J. Trends Pharmacol. Sci. 1995, 16,391-398; (b) Eglen, R. M.; Hegde, S. S. Exp. Opin. Invest. Drugs, 1996, 5, 373-388.

[12]. Bureau, R.; Daveu, C.; Lemaitre, S.; Dauohin, F.; Landelle, H.; Lancelot, J. C.; Rault, S. J.Chem. Inf. Comput. Sci. 2002, 42, 962-967.

[13]. Schaus, J. M.; Thompson, D. C.; Bloomquist, W. E.; Susemichel, A. D.; Calligaro, D. O.;Cohen, M. L. J. Med. Chem. 1998, 41, 19-1955.

[14]. Curtet, S.; Soulier, J. L.; Zahradnick, I.; Giner, M.; Berque-Bestel, Fischmeister, R.;Langlois, M. J. Med. Chem. 2000, 43, 3761-3769.

[15]. Lopez-Rodriguez, M. L.; Benhamu, B.; Viso, A.; Morcillo, M. J.; Murcia, M.; Orensanz, L.;Alfaro, M. J.; Martin, M. I. Bioorg. Med. Chem. 1999, 7, 2271-2281.

[16]. Salama, H. M.; Labouta, I. A.; Moustafa M. A.; Moustafa. Alex. J. Pharm. Sci. 1990, 4, 44-46.

[17]. Bonde, C. G.; Gaikwad, N. J. Bioorg. Med. Chem. 2004, 12, 2151-2161.

[18]. Kucukguzel, S. G.; Oruc, E. E.; Rollas, S.; Sahin, F.; Ozbek, A. Eur. J. Med. Chem. 2002,37, 197-206.

[19]. Capan, G.; Ulusoy, N.; Ergenc, N.; Kiraz, M. Monatsh. Chem. 1999, 130, 1399-1407.

[20]. Ergenc, N.; Capan, G. II Farmaco. 1994, 49, 133-135.

[21]. Bhatt, J. J.; Shah, B. R.; Shah, H. P.; Trivedi, P. B.; Undavia, N. K.; Desai, N. C. Indian J.Chem. 1994, 33B, 189-192.

[22]. Bukowski, L.; Janowiec, M.; Zwolska-Kwiek, Z.; Andrezejczyk, Z. Pharmazie 1998, 53,373-376.

[23]. Barreca, M. L.; Chimirri, A.; Luca, L. D.; Monforte, A.; Monforte, P.; Rao, A.; Zappala, M.;
[24]. Balzarini, J.; De Clercq, E.; Pannecouque, C.; Witvrouw, M. Bioorg. Med. Chem. Lett. 2001,11, 1793-1796.
[25]. Andres, C. J.; Bronson, J. J.; Andrea, S. V. D.; Deshpande, M. S.; Falk, P. J.; Grant-Young,
[26]. K.A.; Harte, W. E.; Ho, H. T.; Misco, P. F.; Robertson, J. G.; Stock, D.; Sun, Y.; Walsh, A.W. Bioorg. Med. Chem. Lett. 2000, 10, 715-717.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Density, Excess Molar Volumes of Water-Ethanol Binary Mixtures at Various Temperatures

Ramesh R. Pawar[1*], Sachin S. Kale[2], Atul S. Kale[3]

[1]Department of Chemistry, M.S.G. College, Malegaon camp, Nashik, Maharashtra, India
[2]Department of Chemistry, A.S.C. College, Navapur, Nandurbar, Maharashtra, India
[3]Department of Chemistry, S.P.H. Mahila College, Malegaon camp, Nashik, Maharashtra, India

ABSTRACT

In the present work Densities of pure ethanol, water and its binary mixtures were measured at temperatures ranges from 293.15 to 313.15 K, over the whole mole fraction range of ethanol. Excess molar volume obtained is negative. Density and excess molar volumes plotted against mole fraction X1 of ethanol.

Keywords : Ethanol, Water, Binary Mixtures, Density, Excess Molar Volume.

I. INTRODUCTION

Water is one of the most basic and important materials in living systems. Despite its apparent molecular simplicity, it has long been considered as complex nature [1]. Ethanol has been widely used in medicine and food. Especially, the interaction between ethanol and water molecules by hydrogen bonding is a central issue [2–4].

Ethanol has been used in the last few years as a distillation container or extractive solvent in the chemical industry, as a carrier or additive in food and pharmaceutical processes, and in antimicrobial applications for medical uses. This molecule contains a hydrophilic hydroxyl group which is available to hydrogen-bond of similar compounds, and a residual end conferring a degree of hydrophobicity on the molecule [5].

Mixtures of water with aliphatic alcohols are of considerable interest from the view point of the existence of some interaction, such as hydrogen bonding between water-which contains an –OH group and can act as a π-type donar – and alcohol molecules, which have one acidic H atom on the –OH group and can act as σ- acceptor [6]. The values of density of pure water is determined in previous work [7]

In this study, results obtained of density and excess molar volume of binary mixture formed by ethanol with water at temperature from 293.15 to 313.15 K are reported.

II. EXPERIMENTAL SECTION

Material:

Ethanol was supplied by Changzhou Yangquan chemical with purity (GC) 99.9%. In all experiment, triple distilled water was used.

Method:

Binary mixture was prepared by knowing masses of each liquid in airtight stoppered glass bottles. The densities of pure liquid and binary mixture of liquids were measured in 15 cm^3 double arm pycnometer [8-11]. This pycnometer was calibrated using conductivity water with 0.9970 cm^{-3} at 25^0C its density. The pycnometer filled with air bubble free experimental liquid was kept in a transparent walled water bath in which the temperature was maintained to attained thermal equilibrium. The position of the liquid level in the two arms was recorded with travelling microscope which read correctly to ± 0.01 mm. [12].

III. RESULT AND DISCUSSION

Density and excess molar volume of ethanol with water at temperatures from (293.15 to 313.15) K are shown in table 2 and in figures 1 and 2. Following equation is used to calculate excess molar volume V^E [13].

$$V^E = \frac{(X_1M_1 + X_2M_2)}{\rho_{12}} - \left(\frac{X_1M_1}{\rho_1}\right) - \left(\frac{X_2M_2}{\rho_2}\right)$$

Where, X_1, X_2 are mole fractions, M_1, M_2 are molecular weights and ρ_1, ρ_2 are density of components 1 & 2 respectively of binary mixtures. ρ_{12} is the mixtures density.

Densities of ethanol with water is determined at temperatures from 293.15 to 313.15 K. the densities of the pure ethanol are shown in table 1 along with the literature values. From table 1 it is seen that there is closeness between experimental values and literature values. The results obtained are satisfactory. The density and corresponding V^E data of the binary systems of ethanol with water is shown in table 2 at different temperatures. Figure1 shows the plots of densities as a function of mole fraction of ethanol in water. Continuous decrease in density at the same rate on addition of ethanol in water is observed. Excess molar volumes, V^E for ethanol in water have been plotted in figure 2 from this fig. V^E Found to be negative.

Table 1. Densities (ρ) of pure ethanol at various temperatures (T/K)

Temp. (T/K)	Density ($\rho \cdot 10^{-3}$(kg·m^{-3})	
	Expt.	Lit.
293.15	0.7892	-
295.15	0.7876	-
298.15	0.7851	0.7850[14]
300.15	0.7835	-
303.15	0.7809	-
305.15	0.7792	-
308.15	0.7768	0.7771[15]
310.15	0.7750	-
313.15	0.7731	0.7731[15]

Table 2. Density (ρ), Excess molar volume (V^E) for various mole fractions (X_1) of ethanol at (293.15 to 313.15) K

T/K	X_1 Ethanol	$\rho \cdot 10^{-3}$ (kg·m^{-3})	$V^E.10^6$ (m^3·mol^{-1})	T/K	$\rho \cdot 10^{-3}$ (kg·m^{-3})	$V^E.10^6$ (m^3·mol^{-1})
293.15	0.0000	0.9975	0.0000	305.15	0.9944	0.0000
	0.0416	0.9816	-0.1962		0.9778	-0.2057
	0.0891	0.9683	-0.4669		0.9628	-0.4624
	0.1436	0.9532	-0.7228		0.9458	-0.6964
	0.2068	0.9348	-0.9200		0.9261	-0.8798
	0.2812	0.9137	-1.0466		0.9044	-1.0026
	0.3698	0.8990	-1.3903		0.8810	-1.0550
	0.4772	0.8677	-1.1086		0.8576	-1.0674

	0.6100	0.8430	-0.9800		0.8327	-0.9428
	0.7788	0.8178	-0.7124		0.8076	-0.6902
	1.0000	0.7892	0.0000		0.7792	0.0000
295.15	0.0000	0.9970	0.0000	308.15	0.9934	0.0000
	0.0416	0.9810	-0.1975		0.9767	-0.2100
	0.0891	0.9675	-0.4666		0.9613	-0.4639
	0.1436	0.9520	-0.7172		0.9438	-0.6912
	0.2068	0.9334	-0.9114		0.9239	-0.8715
	0.2812	0.9122	-1.0379		0.9019	-0.9919
	0.3698	0.8892	-1.0902		0.8785	-1.0450
	0.4772	0.8660	-1.0982		0.8551	-1.0583
	0.6100	0.8412	-0.9708		0.8300	-0.9309
	0.7788	0.8161	-0.7070		0.8049	-0.6762
	1.0000	0.7876	0.0000		0.7768	0.0000
298.15	0.0000	0.9963	0.0000	310.15	0.9927	0.0000
	0.0416	0.9802	-0.2011		0.9760	-0.2114
	0.0891	0.9662	-0.4667		0.9603	-0.4622
	0.1436	0.9502	-0.7115		0.9424	-0.6842
	0.2068	0.9312	-0.9017		0.9224	-0.8649
	0.2812	0.9099	-1.0285		0.9004	-0.9874
	0.3698	0.8868	-1.0807		0.8768	-1.0381
	0.4772	0.8635	-1.0908		0.8533	-1.0507
	0.6100	0.8387	-0.9622		0.8283	-0.9277
	0.7788	0.8136	-0.7053		0.8032	-0.6778
	1.0000	0.7851	0.0000		0.7750	0.0000
300.15	0.0000	0.9958	0.0000	313.15	0.9916	0.0000
	0.0416	0.9796	-0.1817		0.9747	-0.2136
	0.0891	0.9653	-0.4451		0.9587	-0.4637
	0.1436	0.9489	-0.6870		0.9404	-0.6830
	0.2068	0.9299	-0.8789		0.9200	-0.8583
	0.2812	0.9084	-1.0052		0.8978	-0.9773
	0.3698	0.8851	-1.0564		0.8743	-1.0342
	0.4772	0.8619	-1.0699		0.8507	-1.0494
	0.6100	0.8370	-0.9480		0.8256	-0.9230
	0.7788	0.8119	-0.6919		0.8005	-0.6732
	1.0000	0.7835	0.0000		0.7723	0.0000

303.15	0.0000	0.9950	0.0000	
	0.0416	0.9786	-0.2059	
	0.0891	0.9639	-0.4660	
	0.1436	0.9471	-0.7024	
	0.2068	0.9275	-0.8855	
	0.2812	0.9058	-1.0084	
	0.3698	0.8826	-1.0635	
	0.4772	0.8593	-1.0745	
	0.6100	0.8344	-0.9485	
	0.7788	0.8093	-0.6954	
	1.0000	0.7809	0.0000	

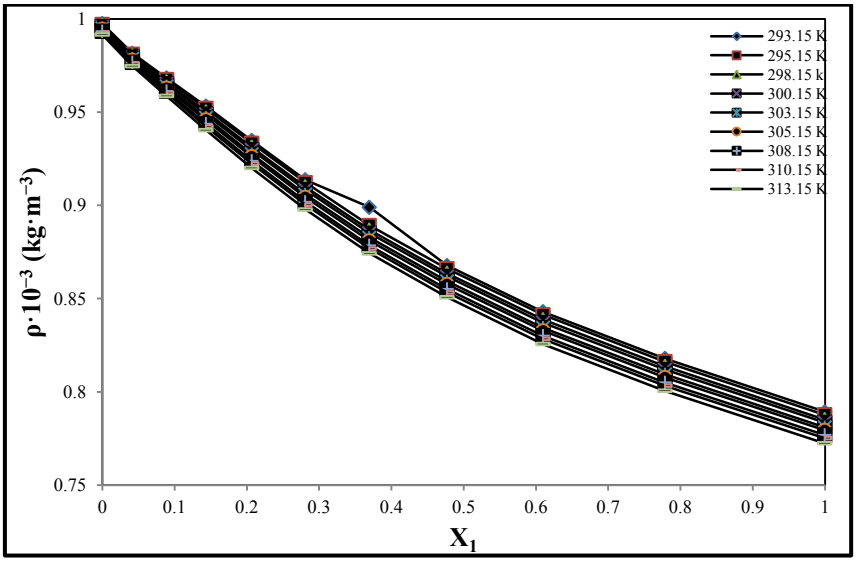


Figure 1. Plot of density (ρ) Vs mole fraction (X_1) of ethanol in water system at 293.15 to 313.15 K.

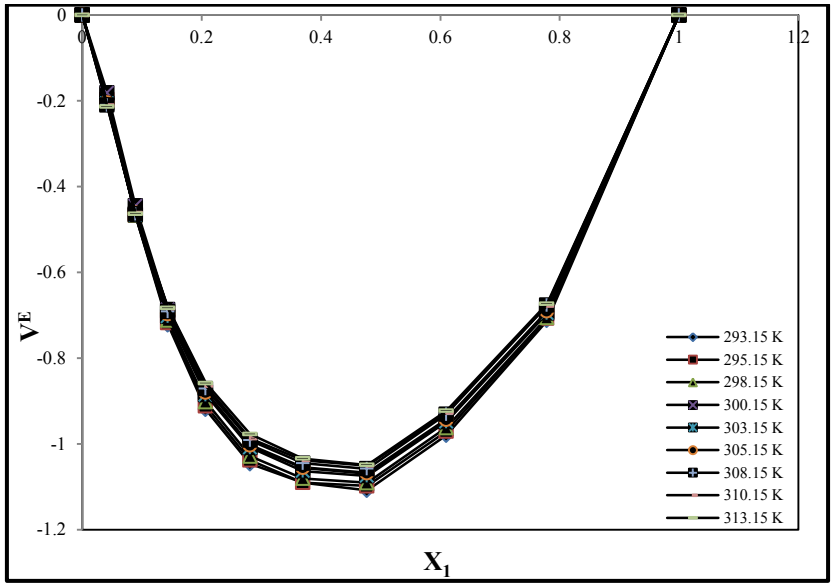


Figure 2. Plot of Excess molar volume (V^E) Vs X_1 for ethanol in water at 293.15 to 313.15 K.

IV. CONCLUSION

Densities, Excess molar volume of pure water, ethanol and in water-ethanol binary mixtures is measured at temperatures from 293.15 to 313.15 K. Continuous decrease in density at the same rate on addition of ethanol in water are observed and excess molar volume is found to be negative.

V. REFERENCES

[1]. C.Q. Sun, X. Zhang, J. Zhou, Y.L. Huang, Y.C. Zhou, W.T. Zheng, Density, elasticity, and stability anomalies of water molecules with fewer than four neighbors, J. Phys. Chem. Lett., 4 (2013) 2565–2570.

[2]. C.J. Benmore, Y.L. Loh, The structure of liquid ethanol: a neutron diffraction and molecular dynamics study, J. Chem. Phys., 112 (2000) 5877–5883.

[3]. C. Talon, M.A. Ramos, S. Vieira, Low-temperature specific heat of amorphous, orientational glass, and crystal phases of ethanol, Phys. Rev. B, 66 (2002) 012201.

[4]. A. Matic, C. Masciovecchio, D. Engberg, G. Monaco, L. Börjesson, S.C. Santucci, R. Verbeni, Crystal-like nature of acoustic excitations in glassy ethano, Phys. Rev. Lett., 93 (2004) 145502.

[5]. O. Redlich, A.T. Kister, Ind. Eng. Chem., 40 (1948) 341.

[6]. Hulya Y., excess properties of alcohol-water systems at 298.15 K, Turkey journal physics, 26, (2002), 243-246.

[7]. R.R. Pawar, S.S. Kale, A.S. Kale and S.B. Nahire, density and excess molar volumes of water-methanol binary mixtures at (293.15 to 313.15) K, International Journal of Current Advanced Research, Volume, 6, (2017), 6657-6659.

[8]. M.Hasan, , D.F.Shirude, , A. P.Hiray, U.B.,Kadam, A.B. Sawant, J Chem Eng Data, 51, (2006) 1922-192.

[9]. P.S.Nikam, A.B. Sawant, J. Chem Data, 42 (1997), 585.

[10]. P.S.Nikam, A.B. Sawant, J molecular liquid, 75, (1998), 119.

[11]. P.S. Nikam, A.B. Sawant, Bull Chem Soc. JPN, 71, (1998), 2061.

[12]. Kaminsky, J. phys Chem (NF), 5, (1995), 154.

[13]. M.A. Tejraj, Thermodynamic interactions in binary mixtures of ethenylbenzene with methanol, ethanol, butan-1-ol, pentan-1-ol, and hexan-1-ol in the temperature range 298.15–308.15 K. J. Chem. Eng. Data, 44, (1999), 1291–1297.

[14]. J.V. Herr´aez, R. Belda, Refractive Indices, Densities and Excess Molar Volumes of Monoalcohols + Water, J Solution Chem., 35, (2006), 1315–1328.

[15]. A.B. Dikko, A. Alkasim, Some Excess Properties of Ternary Liquid Mixture of Water, Methanol, Ethanol at Several Temperatures, IJRRPCS., 1, (2014), 8-12.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Versatile Synthesis of Ni Nanoparticles by Solution Combustion Method

Sandip V. Wakchaure, Brijesh Gaud
Department of Chemistry, JSM'S SGAS&GPC College Shivle Murbad Thane, Maharashtra, India

ABSTRACT

We report here a simple solution combustion synthesis of Nickel oxide (NiO) nanoparticles by using glycine as a fuel and nickel nitrate hexahydrate (Ni(NO3)2.6H2O) as an oxidizer and it's reduction to nickel nanoparticles by using hyderogen gas. The morphological structural properties of nanoparticles were investigated by scanning electron microscopy (SEM), Xray diffraction (XRD), Fourier transform infrared (FT-IR) and UV-Visible spectroscopic technique. The solution combustion-synthesized Ni nanoparticles have a crystalline size of ~ 20 nm. The agglomeration of fine particles with particle size in the range of 30~50 nm is seen by SEM images. The FT-IR spectrum shows absorption band at ~ 466 cm-1 for Ni nanoparticles. UV-Vis. Spectroscopy shows absorption band for NiO at ~ 350 nm and Ni at 400 nm. Ni nanoparticles are used in different fields like CNT synthesis catalysis supercpacitors etc.

Keywords: Solution combustion, NiO, Glycine, Catalyst, CVD, H2 gas, Nanoparticles

I. INTRODUCTION

From literature survey we learnt that, Nanoparticles of transition metals like Ni, Co, Fe etc. Have been studied by several scientists in the last few years. Nanoparticles of transition metals are getting continuous importance for various application such as catalysts (Patil K C 1997) super capacitors, gas sensors, biosensors, supercapacitors, Several researchers have prepared NiO by different method like sol-gel (C N R Rao 1963) surfactant-mediated synthesis (C N R Rao 1994) thermal decomposition (Rao K J and Ramesh P D 1995) solution combustion synthesis (S. Balmurgan and A.J. Linda Phillip 2016) and so on. However to the best and most commonly used laboratory synthesis is solution combustion method. In solution combustion method different combination of fuels and oxidizers are used oxidizers like metal nitrate, metal chloride metal sulphates etc., fuel like glycine, urea, citric acid, oxalic acid, glucose, sucrose, aniline.

Current report is solution combustion synthesis using nickel nitrate hexahydrate as an oxidizer and glycine as a fuel. The characterization study of prepared NiO and Ni nanoparticles was done by SEM, XRD, FTIR, UV-Vis spectroscopy.

II. MATERIALS AND METHODS:

Synthesis of Nickel oxide and Ni nanoprticles Very small particles of metals such as Ni, Co, Fe are known for their catalytic role in growth of CNT (Schwarz et al., 1995). Ni catalyst was prepared by thermal decomposition method. Nickel nitrate hexahydrate and glycine were mixed at fixed 1:1 molar ratio in 25ml distilled water and stir for 10 minutes. (Chatterjee et al., 2003). The solution was then kept in pre heated muffle furnace at 350oC at the flash point of glycine. The decomposition of glycine is highly exothermic and large amounts of ammonia and carbon dioxide are liberated and fine Nickel oxide was obtained. Nickel oxide produced in grind with hand pastle to get fine powder. Nickel oxide obtained after grinding is blackish in colour and sonicated. Here NO3- in metal nitrate act as oxidizer and an organic compound that has carboxylate and/or amine (i.e. glycine) act as fuel. This is an autocatalytic and self-propagating reaction utilizing exothermic redox decomposition of fuel and oxidizer. Residual energy of combustion (reaction enthalpy) is used to crystallize the particle. The explosive gas blows off and material resulting into ultra-fine crystallite powder. The Nickel oxide was reduced in CVD furnace by H2 at 600oC for 2 h yielding a very fine metal nanoparticle which was used as catalyst to grow CNTs by CVD (Turano et al., 2006). The prepared NiO and Ni

sonicated with ethyl alcohol for 20 min. further studied by using technique like SEM, XRD, FTIR, UV- Vis spectroscopy.

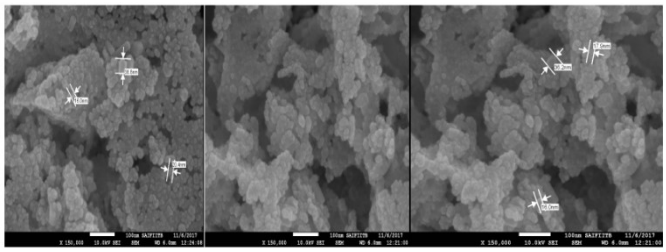


Figure 1. SEM images for Ni nanoparticles

III. RESULTS AND DISCUSSIONS

SEM and XRD Study:

$D = 0.94\lambda/\beta½COS\theta$ Where λ is wavelength of XRD radiation β is the full width at half maximum of the peak corresponding to the plane. θ is the angle obtained from 2θ value corresponding to the XRD pattern. Crystalline size obtained from the sharp peak at 2θ = 44.77 is 59.15 from the powdered XRD.

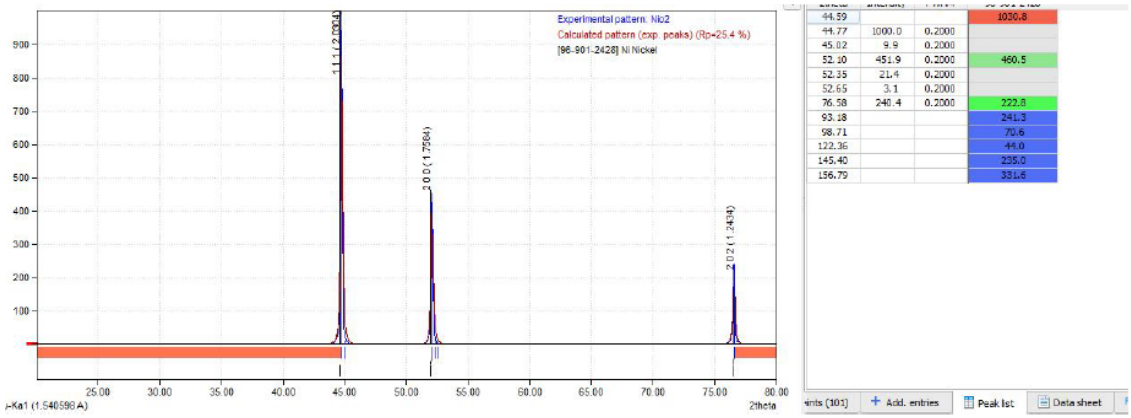


Figure 2. Ni nanoparticle XRD image

UV-Vis and FTIR Study

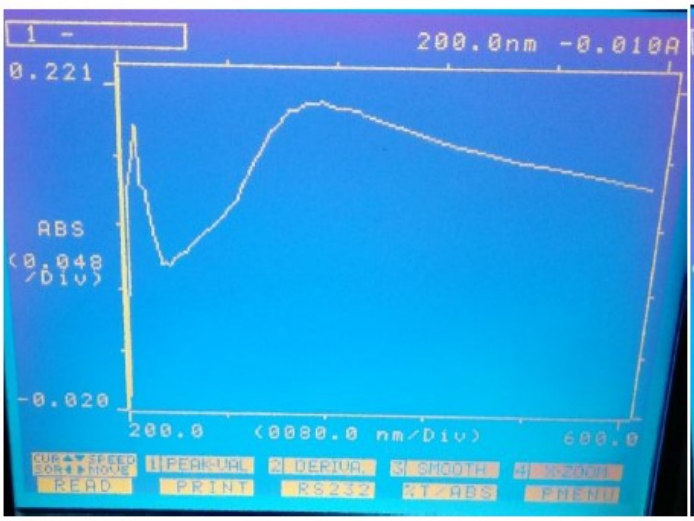


Figure 3.1. UV-Vis spectrum for NiO

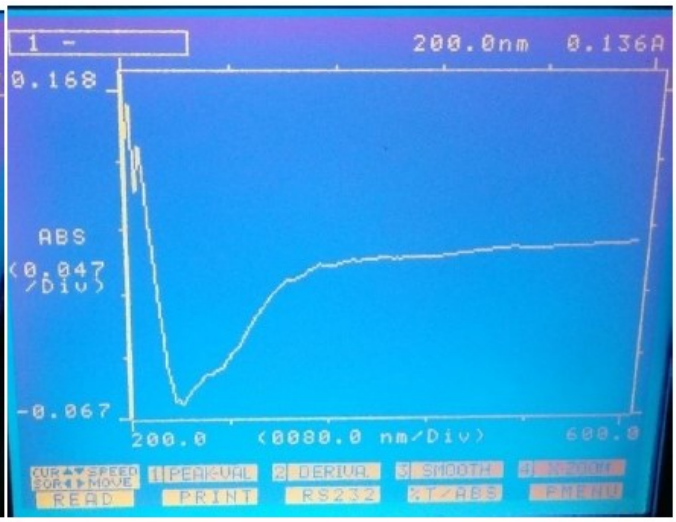


Figure 3.2. UV-Vis spectrum for Ni

The optical absorption behavior of the prepared NiO and Ni was examined in the UV-visible absorption region (200-600 nm), and their typical absorption spectrum is depicted in Figure 3.1 and Figure 3.2 for Nio two UV absorption peaks at ~209 and ~350 nm which indicate presence of NiO

from literature study. In Figure 3.2 maximum absorption observed at 400 nm indicates presence of Ni nanoparticle, further presence of Ni nanoparticles is supported by FT-IR. Peak for Ni nanoprticle is at around 466 cm-1. Images taken during the synthesis of Ni nanoparticles from nickel nitrate hexahydrate and glycine are given in Figure 5.1, 5.2 and 5.3.

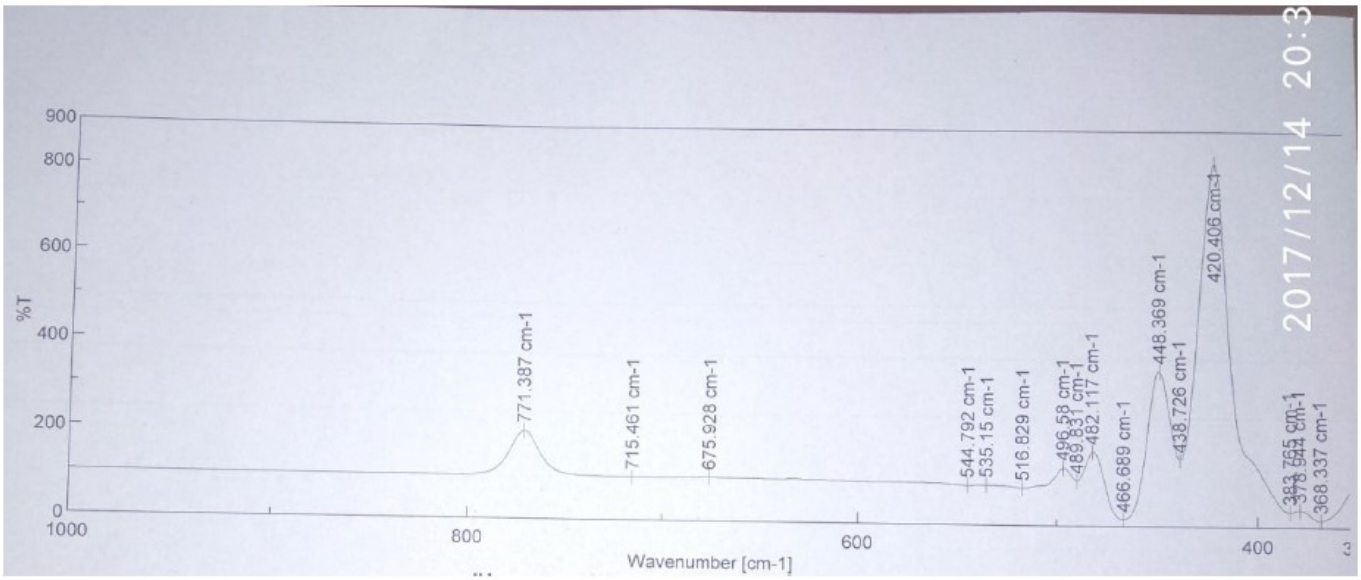


Figure 4. FT-IR spectrum for Ni nanoparticle

Images for NiO and Ni Synthesis

Figure 5.1. Flame at ignition point

Figure 5.2. NiO nanoparticles

Figure 5.3. Ni nanoparticles

IV. CONCLUSION

Ni nanoparticles were successfully synthesized by solution combustion method by using glycine as fuel with good yield 8-10%. The synthesized nanoparticle were highly pure and almost homogeneous in size ranging in between 20-40 nm. The prepared nanoparticle will be suitable for potential application in catalysis, supercapacitors, biosensors, electrochromic devices. In further investigation we are using synthesized Ni nano particles for the production of CNT by CVD and plant oil as precursor.

V. ACKNOWLEDGMENTS

CVD, UV- Vis spectroscopy FT-IR is from Birla College Kalyan. XRD, SEM analysis is from SAIF department IIT Powai Mumbai.

VI. REFERENCES

[1]. A. Kopp Alves et al. Novel Synthesis and Characterization of nanostructured material , Engineering Materials (2013) chapter 2 Combustion Synthesis

[2]. C. N. R. (1994) Chemical approaches to the synthesis of inorganic materials (New Delhi:Wiley Estern Ltd.)
[3]. N. R. Rao and B. Raveau, (1995) Transition Metal O, 2nd edn, Wiley-VCH, Germany.
[4]. S. Balamurgan, A. J. Linda Philip. R. S. Vidya (2016). A Versatile Combustion synthesis and properties of Nickel oxide (NiO) particles
[5]. Chatterjee, A. K., Sharon, M., Banerjee, R. and Spallart M. N. (2003). CVD synthesis of carbon nanotubes using a finely dispersed cobalt catalyst and their use in double layer electrochemical capacitor. ElectrochemicaActa, 48: 439-3446.
[6]. Che, G. B., Lakshmi, B. Martin, C. R., Fisher, E. R. and Ruoff, R. S. (1998). Chemical vapor deposition based synthesis of carbon nanotubes and nanofibers using a tamplate method. Chemistry of Materials, 10 (1): 260-267.
[7]. Sharanabasva V. Ganachari, Ravishankar Bhat, Raghunandan Deshpande (2012).Synthesis and characterization of Nickel oxide nanoprticles by self-propagating low temperature combustion
[8]. Patil, K.C., Mimani, T. (2001). Solution combustion synthesis of nanoscale oxides and their composites. Mater. Phys. Mech. 4, 134-137.
[9]. Mimani, T., Ghosh, S. (2000). Combustion synthesis of cobalt pigments: blue and pink. Curr. Sci. 78, 892-896.
[10]. Muhammad Imran Din and Aneela Rani (2016). Recent Advances in the Synthesis and Stabilization of Nickel and Nickel Oxide Nanoparticles:nA Green Adeptness. International Journal of Analytical Chemistry.
[11]. Li, W. Z., Xie, S. S., Qian, L. X., Chang, B. H., Zou B. S and Zhoi, W. Y. (1996). Largescale synthesis of aligned carbon nanotubes. Science, 274: 1701-1703.
[12]. Ndungu, P., Godongwana Z.G., Petrik, L.F., Nechaev A., Liao S. and Linkov, V. (2008). Synthesis of carbon nanostructured materials using LPG. Journal of Microporous and Mesoporous Materials, 116 (1): 593-600.
[13]. Pradhan, D., Sharon, M., Kumar, M. and Ando, Y., (2003). Nano-Octopus: A New Form of Branching Carbon Nanofiber. Journal of Nanoscience and Nanotechnology, 3(3): 215-217.
[14]. Pederson, M.R. and Broughton, J.Q. (1992). Nanocapillarity in fullerene tubules. Phys. Rev. Lett. 69: 2689.
[15]. Schwarz, J. A., Contescu, C. and Contescu A. (1995). Methods for Preparation of Catalytic Materials. Chem. Rev., 95 (3): 477–510.
[16]. Sezgin, S., Ates, M., Parlak, Elif A. and Sarac A. S. (2012). Scan Rate Effect of 1-(4-methoxyphenyl)-1H-Pyrrole Electro-coated on Carbon Fiber: Characterization via Cyclic Voltammetry, FTIR-ATR and Electrochemical Impedance Spectroscopy. Int. J. Electrochem. Sci., 7: 1093-1106.
[17]. Sharon, M., Mukhopadhyay, K., Yase, K., Iijima, S., Ando, Y. and Zhao X. (1998). Spongy Carbon nanobeads-a new material. Carbon, 36(506): 507-511.
[18]. Sharon, M. and Mukhopadhyay, K. (1996). Glassy Carbon from camphor - A Natural Source. Materials Chemistry and Physics, 49(2): 105-109.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Development and Validation of Stability Indicating RP-HPLC Method on Core Shell Columnfor Determination of Degradation and Process Related Impurities of Macitentan- Anti-hypertension Drug

Shashikant B. Landge[1], Sanjay A. Jadhav[1], Sunil B. Dahale[1] ,Rajendra S. Shinde[1], Kunal M. Jagtap[1], Saroj R. Bembalkar[2], Vijayavitthal T. Mathad[1*]

[1]Department of Process Research and Development, Megafine Pharma Lakhmapur, Dindori, Nashik, Maharashtra, India
[2]Department of Chemistry, Deogiri College, Aurangabad, Maharashtra, India

ABSTRACT

A core shell chromatographic column was used to separate the nine process related and degradation related impurities(Imp-1 to Imp-9) of Macitentanis described in this article. The chromatographic separation was achieved on a Sigma-Aldrich's 'Ascentis Express ® C18 (4.6 mm x 100 mm, 2.7 µ)' HPLC column with a runtime of 35 min. Forced degradation study was carried out under acidic, alkaline, oxidative, photolytic, and thermal degradation conditionsto demonstrate the stability-indicating nature of developed RP-HPLC method.The methodology consists of mobile phase-A as aphosphate buffer and mobile phase-B as a mixture of acetonitrile andmethanol. The column oven temperature was set at 45°C, injection thermostat was set at 5°C, and photodiode array detector (PDA) was set at 215 nm.Adeveloped method was validated as per ICH guideline and found rapid, specific,precise, sensitive,androbust. The proposed RP-HPLC method was successfully applied to the analysis of drug substance and drug product of Macitentan.

Keywords: Macitentan, Core-Shell HPLC Column, RSD and Validation, Stability Indicating.

I. INTRODUCTION

Macitentan is an antagonist drug chemically known as N-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-4-pyrimidinyl]-N'propylsulfamide and sold under the brand name "Opsumit" to treat the people with pulmonary arterial hypertension (PAH), a chronic, life-threatening disorder which severely compromises the function of the lungs and heart. It is an endothelin receptor antagonist (ERA) approved for the treatment of pulmonary arterial hypertension (PAH). Macitentan is a dual ERA, meaning that it acts as an antagonist of two endothelin (ET) receptor subtypes, ETA and ETB[1].However, Macitentan has a 50-fold increased selectivity for the ETA subtype compared to the ETBsubtype [2].The drug received approval from the U.S. Food and Drug Administration (FDA) on October 13, 2013[3].

An analytical method development to determine the quality of product is a critical task during the synthesis of product in generic companies because there is continuous improvement in the process by usingnew reagents, intermediates or by changing the route of synthesis. To develop the robust and selective analytical method nine process related impurities have been discovered during the synthesis of product by process development laboratory (Imp-1 to Imp-9)(**Figure1**).

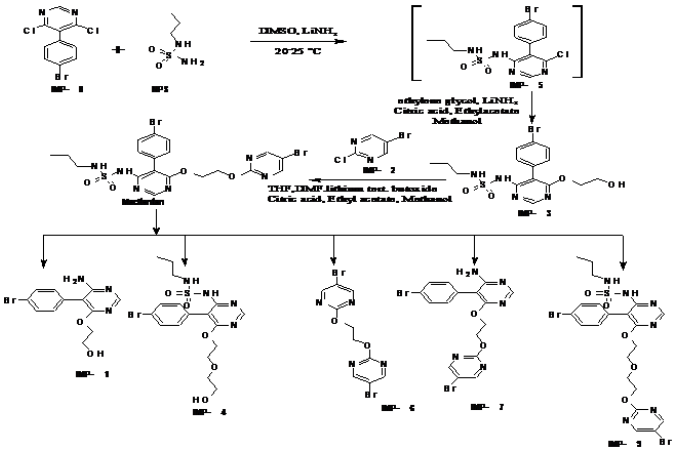


Figure 1. Process Related Impurities of Macitentan

Literature survey revealed that the Macitentan and/ or its metabolites were studied in human plasma by liquid chromatography-mass spectrometry method for theirpharmacokinetics[**4-5**].However, these papers were restricted to the determination of Macitentan and the details of process-related impurities and degradation related impurities formed under the stress conditions are not discussed.Few more analytical papers were also reported describing the method for determination of Macitentan content and not for the process-related impurities and degradation related impurities formed under the stress conditions employed[**6-8**].One of the articles reported on Quality by Design (QbD) HPLC method development of Macitentan lacks the forced degradation study and covers the limited number of impurities as compared to this article.Moreover, the column used in this article is porous and 3µ which often gives the high backpressure during the analysis[**8**].Further, Macitentan is not yet official in any of the pharmacopoeia andas per the requirements of various regulatory authorities, the impurity profile study of drug substance and drug product must be carried out using a suitable analytical method in the final drug product. Hence, we felt the need for the development of a selective, fast, and stability-indicating HPLC method on core shell column.

To the best of our knowledge no method on core shell column has been reported for the determination of Macitentan and its potential process related impurities in the drug substance and drug product for regular and stability study analysis in quality control laboratory.In present article the core shell chromatography column has been used to separate the nine process related impurities. The silica particle used in UPLC and HPLC column are porous in nature andsub-2µ and sub-5µ respectively. These columns give good resolution, speed, and sensitivity but same time it gives high backpressure[**9-11**].Hence core shell columns have been preferred to overcome these limitations. The present article describes the method development and method validation for determination of Macitentan and its process related impurities in bulk and dosage form using the core shellHPLC column [**12-13**].The core-objective of this research work was to develop a specific, precise, sensitive, and rapid stability-indicating RP-HPLC method for the determination of process and degradation related impurities of Macitentan. Thedeveloped method was successfully validated according to the USP<1225>Validation of Compendial procedures and ICH Q2 (R1) guideline[**14-15**].

II. EXPERIMENTAL

2.1. Materials and Reagents

The reagents like, ammonium dihydrogen orthophosphate, hydrochloric acid, sodium hydroxide, and hydrogen peroxide, triethylamine were all of AR grade, procured from Merck (India). The gradient grade acetonitrile and methanol was procured from J.T. Baker, Mumbai, India. HPLC grade water obtained from Millipore system (Millipore Inc., USA). The test sample of Macitentanand its potential process related impurities (Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, and Imp-9) were received from synthetic laboratory of Megafine Pharma (P) Ltd, Nashik, India.

2.2. Instrumentation and Chromatographic Conditions

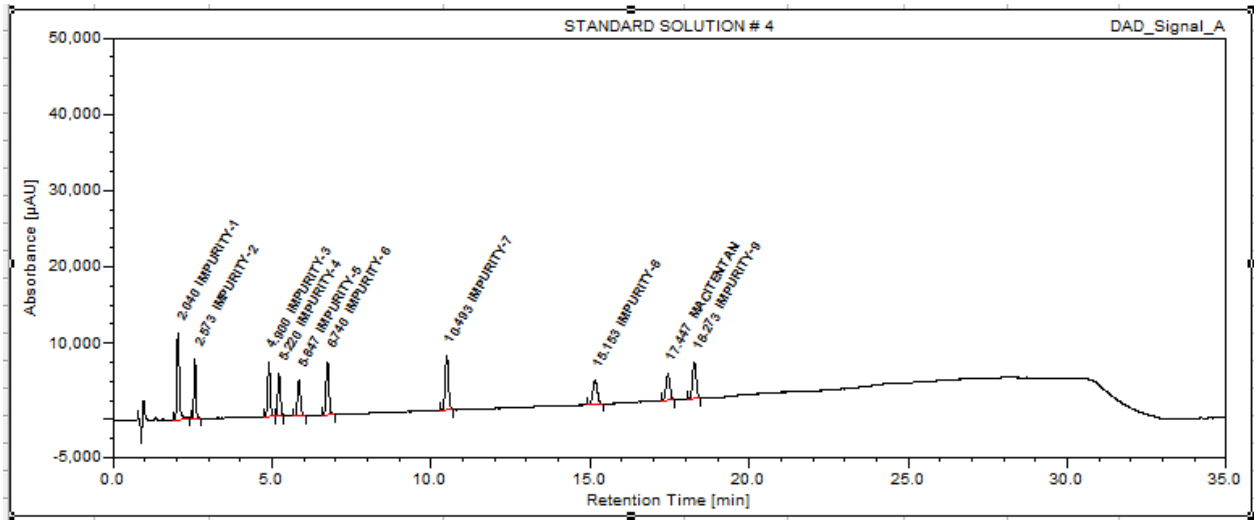
High Performance Liquid Chromatography (HPLC) equipped with photodiode array detector (1260, Agilent Technologies, Germany) was used for analytical method development and analytical method validation. 0.05M ammonium dihydrogen orthophosphate buffer was prepared bydissolving 5.75±0.10g ammonium dihydrogen orthophosphate in 1000 mL of waterand by adjusting pH 5.5 with triethylamine, further this buffer was filtered through 0.45µm membrane filter (0.45µ, Millipore) and degassed in ultrasonic bath prior to use as mobile phase A. 70:30 v/v,Acetonitrile and methanol was used as mobile phase B. Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ) HPLC column thermostated at 45°C was used for the separation.The flow rate and injection volumes were 1.0 mL min^{-1} and 15µl respectively and injection thermostat was set at 5°C.The analysis was carried out under the gradient condition as time $_{(min)}$/A (v/v): B (v/v);$T_{0.01}$.60/40, $T_{25.0}$.35/65,$T_{29.0}$/35/65,$T_{31.0}$/60/40and $T_{35.0}$/60/40. The data was acquired at 215 nm for 35 min and processed by using Chromeleon software Ver. 6.80.

2.3. Preparation of Solutions and Analytical Procedure

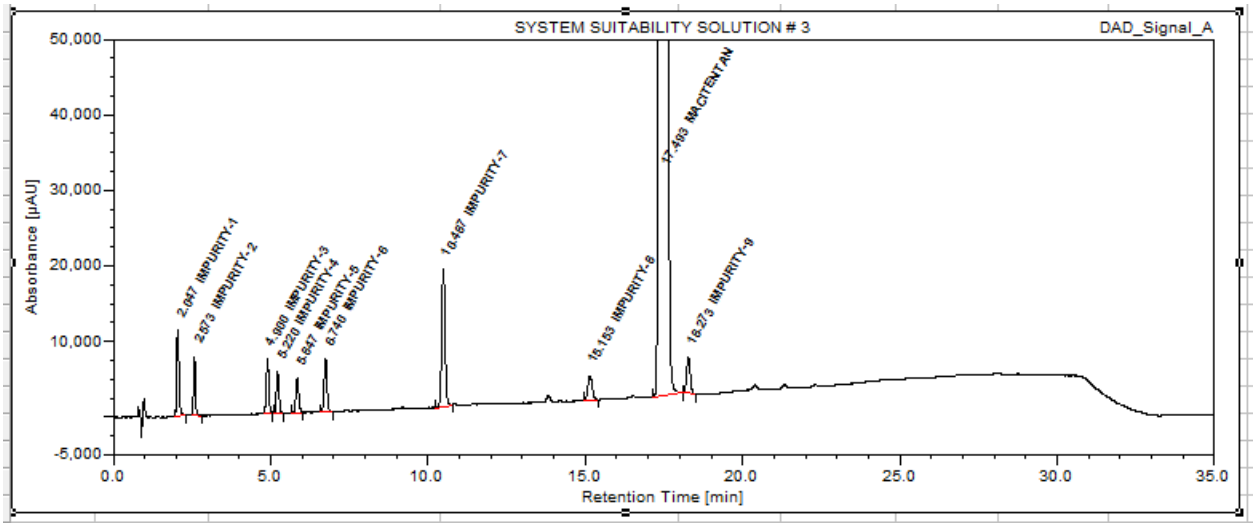
The diluent for analysis was prepared by mixing water and acetonitrile in the ratio of 50:50 (v/v).The stock

solutions of each impurity (Imp-1 to Imp-9) at concentration about75 µg $mL^{-1}$was prepared in diluent and further diluted to prepare the standard solution for quantification of impurities(**Figure2a**). The specification limits used for study was 0.15% for the related substances. Macitentan standard solution (500 µg mL^{-1}) spiked with all impurities at a specification level (w/w) was used as system suitability test (SST)(**Figure 2b**).The test sample solution having concentration of 500 µg mL^{-1} was prepared for the determination of related substances. The blank, system suitability test, 6 replicates of standard and test solution were injected separately. The resolution NLT 2.0, between Macitentan and Imp-9 and %RSD, NMT 5.0% for areas of six replicate injections of standard solution were set as system suitability criteria.

a) HPLC Chromatogram of Standard Solution:



b) HPLC Chromatogram of System Suitability Test Solution (SST):



c) HPLCChromatogram of Test Solution Spiked with Impurities at 1.0%w/w:

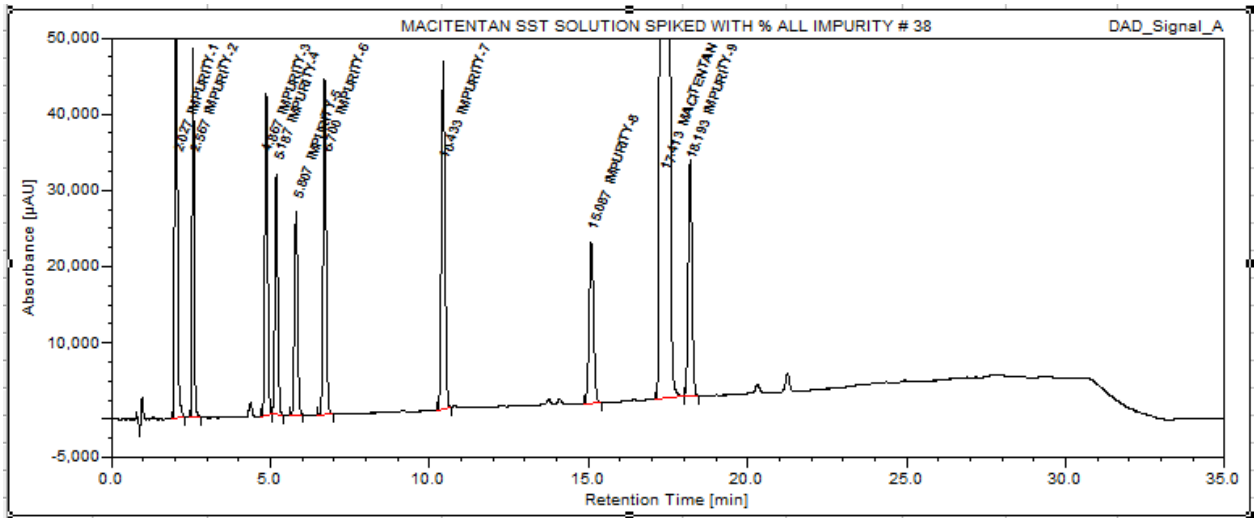


Figure 2. The chromatograms of Core shell column (a) standard solution (b) SSTand (c) test solution spiked with Impurities at 1.0%w/w

2.4. Characterization of Impurities

2.4.1. Fourier Transform Infrared Spectroscopy (FT-IR).FT-IR spectra were recorded for all the nine process-related and degradation impurities on Perkin Elmer model-spectrum-100 (California, USA) instrument using KBr pellet method.

2.4.2. ^{1}H NMR Spectroscopy.The ^{1}H NMR spectra were recorded on Bruker AV400 (400MHz) spectrometer using suitable solvent and tetramethylsilane (TMS) as internal standard.

2.4.3. Mass Spectrometry (MS).Mass spectra were recorded by using ESI source mass spectrometer equipped with a single quadrupole mass analyzer (Shimadzu LCMS-2020coupled with a Shimadzu UFLC Nexera, Japan). Ions were detected in electron spray ionization with positive/or negative ion mode (Event). Spectra were acquired from m/z 80 to 800 in scan mode.

All impurities (Imp-1 to Imp-9) were characterized using MS, FT-IR, and NMR spectroscopic techniques. The mass, FT-IR spectral data and ^{1}H NMR chemical shift values of these impurities are presented in **Table 1.**

Table 1. Mass, FTIR Spectral Data and 1H NMR Chemical Shift Value

Name of Impurity	**Mass value (m/z)** $(M+H)^+$	**FT-IR (KBr) absorption bands (cm^{-1})**	**^{1}H NMR Chemical shift values, δ in ppm, (multiplicity, integration)**
1) Imp-1	312.0	3463.65, 3443.03, 3341.27, 3136.68, 1640.12, 1578.71, 1432.65	4.44 (s, 1H), 4.23-4.24 (t, 2H), 3.56-3.57 (t, 2H), 6.40 (s, 2H)7.59-7.62 (d, 2H), 7.27-7.29 (d, 2H), 8.15 (s, 1H)
2) Imp-2	195.0	2935.40, 2965.51, 1597.08, 1752.30, 1193.71.	8.67 (s, 2H)

3) Imp-3	433.0	3331.18, 3502.85, 2983.98, 1572.04, 1334.78, 1163.11, 1064.74.	0.91-0.94 (t, 3H), 1.52-1.61 (m, 2H), 2.56-2.59 (t, 1H, OH), 2.92-2.97 (q, 2H), 3.81-3.84 (q, 2H), 4.46-4.48 (t, 2H), 5.59-5.62 (t, 1H, NH), 6.91 (bs, 1H, NH), 7.17-7.20 (dd, 2H), 7.62-7.65 (dd, 2H), 8.45-8.46 (s, 1H),
4) Imp-4	477.0	3310.0, 1570.0, 1430.0, 1340.0, 1170.0, 1080.0, 836.0.	0.77-0.82 (t, 3H), 1.36-1.48 (h, 2H), 2.78 (t, 2H), 3.35-3.42 (m, 4H), 3.60-3.63 (t, 2H), 4.39-4.42 (t, 2H) 4.58 (bs, 1H), 7.24-727 (d, 3H), 7.61-7.64 (d, 2H), 8.49 (s, 1H), 9.83 (s, 1H).
5) Imp-5	407.0	3265.59, 2966.62, 1543.10, 1340.57, 1165.04.	0.91-0.95 (t, 3H), 1.53-1.62 (m, 2H), 2.94-2.99 (q, 2H), 5.50-5.53 (NH) (t, 1H), 6.92 (NH), (bs. 1H), 7.16-7.19 (dd, 2H), 7.69-7.72 (dd, 2H), 8.65 (s, 1H).
6) Imp-6	377.0	3049.72, 1568.27, 1433.06, 1320.57, 1057.12.	4.73 (t, 4H), 8.52 (s, 4H)
7) Imp-7	468	3391.74, 3306.44, 3167.83, 1643.85, 1549.38, 1575.72, 1453.43, 1307.16, 1148.64, 1066.20, 791.58.	4.59-4.68 (m, 4H), 4.81(s, 2H), 7.19-7.22 (dd, 2H), 7.50-7.53 (dd, 2H), 8.22 (s, 1H) 8.48(s, 2H).
8) Imp-8	305.0	1546.93, 1508.27, 1371.69, 1224.95, 1070.70, 804.63	7.12-7.20 (m, 2H), 7.63-7.66 (m, 2H), 8.78 (s, 1H).
9) Imp-9	633.0	3292.38, 2972.84, 1569.47, 1556.17, 1431.43, 1312.95, 1172.29, 1087.81, 835.08	0.91-0.963 (t, 3H), 1.52-1.64 (h, 2H), 2.92-2.99 (q, 2H), 3.74-3.78 (q, 4H), 4.39-4.52 (m, 4H), 5.63-5.67 (t, 1H), 6.96 (s, 1H), 7.21-7.24 (dd, 2H), 7.60-7.63 (d, 2H), 8.46 (s, 1H), 8.53 (s, 2H).

III. RESULTS AND DISCUSSION

3.1 Development of Chromatographic Conditions

3.1.1 Optimization of Chromatographic Conditions by Using Core Shell Column

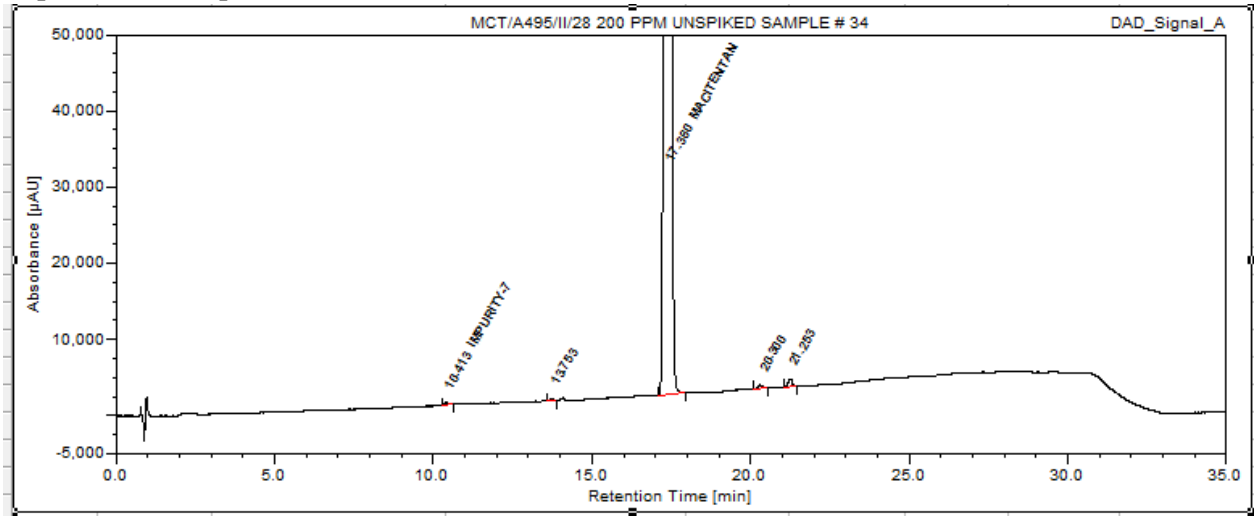
The objective of method development was to separate Macitentan and its process and degradation related impurities (Imp-1 to Imp-9) in a short run time with good resolution and good peak shape. Theresolution between Macitentan and Imp-9 was critical during the method development andhence selection of stationary phase was an important criterion during method development. The silica particle present in conventional HPLC column are porous in nature which results in higher back pressure, low resolution and higher run time of method. Therefore to reduce the run

time and backpressure and to maintain the good peak shape and resolution of impurities,the preference was given to core shell HPLC column than the conventional HPLC column.In core shell columns the modified silica particle having particle size of 2.7µ are used. Out of 2.7µ, 1.7µ is a solid core and 1.0µ is diffusion core/path. In core shell columns mobile phase is passing through only 1.0µ diffusion path whereas in conventional HPLC column mobile phase is passing through 3µ/5µ diffusion path.The core shell columns gives good peak shape, good resolution and theoretical plates due to less diffusion path to mobile phase.

Initial method development trials were conducted on different stationary phases like C_8, C_{18}, Phenyl-hexyl, andBiphenyl along with the optimization of other chromatographic conditions like detection of wavelength, the type, and quantity of organic/inorganic buffer, pH of mobile phase, thermostat, and column oven temperature. Every time system suitability criteria were evaluated during the different trial runs of method development to ensure the strength of developed method. Gradient mode was preferred than the isocratic mode to achieve the good resolution between all the impurities. We explored different core shell columns such as Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ), Kinetex ® C_{18} (4.6 mm x 100 mm, 2.7µ), Kinetex ® Phenyl-hexyl (4.6 mm x 150 mm, 5µ), Kinetex ® C_8 (4.6 mm x 150 mm, 5µ), and Kinetex ® Biphenyl (4.6 mm x 150 mm, 5µ) during the development[**16**].

Among these columns satisfactory peak shape and good resolution of Macitentan and its process and degradation related impurities were achieved on Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ) column with 35min run time, column flow rate 1.0 mL min^{-1}, λ 215nm, column oven temperature 45°C, injection thermostat 5°C and mobile phase consisting of phosphate buffer and combination of acetonitrile and methanol as a solvents.It was like a UPLC performance on conventional HPLC by using core shell column/technique. The typical chromatograms obtained from the analytical method development on core shell HPLC column are depicted in (**Figure 2c**) and (**Figure 3**)**.**

a) Un-Spiked Test Preparation:



b) Spiked Test Preparation:

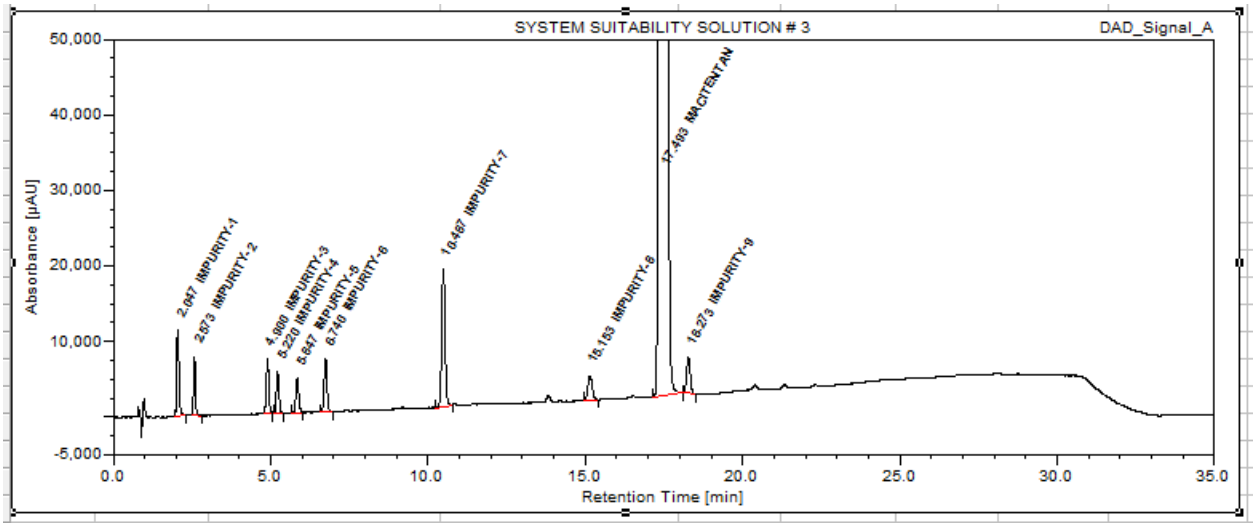


Figure 3. Typical HPLC chromatograms of; a) Macitentan, un-spiked test preparation, b) Macitentan spiked test preparation (0.15%w/w) with known impurities (Imp-1 to Imp-9)

3.1.2System Suitability Criteria

There was a critical resolution between Macitentan and Imp-9, hence a resolution criterion was set not less than 2.0.Other system suitability criteria were set as column efficiency/theoretical plates should not be less than 2,000, tailing factor should not be more than 2.0 and %RSD for six replicate injections of standard solution should not be more than 5.0%. The results of system suitability criterion are depicted in **Table 2**.

Table 2. System Suitability Test Results

Compound	Selectivity (α)	Resolution (R_s)	Tailing factor (T)	Theoretical Plates	RRT
Imp-1	0.533	-	1.33	3755	0.12
Imp-2	2.327	4.45	1.15	9733	0.15
Imp-3	0.32	18.42	1.09	17460	0.28
Imp-4	0.627	2.14	1.09	18870	0.30
Imp-5	0.893	3.96	1.04	19968	0.34
Imp-6	3.753	5.43	1.11	26860	0.39
Imp-7	4.66	20.95	1.05	47625	0.60
Imp-8	2.294	20.90	1.04	56501	0.87
Macitentan	0.826	9.51	1.05	102478	1.00
Imp-9	-	3.43	1.05	110051	1.05

RRT (Relative retention time)

3.2 Validation

3.2.1 Specificity (Selectivity)

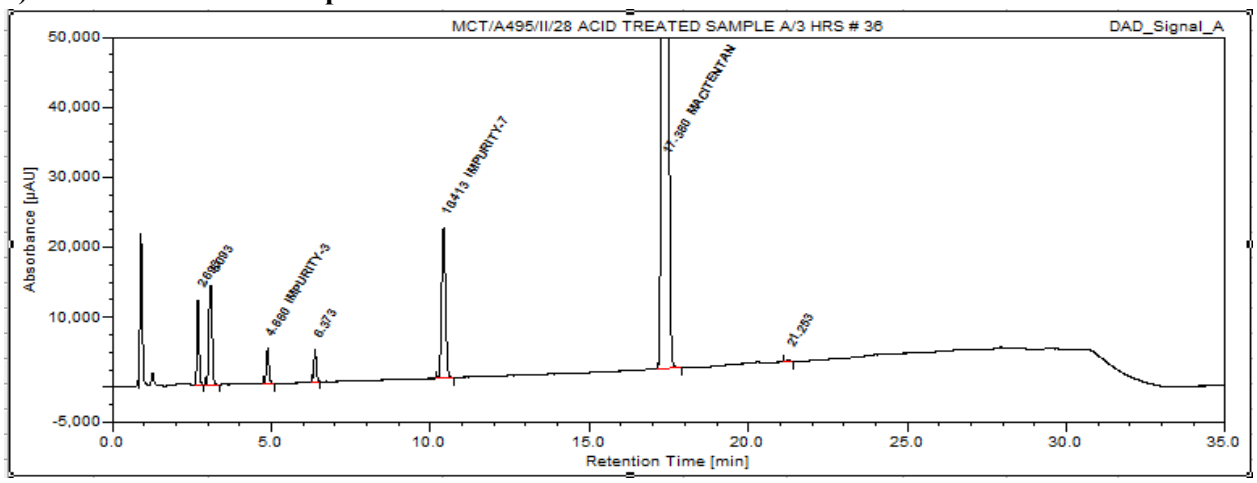
A forced degradation study was performed on Macitentanto provide an indication of the stability-indicating property and specificity of the proposed method.The specificity of developed RP-HPLC method for Macitentan was determined in presence of its impurities (Imp-1 to Imp-9) and degradation products. Aphotodiode array detector was employed to check and ensure the homogeneity and purity of Macitentan peak in all the stressed sample solutions. The stress conditions employed for the degradation study included light (1.2 million lux hours), heat (105°C), acid hydrolysis (5M HCl), base hydrolysis (1M NaOH) and oxidation (30%v/v H_2O_2). For heat sample was exposed for 4 days, for acid and base samples were treated for 3 hr and 40 minutes respectively at RT, whereas for oxidation sample was treated for 44 hr. The degradation was observed in acid, alkali, and peroxide degradation conditions. The mass balance was calculated for all the stressed samples. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error[**17-19**].The results of forced degradation study are given in **Table 3** and**Figure 4.**

Table 3. Forced Degradation Results

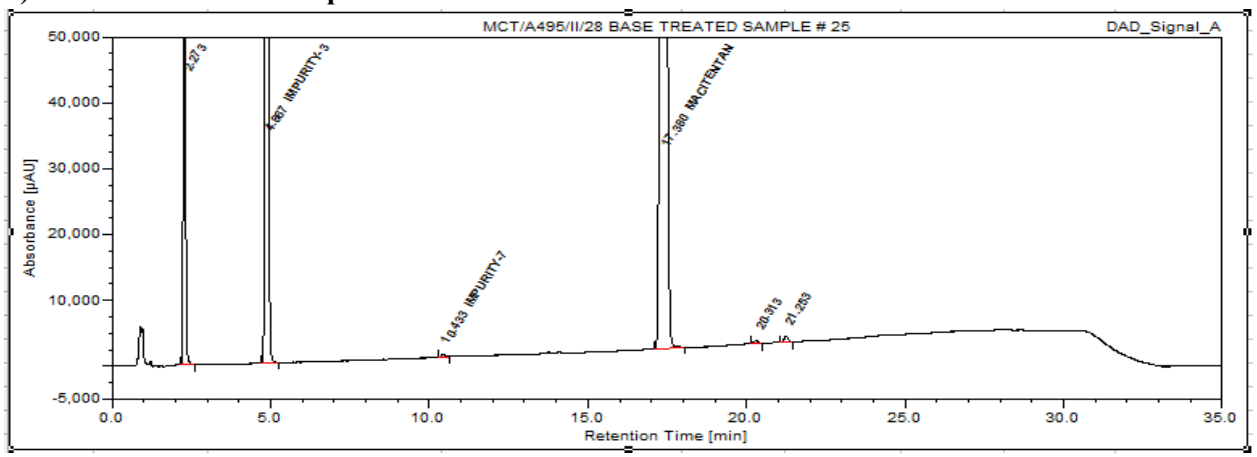
Stress condition	% of Macitentan	%of degredants	Observation and mass balance	Peak purity
Un-Treated	98.3	-	-	1.0000
Acid hydrolysis (5M HCl, 3 h at Room Temp.)	91.46	9.54	Major unknown degradation product (2.57%) formed (Mass balance: 97.69%)	1.0000
Base hydrolysis (1M NaOH, 40 min, at Room Temp.)	83.61	16.39	Major unknown degradation product (10.05%) formed (Mass balance: 97.88%)	1.0000
Oxidation (30% H_2O_2, 44 h. at Room Temp.)	96.50	3.50	Major unknown degradation product (3.13%) formed (Mass balance: 99.82%)	1.0000
Thermal (105°C, 4-days)	100.56	Nil	No any known and unknown degradation product formed (Mass balance: 100.71%)	1.0000
Photolytic as per ICH	99.85	Nil	No any known and unknown degradation product formed (Mass balance: 100.00%)	1.0000

Mass balance = % assay + % sum of all impurities + % sum of all degredants.

a) Acid Treated Test Sample



b) Base Treated Test Sample



C) Peroxide Treated Test Sample

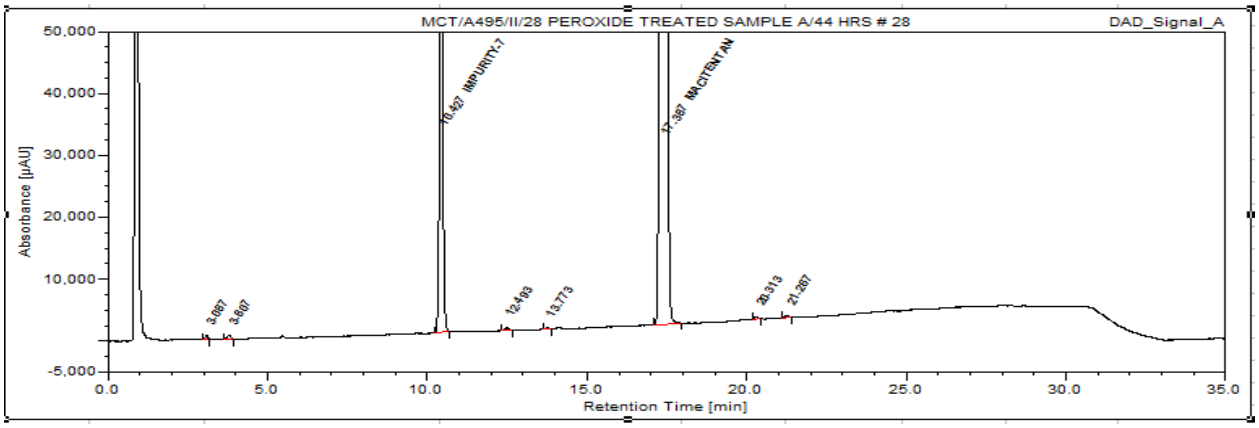


Figure 4. Typical HPLC chromatograms of forced degradation study; a) acid treated test sample, b) Base treated test sample, and c) Peroxide treated test sample.

3.2.2 Linearity

The linearity study of Macitentan and its related impurities was performed by using the six levels of linearity ranging from LOQ to 250% (LOQ, 0.187 µg mL^{-1}, 0.375 µg mL^{-1}, 0.75 µg mL^{-1}, 1.125 µg mL^{-1} and 1.50µg mL^{-1}) with respect to the specification level . The linearity plot was drawn for peak areas versus different concentrations of Macitentan and its related impurities. The linear regression data for all the components tested is presented in**Table 4**.

Table 4. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ) data

Component	LOD /LOQ results		Linearity
	LOQ µg/ml, (% w.r.t.)[c]	LOD µg/ml, (% w.r.t.)[c]	
Imp-1	0.190 (0.038)	0.063 (0.013)	1.00000
Imp-2	0.188 (0.038)	0.063(0.013)	1.00000
Imp-3	0.187 (0.037)	0.062(0.012)	0.99998
Imp-4	0.178 (0.036)	0.059(0.012)	0.99999
Imp-5	0.188 (0.038)	0.063(0.013)	0.99995
Imp-6	0.189 (0.038)	0.063(0.013)	0.99989
Imp-7	0.186 (0.037)	0.062(0.012)	0.99994
Imp-8	0.189 (0.038)	0.063(0.013)	0.99994
Macitentan	0.129 (0.026)	0.043(0.009)	0.99982
Imp-9	0.188 (0.038)	0.063(0.013)	0.99992

[c] LOD LOQ values are in % with respect to test concentration of 500 µg/ml

3.2.3 Limits of Detection and Quantification(LOD and LOQ)

The limits of detection (LOD) and the limit of quantification (LOQ) of Macitentan and its process related impurities (Imp-1 to Imp-9) were estimated by calibration curve method [standard deviation of the response (σ) and the slope (S)], as per the ICH Q2 (R1) guideline. The values of LOD and LOQ for impurities and Macitentan were found in the range of 0.012%-0.013% and 0.036%-0.038% respectively. The precision was studied at the LOQ level by injecting six replicate injections of Macitentan and its related impurities, followed by the calculation of %RSD of the peaks areas. The %RSD of LOQ precision was found <10.0%. The results are depicted in **Table 4.**

3.2.4 Precision

A standard solution of Macitentan was injected for six times to determine the system precision of the method and %RSD was calculated for Macitentan and its all process related impurities. The %RSD of system precision was found in between 0.28%to 2.18%. For method precision six separate test sample solutions of Macitentan were prepared by spiking the related impurities (Imp-1 to Imp-9) at specification level. The %RSD (n = 6) for each related impurities was evaluated and found in between 0.72% to 2.44 %. For intermediate precision, similar procedure of method precision was carried out by a different analyst, on different instrument and on a different day with different lot of column. The %RSD of results for intermediate precision study was calculated and compared with the method precision results.

3.2.5 Accuracy (Recovery)

Macitentan sample solutions were spiked with all related substances at four different concentration levels, LOQ, 50,100, and 150% at specified limitin triplicate and theses spiked sample solutions were analyzed to determine the recovery of analytical method. The recovery of all these related substances were found to be in-between the predefined acceptance criterion, 80.0-120.0% and the data is given in **Table 5**.

Table 5. Accuracy Data of Related Substances

Component	Recovery results (Mean % Recovery[a]± %RSD)			
	LOQ level ; amount (%w/w)	50% of specification level[b]; amount (%w/w)	100% of specification level[b]; amount (%w/w)	150% of specification level[b]; amount (%w/w)
Imp-1	96.30±4.40	99.51±3.95	101.38±1.80	104.23±1.00
Imp-2	105.48±4.50	102.28±2.05	101.16±1.72	100.91±0.79
Imp-3	106.43±4.00	100.91±0.78	96.54±1.88	96.07±1.36
Imp-4	102.94±5.71	100.02±2.49	95.61±2.00	96.15±1.81
Imp-5	107.33±1.43	103.16±1.54	99.09±2.41	99.40±0.70
Imp-6	102.78±2.70	96.37±1.60	98.40±1.75	98.65±0.79
Imp-7	112.25±8.28	106.85±3.28	97.68±2.17	100.15±0.69
Imp-8	109.06±3.72	104.04±2.60	100.68±1.79	101.62±2.04
Imp-9	97.17±0.05	95.38±3.66	96.03±2.54	95.72±1.66

[a]% Recovery average of three determinations.
[b]0.15% of all related substances

3.2.6 Stability of Analytical Solution

To determine the stability of sample solution, Macitentan spiked with all related impurities at specified level were prepared and analyzed immediately and at after different time intervals up to 12 hrs. A sample cooler temperature was maintained at about 5°C. The result from these studies indicates that the sample solution is unstable and need to be injected freshly or within 8 hrs. at cooler temperature.

3.2.7 Robustness

The chromatographic conditions were deliberately altered to evaluate the robustness of developed method. The resolution between closely eluting peak pair i.e. Macitentanand Imp-9 was evaluated on altered chromatographic conditions.To study the effect of flow rate on the resolution the flow rate of mobile phase was altered by 0.1 units i.e. from 0.9 to 1.1 mL $min^{-1}$from 1.0 mL min^{-1}. The effect of column oven temperature on resolution was studied at 43°C and 48°Cinstead of 45°C whereas all other mobile phase components were held constant as described above. The tailing factor of Macitentanwas less than 2.0and the resolution between Macitentanand Imp-9 was greater than 2.0 in all the deliberately varied chromatographic conditions indicates that the robustness of the method.

3.2.8 Application of the Method

The analysis of bulk drug sample indicated that the method is specific and selective for determination of related substances in the bulk drug samples. The developed method is capable for quantitative analysis of Macitentanbulk drug and in a pharmaceutical dosage formand the data is given in **Table 6**.

Table 6. Results of Analysis of Bulk Drug Batches.

Component	Bulk drug sample batches		
	Batch No.1	Batch No.2	Batch No.3
Imp-1	ND	ND	ND
Imp-2	ND	ND	ND
Imp-3	ND	ND	ND
Imp-4	ND	ND	ND
Imp-5	ND	ND	ND
Imp-6	ND	ND	ND
Imp-7	0.02	0.02	0.02
Imp-8	ND	ND	ND
Macitentan	99.82	99.86	99.79
Imp-9	ND	ND	ND

ND: Not detected

IV. CONCLUSION

This is the first method reported in literature for the separation and quantification of Macitentanand its process related and degradation related impurities on core shell column. The RP-HPLC method is specific, linear, sensitive, accurate, precise, and robust. Moreover, the developed method was found to be more selective and rapid with respect to short runtime and low back pressure as compared to conventional HPLC column method. This method is validated as per ICH Q2 (R1) guideline. The developed method is stability indicating method which can be used for the analysis of routine and stability samples of Macitentan drug substance and drug products.

V. ACKNOWLEDGEMENTS

The authors wish to thank the management of MegafinePharma (P) Ltd., for supporting this work. The author is also thankful to colleagues in the division of Research and Development of MegafinePharma (P) Ltd for their cooperation in carrying out this work.

VI. REFERENCES

[1]. I.S. Hong, H.V. Coe, L.M. Catanzaro, "Macitentan for the Treatment of Pulmonary Arterial Hypertension," The Annals of Pharmacotherapy, Vol.48,(2014). Pp-1-10.

[2]. M. Iglarz, C. Qiu, W. Fischli, K. Morrison, C. Binkert, M. Clozel, P. Hess, B. Capeleto, S. Buchmann, C. Boss, M.H. Bolli, T.Weller, A.Treiber, J. Gatfield, "Pharmacology of Macitentan, an Orally Active Tissue-Targeting Dual Endothelin Receptor Antagonist," Journal of Pharmacology and Experimental Therapeutics, Vol.327(3), 2008, pp-736-745.

[3]. Actelion receives US FDA approval of Opsumit (Macitentan) for the treatment of pulmonary arterial hypertension. Actelion. Retrieved 22 October 2013.

[4]. L.Yu , Y. Zhou , X. He , H. Li , H. Chen, W. Li ,"Simultaneous Determination of Macitentan and Its Active Metabolite in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry," Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, Vol.07 (53), 2015, pp-358-363.

[5]. M. Purushothaman, R. Subramanian, "Bio analytical Method Validation for Determination of

Macitentan in K2EDTA Human Plasma by LC-MS/MS," International Journal of ChemTech Research, Vol.10 (10), 2017, pp-752-761.

[6]. A. Unnisa, S. Sadath Ali, S. Santosh Kumar, "Development and Validation of RP-HPLC-PDA Method for the Estimation of Macitentan in Bulk and Tablet Dosage Forms," Indo American Journal of Pharmaceutical Research, Vol.04 (09), 2014, ISSN No. 2231-6876.

[7]. M. Ahmed, B. M. Deepak, S. Shetty, I.J. Kuppast, S.M. Anilkumar, M.C. Ravi, "RP-HPLC method development and validation for estimation of Macitentan in tablet dosage form," World Journal of Pharmacy and Pharmaceutical Sciences, Vol.04 (1), 2014, pp-881-887.

[8]. D. Lakshmi, P. Hitesh Kumar, M. Praveen, S. Venkatesh, TVS. Prakash Reddy, G. Manish, J. Jayachandran, "Quality by Design Based HPLC Method Development of Macitentan and its Related Compounds in Bulk Drugs," Journal of Pharmaceutics and Drug Delivery Research, Vol.05 (6), 2016, doi: 10.4172/2325-9604.

[9]. S. A.C. Wren, P. Tchelitcheff, "Use of ultra-performance liquid chromatography in pharmaceutical development",Journal of Chromatography A, Vol. 1119, 2006, pp-140-146.

[10]. L. R. Snyder, J. J. Kirkland, and J. L. Glajch, "Practical HPLC Method Development", 2nd Edition, 1997.

[11]. L. R. Snyder, J. J. Kirkland, and J. W. Dolan, "Introduction to Modern Liquid Chromatography," 1997.

[12]. J. M. Cunliffe, T. D. Maloney, "Fused-core particle technology as an alternative to sub-2-micron particles to achieve high separation efficiency with low backpressure", Journal of Separation Science, Vol. 30(18), 2007, pp-3104-3109.

[13]. V. F. Samanidou, "Core-shell particle technology in pharmaceutical analysis,"Pharmaceut Anal Acta, 4:e148. doi:10.4172/2153-2435.1000e148

[14]. The United States Pharmacopeia, "Validation of Compendial Methods", USP38 NF-33, Chapter < 1225 >, Year2015.

[15]. "Validation of Analytical Procedure: Text and Methodology", International conference on Harmonization (ICH), Q2 (R1) 2005.

[16]. http://www.phenomenex.com/Kinetex/CoreShellTechnology

[17]. M. Bakshi and S. Singh, "Development of validated stability-indicating assay methods-critical review",Journal of pharmaceutical and biomedical analysis, Vol. 28, 2002, pp- 1011-1040.

[18]. J. T. Carstensen and C. T. Rhodes, "Drug Stability Principles and Practices," 3rd Edition, Marcel Dekker, New York, 2000.

[19]. J. Ruan, P. Tattersall, R. Lozano, and P. Shah, "The role of forced degradation studies in stability indicating HPLC method development", American Pharmaceutical Review, Vol. 9, No. 1, 2006, pp-46-53.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Computer Aided Drug Design

Dr. S. S. Harak*, D. R. Mali, Dr. S. V. Amrutkar
Department of Pharmaceutical Chemistry, Gokhale Education Society's Sir Dr. M.S. Gosavi College of Pharmaceutical Education & Research, Prin.T.A.Kulkarni Vidyanagar, Nashik, Maharashtra, India

ABSTRACT

In the fast pace world fast pace development of drug is essential. This has been boosted by Computer aided drug design(CADD). The methodology has been cost-effective reducing the labour and time of design and discovery by almost fifty percent. The paper discusses mainly those approaches of CADD mainly developed based the structure of macromolecule protein.

Keywords: CADD, Structure Based Drug Design, Docking, Homology Modelling

I. INTRODUCTION

The field of medicinal chemistry basically deals with discovery newer drugs for the benefit of the general populace. These should reach them with easy availability and at economic prices. However in the past drug discovery and developing a new medicine is/was assumed to be a long, complex, costly and highly risky process that had few peers in the commercial world. But by the introduction of computer-aided drug design (CADD) approaches the scenario has changed. During the 1980s, the ability to rationally design drugs using protein structures was an unrealized goal for many structural biologists. However, now the human genome project has made available a substantial amount of sequence data that can be used in various drug discovery projects. Additionally, increasing knowledge of biological structures, as well as increasing computer power has made it possible to use computational methods effectively in various phases of the drug discovery and development has become the major subject of research for many academic laboratories. It is being widely used in the pharmaceutical industry to accelerate the process. The use of computational tools in the lead optimization phase of drug development leads to substantial cost benefit.

In the earlier scenario it took on 10-15 years and US $500-800 million to introduce one drug in the market, with synthesis and testing of lead analogs being highest cost areas.The greatest cost benefit was achieved in application of computational tools in hit-to-lead optimization which covers a wider chemical space while reducing the number of compounds that must be synthesized and tested in vitro. The computational optimization of a hit compound involves a structure-based analysis of docking poses and energy profiles for hit analogs, ligand-based screening for compounds with similar chemical structure or improved predicted biological activity, or prediction of favorable affinity or optimize drug metabolism and pharmacokinetics (DMPK) or absorption, distribution, metabolism, excretion, and the potential for toxicity (ADMET) properties. The comparably low cost of CADD compared with chemical synthesis and biological characterization of compounds make these methods attractive to focus, reduce, and diversify the chemical space that is explored. Today CADD has become an effective and indispensable tool in therapeutic development. The importance of in silico tools is greater than ever before and has advanced pharmaceutical research.[1]

Methods

The two methodologies involved are structure based drug design and ligand based drug design.

II. STRUCTURE BASED DRUG DESIGN

The structure based drug design is the best suited at present for emerging diseases/disorders. If the three-dimensional structure of a disease-related drug target is known, the most commonly used CADD techniques are structure-based. In SBDD the therapeutics are designed

based on the knowledge of the target structure. Two commonly used methods in SBDD are molecular docking approaches and de novo ligand (antagonists, agonists, inhibitors, etc. of a target) design. Molecular dynamics (MD) simulations are frequently used in SBDD to give insights into not only how ligands bind with target proteins but also the pathways of interaction and to account for target flexibility. This is especially important when drug targets are membrane proteins where membrane permeability is considered to be important for drugs to be useful. Successes have been reported for SBDD and it has contributed to many compounds reaching clinical trials and get FDA approvals to go into the market. Examples include Saquinavir[2] and Amprenavir[3] which were developed targeting HIV-1 protease based on SBDD. Also Dorzolamide[4] is a carbonic anhydrase II inhibitor was also sought on SBDD approach. SBDD methods rely on the protein structure and in the cases where the target structure is not possible to be determined by experimental methods, computational methods become useful. Several methods have been used for protein structure prediction including homology modeling, threading approaches, and ab initio folding.

III. HOMOLOGY MODELING

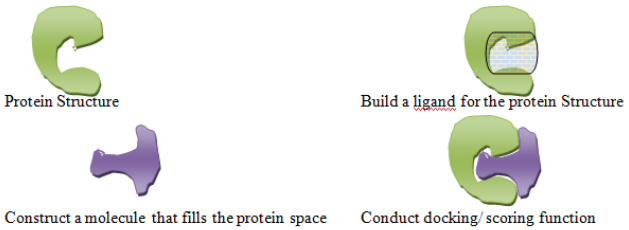
Homology modeling is a popular computational structure prediction method for obtaining the 3D coordinates of structures. Here we first use NCBI Basic Local Alignment Search Tool (BLAST) to identify a homologous protein structure on which model for the target structure is built using comparative modeling algorithms. The models built are evaluated and refined for stereochemistry. Once the models are verified to be acceptable in terms of their stereochemistry, they are then evaluated using 3D profiles or scoring functions that were not used in their generation.

Examples include homology modeling of HIV protease from a distantly-related structure has been used in the design of inhibitors for this structure.[5] Also, structure prediction of M antigen by homology modeling has given insights into its function by revealing that the structures and domains are similar to fungal catalases.[6] Homology servers used are SWISS-MODEL, MODELLER, 3D-JIGSAW, HHpred, etc.

IV. DOCKING AND SCORING

Docking of small molecules to receptor structures has become increasingly important in the context of drug discovery. Generally speaking, docking is carried out using a computer program in order to dock computer-generated representations of small molecules to a receptor (or to a user-defined part thereof, e.g. the active site of an enzyme), followed by evaluation of the molecules with respect to complementarities in terms of shape and properties, such as electrostatics. Good complementarities of a molecule indicate that the molecule is potentially a good binder. The outcome of a docking exercise normally includes some sort of affinity prediction for the molecules investigated, yielding a relative rank-ordering of the docked compounds with respect to affinity.

V. DE NOVO LIGAND DESIGN



It involves fragment-based approach for designing of ligand wherein assembling of different fragments of drug-like molecules is done to develop new ligand. The approach can be restricted by complexity of the molecule predicted. When a high resolution target structure is available, ligand growing programs such as biochemical and organic model builder (BOMB) can be used to design ligands that bind to the target without using ligand databases.[7,8] Using BOMB it is possible to grow molecules by adding substituents into a core structure. Examples include designing inhibitors for Escherichia coli RNS polymerase and inhibitors for Enterococcus faecium ligase VanA using hydroxyethylamine as the base template structure.[9]

VI. LEAD OPTIMIZATION AND ADME

Once the target structure/s is determined the next step includes lead optimization. Lead optimization indicates effectiveness of promising hits with the desired pharmacological profiles to reach the required affinity,

pharmacokinetic properties, drug safety, and ADME properties. Software includes QikProp, an ADME program offered by Schrodinger.

VII. ACKNOWLEDGEMENT

We are grateful to Gokhale Education Society members for their continuous encouragement and support for the preparation of this paper.

VIII. REFERENCES

[1]. Leelananda, S.P.; Lindert, S.; Computational methods in drug discovery, Beilstein J Org Chem. 2016; 12: 2694-2718.
[2]. Craig, J. C.; Duncan, I. B.; Hockley, D.; Grief, C.; Roberts, N. A.; Mills, J. S. Antiviral Res. 1991, 16, 295-305.
[3]. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. J. Am. Chem. Soc. 1995, 117, 1181-1182.
[4]. Talele, T. T.; Khedkar, S. A.; Rigby, A. C. Curr. Top. Med. Chem. 2010, 10, 127-141.
[5]. Clark, D. E. Expert Opin. Drug Discovery 2006, 1, 103-110.
[6]. Blundell, T.; Carney, D.; Gardner, S.; Hayes, F.; Howlin, B.; Hubbard, T.; Overington, J.; Singh, D. A.; Sibanda, B. L.; Sutcliffe, M. Eur. J. Biochem. 1988, 172, 513-520
[7]. Jorgensen, W. L.; Acc. Chem. Res. 2009, 42, 724-733
[8]. Agarwal, A. K.; Johnson, A. P.; Fishwick, C. W. G. Tetrahedron 2008, 64, 10049-10054.
[9]. Sova, M.; Cadez, G.; Turk, S.; Majce, V.; Polanc, S.; Batson, S.; Lloyd, A. J.; Roper, D. I.; Fishwick, C. W. G.; Gobec, S. Bioorg. Med. Chem. Lett. 2009, 19, 1376-1379.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Translocation of Cyclophosphamide by Using Multi-Walled Carbon Nanotubes Into Mammalian Cancer Cells

P. S. Uttekar, V. V. Chopade
P. E. Society's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India

ABSTRACT

The aim of the present work was to prepare cyclophosphamide loaded MWCNTs for target drug delivery of Cyclophosphamide (CP), an Anti-Cancer drug. Experimental designing was done by using the Ultrasonic probe sonicator, PCI Analytics DP-120 under continuous stirring. Translocation of cyclophosphamide was done on functionalized MWCNTs. Functionalization of MWCNTs was done by Acid Purification of pure MWCNTs & conjugation of FA-EDA with oxidized MWCNTs.The loaded MWCNTs were characterized for various parameters including the MTT assay,DSC,SEM,FTIR, Zeta Potential,NMR,Particle Size Analysis, XRD, Stability Study & poly dispersive Index.it is worth that the cyclophosphamide loaded MWCNTs showed better targeting action.

Highlights:

Functionalization of MWCNTs.

Loading of CP with FA-EDA-MWCNTs

Characterization of Cyclophosphamide anchored CNTsfor various parameters.

Keywords: Multiwall Carbon nanotubes (MWCNTs), cyclophosphamide, Functionalization of MWCNTs, Translocation.

I. INTRODUCTION

Cancer ranks amongst the top three killers in modern society, next to heart and cerebrovascular diseases. In 2009, according to World Health Organization (WHO), approximately eight million people died from cancer worldwide. In 1991, Sumio Iijima identified a new structural form of this allotrope (The discovery of the third allotropic form of carbon fullerene), the cylindrical fullerene and named them as carbon nanotubes (CNTs) [1,45].CNTs are graphene sheets rolled into a seamless cylinder that can be open ended or capped, having a high aspect ratio with diameters as small as 1 nm and a length of several micrometer2.Carbon nanotubes are allotropes of carbon with a cylindrical nano structure. Nanotubes have been constructed with length to diameter ratio of upto 132000000:1, significantly larger than for any other material. These cylindrical carbon molecules have unusual properties, which are essential for nano technology, electronics, optics & other fields of materials, science & technology.

Their name is derived from their long, hollow structure with the walls forms by 1-atom-thick sheets of carbon, called graphene. These sheets are rolled at specific & discrete ("chiral") angles ,& the combination of the rolling angle & radius decides the nanotubes properties; for example, whether the individual nanotube shell is a metal or semiconductor. Nanotubes are categorized as single –walled nanotubes (SWNTs) & multi-walled nanotubes (MWCNTs). Individual nanotubes naturally align themselves into "ropes" held together by Vander Waals forces.

Applied quantum chemistry, specifically, orbital hybridization best describechemical bonding in nanotubes. The chemical of nanotubes is composed entirely of sp2 bonds, similar to those of graphite. These bonds, which are stronger than the sp bonds found in alkanes, provide nanotubes with their unique strength.
Carbon atoms have 3 allotropic forms:

- ✓ Diamond-sp3 hybridization
- ✓ Graphite –sp2 hybridization
- ✓ Fullerenes-sp2 hybridization

CP is widely used in cancer chemotherapy, mostly in combination with other anti-neoplastic agents, and as a immunosuppressant. CP belongs to the group of alkylating agents and is a pro drug that is activated via 4-hydroxylation by cytochromeP450s to generate

alkylating nitrogen mustards. The resultant mustards can alkylate DNA to form DNA-DNA cross-links, leading to inhibition of DNA synthesis and cell apoptosis (7).

With more than 10 million new cases every year, cancer is one of the most devastating diseases. Though the current treatments of cancer by surgery, radiation, and chemotherapy are successful in several cases; however, these curative methods are likely to kill healthy cells and cause toxicity to the patient 8, 13. Many patients who succumb to death due to cancer do not die as a result of the primary tumor, but because of the systematic effects of metastases on the other regions away from the original site. The main problem associated with the various chemotherapeutic agents is the lack of selectivity towards cancerous cells. This problem can be overcome by using CNTs as a Nano carrier for anticancer drug.

II. MATERIALS AND METHODS

2.1. Materials.

2.2. Carbon nanotubes were procured from Applied Science innovations Pvt ltd, pune, Cyclophosphamide was procured from Emcure Pune, folic Acid, N-Hydroxisuccinimide , N,N'-Dicyclohexylcarbodiimide & Di-ter butyl di-carbonate (t-boc) were procured from Sisco Research Lab.Pvt.Ltd, Ethylene Diamine was supplied by Thermo Fisher Scientific India Pvt.Ltd, MTT Reagent from Sigma Aldrich, Mumbai & MCF -7 Cells from NCCS pune.

2.3. Functionalizationof MWCNTs.

2.3.1 Acid Purification of Pure MWCNTs:

Acid treatment will used for removal of catalytic and amorphous impurities from the unpurified MWCNTs.Firstly, the unpurified MWCNTs (pristine MWCNTs) (500 mg) treating in a microwave oven at 400±2°C for 2 hr. The microwave treated MWCNTs (500 mg) will reflux with a 200 ml mixture of concentrated Nitric and Sulphuric acid (HNO_3: H_2SO_4 :: 1:3 ratio) in a flat bottom flask (equipped with the reflux condenser and thermometer) with continuous magnetic stirring at 120 ± 5°C for 24 hr, upon completion of reaction, the mixture was washed with cold distilled water to remove the residual acid and then ultra-centrifuged (20,000 rpm for 15 min) untill the supernatant of the mixture represent the pH=7 which exhibit the no acidity in the suspension. The sample was then dried in a vacuum oven at 80°C for 4 h [19,20]

2.3.2 Conjugation of Folic Acid-Ethylenediamine with Oxidized MWCNTs (FA-EDA-MWCNTs):

Step I: Preparation of FA-NHS Ester

Folic acid (FA) (1 gm) was dissolved in dimethyl sulfoxide (40 ml) and triethylamine (0.5 ml) in a reaction vessel. Then, N-Hydroxy Succinimide (NHS) (520 mg) and Di-Cyclohexyl Carbodimide (500 mg) was added with continuous magnetic stirring at room temperature in dark for 18 hr. The mixture was filtered to remove precipitated side product dicyclohexyl urea, triethyl amine (TEA) was removed by evaporation under reduced pressure and remaining product was stored at -20°C. The collected product was characterized by FTIR spectroscopy. [21]

Step II: Conjugation of FA-NHS Ester to Ethylene Diamine

The FA-NHS (150 mg) active ester was mixed with Ethylene diamine (75 ml) in DMSO (15 ml) in the presence of tri-ethyl amine (0.5 ml) with continuous magnetic stirring at 100 rpm for 24 hr at room temperature. The unconjugate ethylene diamine (EDA) was removed, filter, dry under vaccum to yield folate conjugate (FA-EDA-NH_2) as a pale yellow solid and detect using UV visible spectrophotometer at ƛmax 363 nm. [21]

Step III : Conjugation of FA-EDA with Oxidized MWCNTs(FA-EDA-MWCNTs)

Oxidized MWCNTs (50 mg) will disperse in DMSO and N-ethyl-N'-(3-dimethyl-aminopropyl)Carbodimide hydrochloride (EDAC) dissolve in DMSO (6.41 mg/ml) was added to it with continuous magnetic stirring (100 rpm) for 6 hr, followed by addition of FA-EDA-NH_2 (4.60 mg/ml). The reaction was continue under vigorous stirring upto 5 days and remaining unconjugated FA-EDA-NH_2 was removed by dialysis, the product was collected, dried and characterized by FTIR spectroscopy. [20,21]

2.3.3 Loading of Cyclophosphamide molecule with CNTS (FA-EDA-MWCNTs/CP):[1]

Cyclophosphamide (30mg) in triethylamine (TEA) was mixed with FA-EDA-MWCNTs (10mg) dispersion in phosphate buffer solution (PBS; pH 7.4) with continuous magnetic stirring upto 48 h at room temperature in dark condition.

III. CHARACTERISATION OF CYCLOPHOSPHAMIDE LOADED MWCNTS

3.1FTIR SPECTROSCOPY:

Utilizing FTIR, functional groups that may be present on particle can be identified. The potassium bromide (KBr) disks with sample were prepared using electrically operated KBr Press Model HP-15. About 1 mg of sample was triturated with about 5 mg of dry KBr and then pressed into the disks. The FTIR spectrum was recorded using Jasco 4100 (TOKYO, JAPAN) with IR resolution software. The scanning range was 4000-400 cm^{-1}.

3.2 U.V.VISIBLE SPECTROSCOPY:

The U.V.Spectroscopy studied by using U.V.Visible Spectrophotometer (Jasco V630).

The U.V.visible spectrum of pure Cyclophosphamide, Amide F-MWCNTs, Cyclophosphamide loaded to MWCNTs and unbound cyclophosphamide molecules were obtained in water as a solvent. Samples were scanned over the range of 200-400 nm by using U.V.Visible Spectrophotometer (Jasco V630) and observe the various peak obtained.

3.3 N.M.R SPECTROSCOPY:

The proton magnetic resonance spectrometry has been extensively used as a fundamental tool for the determination of structure of the synthesized compounds.

The H^1 NMR spectra of the synthesized compounds such as Oxidised MWCNTs, Amide Functionalized MWCNTs and Loaded Cyclophosphamide on F-MWCNTs were recorded in DMSO (unless specified) with TMS as internal reference (chemical shift in δ, ppm) using MERCURY VARIAN 500 MHz instrument and MERCURY VARIAN 300 MHz instrument.

3.4 SCANNING ELECTRON MICROSCOPY (SEM):

The detailed surface characteristics of the pure Carbon Nanotubes and Carbon Nanotubes loaded on Cyclophosphamide were observed by using a JEOL Scanning Electron Microscope (Model: JSM 6360 A, Japan). SEM was the most commonly used method for characterizing particulate drug delivery system. SEM was used to determine surface topography, texture and to examine the morphology of fractured surface.

The samples were attached to the specimen holder to aluminium stab by using a double coated adhesive tape and gold coated (20 nm thickness) under vacuum using sputter coater (Model : IB-2, Hitachi, Tokyo, Japan) during gold coating process the samples were exposed to vacuum for 5-10 min at 40 mA and investigate a accelerating voltage of 15 kV and 10 kV was applied and the image was photographed by Asia Pentex Camera. Particle surfaces were evaluated at different magnification of 10X,100X,1000X and 3000X.[25]

3.5P-X.R.D. SPECTROSCOPY:

Powder X-Ray Diffraction (P-XRD) measurement of pure Cyclophosphamide and cyclophosphamide loaded on functionalized Multi-Walled Carbon Nanotubes (MWCNTs) was performed by using Philips (PW 3710) Expert Pro MPD Diffractometer (PAN Analytical Inc, Germany) with resolution of 0.001 A°.

The sample of 10 mg was sprinkled on vacuum grease applied glass slide to make a layer having a thickness of 0.5 mm and slick the sample to slide before measurement. The samples were radiated using a Copper target tube. Scanning angles ranged from 5° to 60° of 2ɵ. The current used was 40 mA and voltage 40 kv.[27]

3.6DIFFERENTIAL SCANNING CALORIMETRY (DSC):

Pure Cyclophosphamide and Cyclophosphamide loaded on Functionalized Multi-Walled Carbon Nanotubes (F-MWCNTs) was analysed for DSC analysis using Differential Scanning Calorimetry equipped with an intra-cooler (DSC METTLER STAR^{e}SW 12.10, Switzerland).

The analysis was carried out on an approximately 2-5 mg of sample sealed in standard pierced aluminium pans (Al -Crucibles, 40 Al). An empty aluminium pan used as reference. An inert atmosphere was maintained by

purging nitrogen gas at a flow rate of 25 ml/min at a scanning rate of 10°C/min from 50°C to 300°C.

3.7PARTICLE SIZE ANALYSIS:

The mean particle size of pure MWCNTs and Cyclophosphamide Loaded on F-MWCNTs were analysed by using (HORIBA Scientific, Nano Particle SZ-100 Series JAPAN) particle size analyzer.

Particle size analysis is performed by dynamic light scattering (DLS) at a scattering angle of 90° at 25 °C using appropriately diluted samples. Exactly 5 mg of pure MWCNTs and Cyclophosphamide loaded on F-MWCNTs were dispersed in 10 ml of deionized water, followed by sonication for 7 min and the resulting suspension was introduced into the measurement chamber. Each sample was measured in triplicate in the analysis. The particle size analysis is the important parameter because it influences the physicochemical properties & biological fate of the nanoparticles after in vivo administration.

3.8POLY DISPERSITY INDEX (PDI):

It is also called as Heterogeneity index. It is a measure of the width of molecular weight distributions.

The PDI of pure MWCNTs and Cyclophosphamide Loaded on F-MWCNTs were analysed by using (HORIBA Scientific, Nano Particle SZ-100 Series JAPAN) particle size analyzer.

3.9Zeta Potential:

Zeta potential is an important parameter to analyze the long-term stability . Zeta potential (ZP) refers to the surface charge of the particles. ZP (±) indicates the degree of repulsion between close and similarly charged particles in the dispersion. This repulsion force prevents aggregation of the particles. Therefore, ZP is a useful parameter to predict the physical stability . Zeta potential is the most important parameter for physical stability of nanoparticles. The higher the electrostatic repulsion between the particles the greater is the stability. ZP value more than +20 mV or less than -20 mV predicts good physical stability of nanoparticle dispersion.

A sample of pure MWCNTs and Cyclophosphamide loaded on F-MWCNTs was extemporaneously diluted in Milli-Q (Millipore Corp., USA) water (1 µl/10 ml) and injected in to the apparatus. The measurements were carried out in the fully automatic mode. Each sample was measured in triplicate in the analysis.

3.10 In-vitro release studies:

The dispersion of FA-EDA-MWCNTs/CP of conjugates were studied in sodium acetate buffer (pH 5.3) and phosphate buffer (pH 7.4) as recipient media using a dissolution method maintaining 37±0.5°C physiological temperature. The MWCNTs conjugates were filled in pre-treated dialysis membrane separately and kept into the releasing media under magnetic stirring at 37±0.5°C . At definite time points, the MWCNTs samples were withdrawn and after each sampling the withdrawn medium was replenished with fresh sink solution maintaining strict sink condition. The drug concentration was determined by UV Visible spectrophotometer. [6]

3.11 EX-VIVO STUDY:- CELL CULTURE STUDY METHYL THIAZOLE TETRAZOLIUM (MTT) ASSAY (Cytotoxicity Assay/ Cell Viability Assay):

The MTT assay is a colorimetric assay for assessingcell metabolic activity. The NAD(P)H dependent cellular oxido-reductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS).[1] Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.

/MTT is a yellow tetrazole, is reduced to purple formazan in living cells.[2] A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergentsodiumdodecylsulfate indiluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually

between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent.

IV. RESULT AND DISCUSSION

4.1 FTIR: The FTIR spectrum was recorded using Jasco 4100 (TOKYO, JAPAN) with IR resolution software. The scanning range was 4000-400 cm^{-1}. FTIR measurements were obtained on a pure sample of Carbon Nanotubes.

I) FTIR of Pure Carbon nanotubes (CNTs)

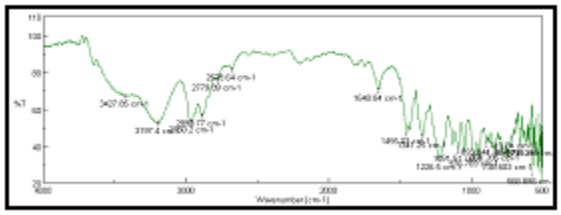


Figure 1. FTIR of Pure CNTS

Broad band at 3427 cm^{-1} and 3197 cm^{-1} is attributed to presence of O-H groups on the surface of pure CNTs Peak at 2960 cm^{-1} and 2890 cm^{-1} shows the C-H stretching of alkane.The characteristic peak at 1648 cm^{-1} suggests the presence of carbon residue on the CNTs surface.

The characteristic Peak at 1091 cm^{-1} shows the C-O stretch of the alkoxy group. [34,37,38]

II) FTIR of Oxidized Carbon nanotubes (O-CNTs)

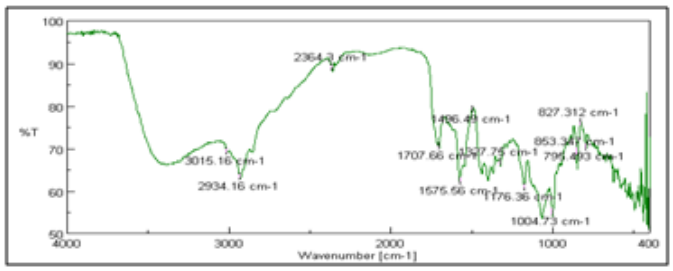


Figure 2. FT/IR OF O-CNTs

Oxidised CNTs shows characteristic peak at 3447 cm-1 and 2934 cm-1 due to O-H and C-H stretching of CH2 functional groups respectively.The characteristic peak at 1707 cm-1 was found for asymmetric stretching of C=O bond due to COOH group.The characteristic peak at 1176 cm-1Shows C-O stretching of the alkoxy group. [38,43]

III) FTIR of Amide Functionalized Carbon nanotubes (CNTs)

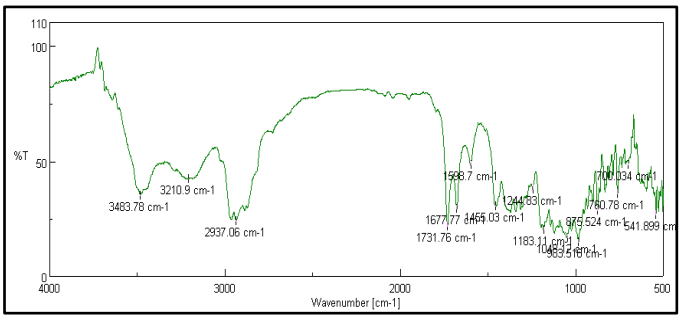


Figure 3. FT/IR of Amide Functionalized-CNTs

Shows Disappearance of the band at 1707 cm-1& corresponding appearance of band with lower frequency 1677 cm-1 assigned to the amide carbonyl (C=O) stretch.

In addition, the presence of the band at 1598 cm-1 and 1183 cm-1, corresponding to (N-H) in plane and (C-N) bond stretching respectively, this confirms that presence of amide functional group.The characteristic peak at 1048 cm-1 was due to C-O stretching of the ether linkage. [38,9,6]

IV) FTIRof Cyclophosphamide loaded on AMIDE F-CNTS

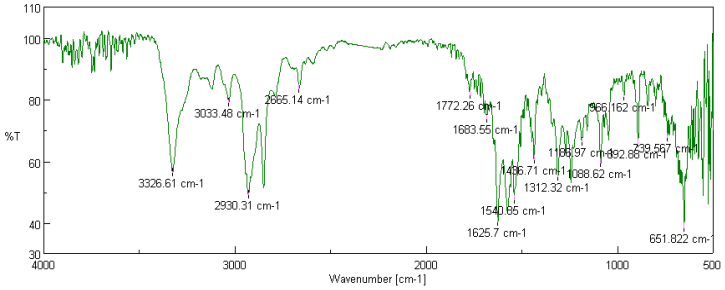


Figure 4. FTIR of FA-EDA-MWCNTs/CP Conjugate

Cyclophosphamide loaded on Amide F-MWCNTs shows the characteristics peak at 3303 cm-1 which is indicates that O-H stretching.The characteristic peak at 3326 cm-1 shows the N-H stretching that mask the N-H stretching peak. The characteristicpeak at 2930 cm-1 shows the C-H stretching. peak at 1683 cm-1 shows the C=O stretch of intra hydrogen bonded quinine, The characteristic peak at 1088 cm-1 shows the C-O bending. [37,44]

2. U.V.Spectroscopy

UV Visible Spectrophotometer (Jasco V630) was used for the characterization purpose. Samples were scanned over the range of 200-400nm.

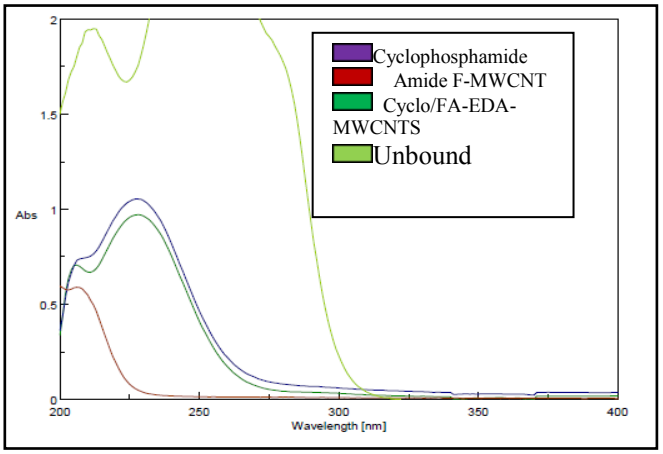


Figure 5. U.V. visible Spectroscopy

UV-visible spectrometry was used to characterize the FA-EDA-MWCNTs/CP conjugates (Figure No-5).The U.V visible spectrum of pure Cyclophosphamide exhibit the typical absorption band of in the range of 216,218,226 nm which can be also observed in conjugated MWCNTs-/CP (FA-EDA-MWCNTs/CP). This confirmed the successful attachment of drug molecule to the surface of amide Functionalized–Multi-Walled Carbon Nanotubes (F-MWCNTs). For further confirmation, we observed the U.V visible spectrum of the supernatant of the centrifuged solution which represents the unbound CP. As results, no absorption band was observed in the 216,218 and 226 nm range.

U.V visible spectroscopy studies showed that CP molecule successfully loaded on the surface of amide functionalized carbon nanotubes.

3) N.M.R.

3.1 ^{1}H-NMR OF OxidizedMWCNTs:-

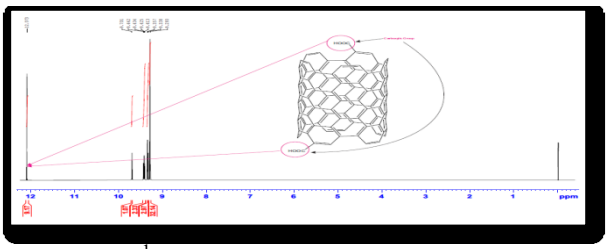


Figure 6. ^{1}H-NMR Spectrum of Oxidized MWCNTs

Table 1. Interpretation of ^{1}H-NMR Spectrum of Oxidized MWCNTs

(δ) ppm	Splitting	Assign	Functional Group
12.07	S	-COOH	Carboxylic acid
9.70	S	-CH	Aromatic
9.43	Dd	-CH	Aromatic
9.33	M	- CH	Aromatic
9.293	S	- CH	Aromatic

The ^{1}H-NMR Spectrum of Oxidized MWCNTs shows in the

(figure 19). The Oxidized MWCNTs shows the chemical shift at 12.073 ppm, which indicates that, the presence of carboxylic acid group on the surface of Oxidized-MWCNTs. The chemical shift between 9.70 to 9.29 ppm which indicates that the presence of aromatic (–CH) group on the surface of Oxidized-MWCNTs.

3.2 ^{1}H-NMR OF Folic Acid-Ethylene Diamine (FA-EDA):-

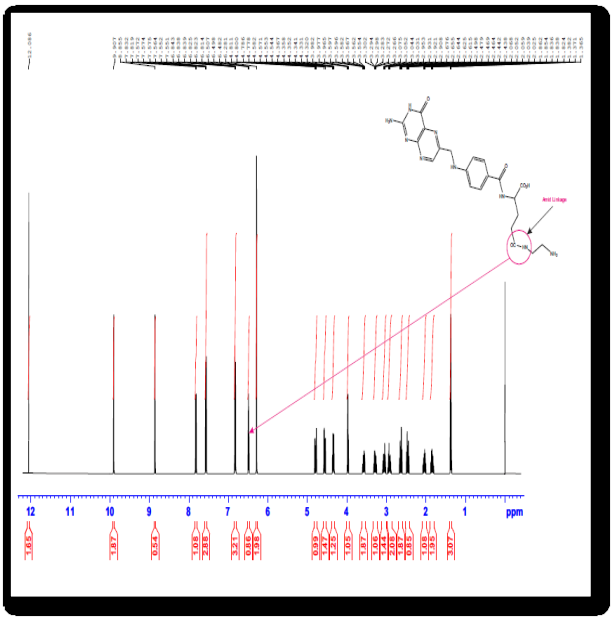


Figure 7. ^{1}H-NMR Spectrum of Folic Acid-Ethylene Diamine (FA-EDA)

Table 2. Interpretation of ^{1}H-NMR Spectrum of Folic Acid-Ethylene Diamine (FA-EDA):-

(δ) ppm	Splitting	No. of Proton	Assign	Functional Group
12.08	s	1	-COOH	Carboxylic acid
9.89	s	1	-NH	Pteridine ring
8.88	s	1	-CH	

7.59	m	1	-CH	Para-amino-benzoic acid
6.88	m	1	-CH	
3.97	t	1	-NH	
6.498	t	1	-CONH	Amide

The characteristic signal of the pteridine ring proton of Folic Acid (FA) shows the chemical shift at 9.89 & 8.88 and aromatic & amine protons of the P-Amino-Benzoic Acid (PABA) of Folic Acid (FA) shows the chemical shift at 7.59, 6.88 & 3.97 ppm [20] (Figure 7)
The characteristic signal of the formation of –CONH (amide bond) between folic acid and ethylene di-amine conjugate shows the chemical shift at 6.498 ppm which indicates that folic acid successfully conjugated with ethylene di-amine.

3.3 ^{1}H-NMR OF Amide F-MWCNTs (FA-EDA-Oxi MWCNTs):

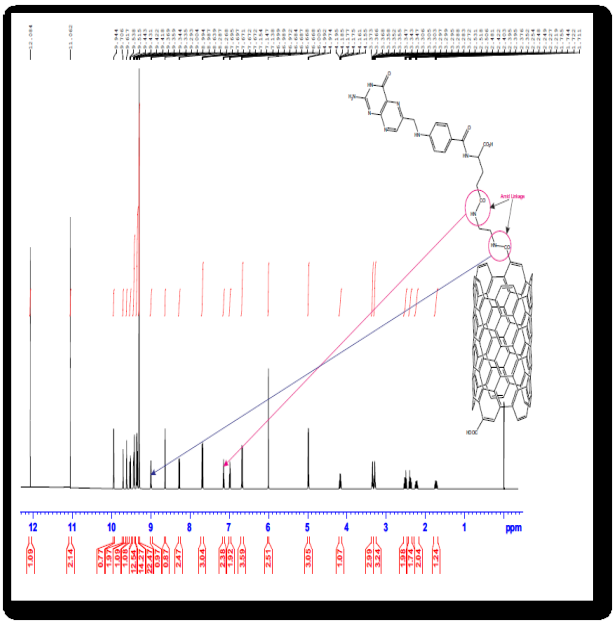


Figure 8. ^{1}H-NMR Spectrum of Amide F-MWCNTs (FA-EDA-Oxi MWCNTs)

Table 3. Interpretation of ^{1}H-NMR Spectrum of Amide F-MWCNTs (FA-EDA-Oxi MWCNTs)

(δ) ppm	**Splitting**	**No. of Proton**	**Assign**	**Functional Group**
12.08	s	1H	-COOH	Carboxylic acid
11.06	s	1H	-COOH	
9.94	S	1H	-NH	Pteridine ring
6.00	s	2H	$-NH_2$	
8.63	s	1H	-CH	
4.98	d	2H	$-CH_2$	
7.68	m	1H	-CH	Para-amino-benzoic acid
6.67	m	1H	-CH	
6.98	t	1H	-NH	
8.994	t	1H	-CONH	Amide
7.154	T	1H	-CONH	

The ^{1}H NMR spectrum of the FA-EDA-MWCNTs conjugate are shown in the (figure 8).

The chemical shift at 12.08 ppm and 11.06 ppm which indicates that the presence of carboxylic acid group on the surface of FA-EDA-MWCNTs conjugate.

The characteristic signal of the pteridine ring proton of Folic Acid (FA) shows the chemical shift at 9.94, 8.63, 6.00 & 4.98 ppm and aromatic & amine protons of the P-Amino-Benzoic Acid (PABA) of Folic Acid (FA) shows the chemical shift at 7.68, 6.98 & 6.67 ppm [20]
The characteristic signal of the formation of two –CONH (amide bond) between folic acid and ethylene di-amine conjugate shows the chemical shift at 7.154 ppm and folic acid-ethylene diamine (FDA-EDA) with oxidized MWCNTs shows the chemical shift at 8.994 ppm which indicates that FA-EDA conjugate successfully conjugated with oxidized MWCNTs.

4.SEM

The morphology of the pure MWCNTs & Cyclophosphamide loaded on amide functionalized MWCNTs were examined by SEM.(Figure no: 9 and 10)

4.1. SEM of Pure MWCNTs:

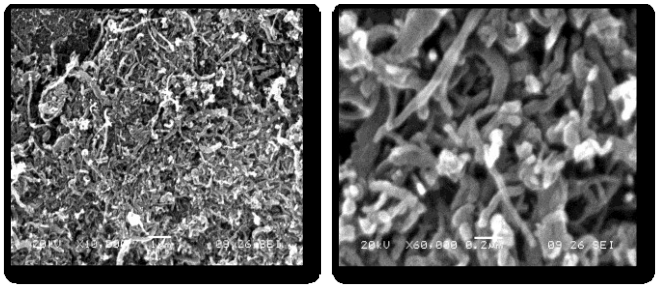


Figure 9. SEM Images of CNTS

4.2. SEM of CNTs/Cyclophosphamide:

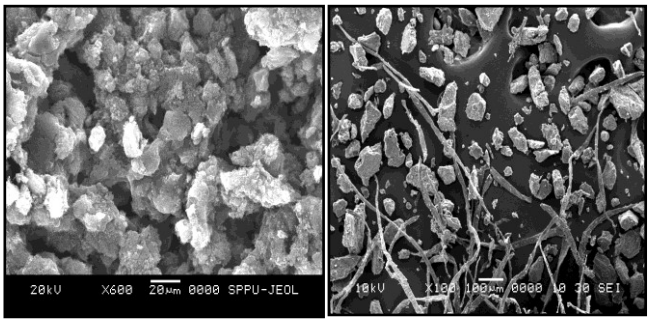


Figure 10. SEM of CNTs/Cyclophosphamide

SEM images shows that the change in the morphology between SEM image of Pure MWCNTs & Loaded MWCNTs/CP.The structure of conjugation can be identified, it shows that clear contrast between the SEM image of Pure MWCNTs & MWCNTs loaded with CP. The SEM image of Pure MWCNTs clearly shows that the CNTs are tubular in shape with open ends and in nanometric size range. Where as after loading process of drug with F-MWCNTs the image obtained was not very clear,the size and shape of tubular CNTs is reduced due to the chemical modification of MWCNTs i.e. loading proces.[39,21]

V. ZETA POTENTIAL

ZP is the useful parameter to predict the physical stability. The higher the electrostatic repulsion between the particles the greater is the stability.ZP value more than +20mV or less than -20mV predicts good physical stability**.**

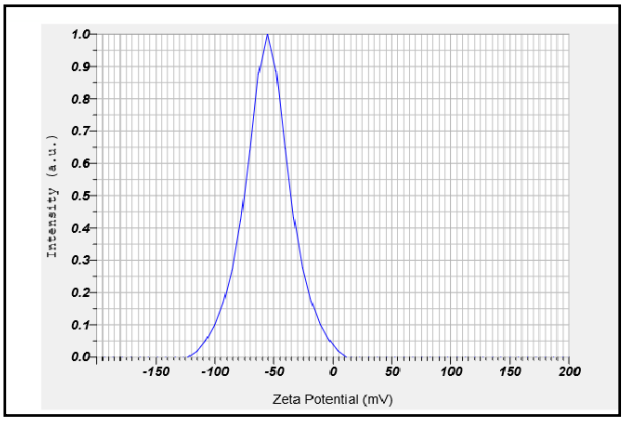


Figure 11. Zeta Potential of Pure CNTs

MWCNTs depicted the slightly negative zeta potential (-55.3mV), which could be due to the generation of acidic functional groups during the oxidation process. On increasing the pH the carboxylic acid group becomes more deprotonated leading to the shift of zeta potential towards negative side. The free COOH group was ionized at alkaline pH & thus negative zeta potential was observed.

5.1 Zeta potential of FA-EDA-MWCNTs/CP:

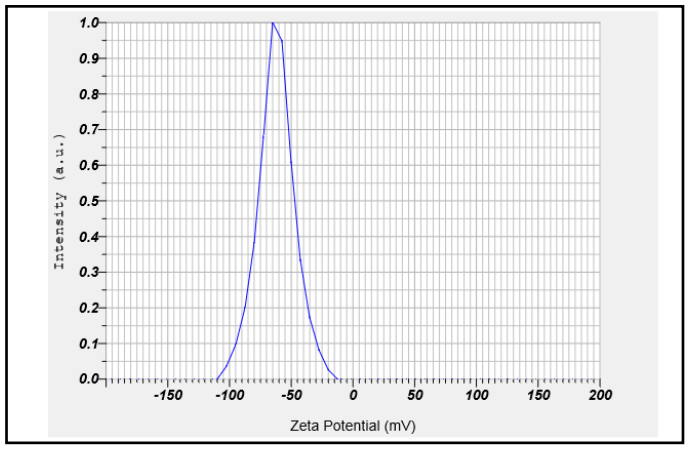


Figure 12. Zeta Potential OfFA-EDA-MWCNTs/CP

The zeta potential of FA-EDA-MWCNTs/CP nano-conjugates was found to be (-61.31 mV) (Figure no 25) as that of oxidized-MWCNTs was found to be (-55.3 mV)(Figure no 12). FA-EDA-MWCNTs/Cyclo nano-conjugates shows negative zeta potential value due to the availability of ionisable groups on the FA-EDA-MWCNTs/Cyclonano-conjugates. These significant changes in zeta potential suggest the loading of Cyclophosphamide molecule on the surface of F-MWCNTs. loading of cationic CPmolecules on FA decorated MWCNTs shows the changes in zeta potential. Value reflected the successful surface modification of the MWCNTs[39,8]

VI. XRD

Powder X-Ray Diffraction (P-XRD) measurement of pure Cyclophosphamide and Cyclophosphamide loaded with functionalized MWCNTs was performed by using Philips (PW 3710) Expert Pro MPD Diffractometer (PAN Analytical Inc, Germany) with resolution of 0.001 A°.

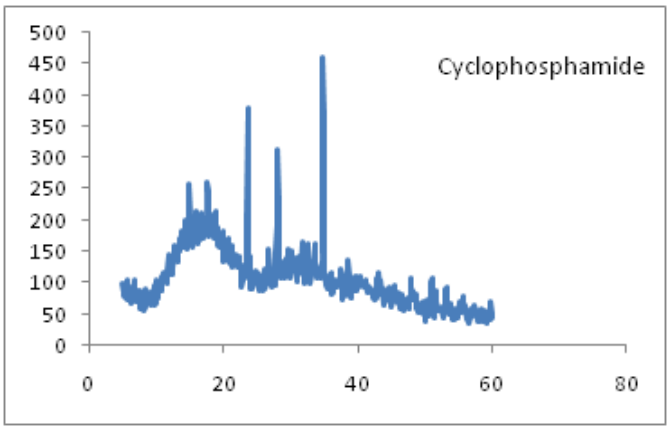


Figure 13. PXRD of Pure Drug

X.R.D analysis of pure Cyclophosphamide shows the peak position in the (Figure No- 13). Cyclophosphamide shows the strong sharp diffraction peak at 2θ angle 35.9°, 23.7°, 16.7°, . X.R.D analysis indicates that no. of sharp diffraction peaks was found; hence structure of CP is crystalline in nature.

6.1XRD of Loaded CNTs/CP:-

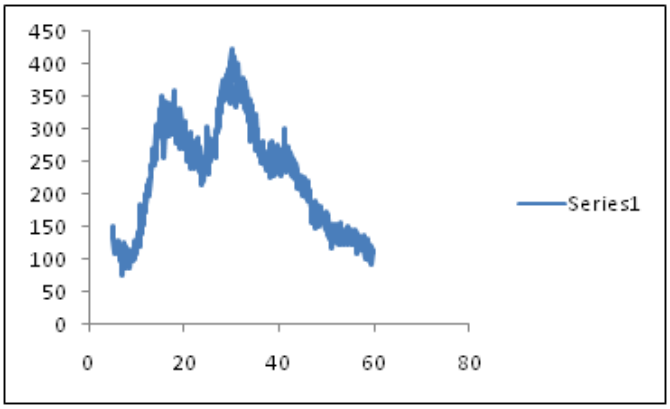


Figure 14. PXRD of Cyclophosphamide loaded MWCNTs

XRD analysis of cyclophosphamide loaded with F-MWCNTs shows the peak in above (figure 14). Cyclophosphamide loaded with F-MWCNTs broad diffraction peak at 2Θ angle 18.8,21.5, peaks were found hence the drug converted into amorphous form, which implies that the drug is dispersed at a molecular level in the MWCNTs surface [37,45].

VII. DSC

7.1D.S.C. OF PURE DRUG (Cyclophosphamide):

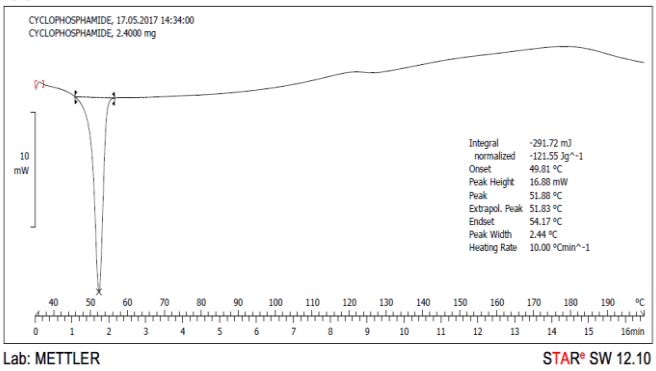


Figure 15. DSC of Pure Cyclophosphamide

The melting point of pure cyclophosphamide was 49-53°C are reported in literature. DSC study shows that sharp endothermic peak of Cyclophosphamide was at 51.88°C observed.(Figure No-15). This is corresponding with the M.P of pure Cyclophosphamide.

7.2 D.S.C. OF loaded CNTs-DRUG (Cyclophosphamide):

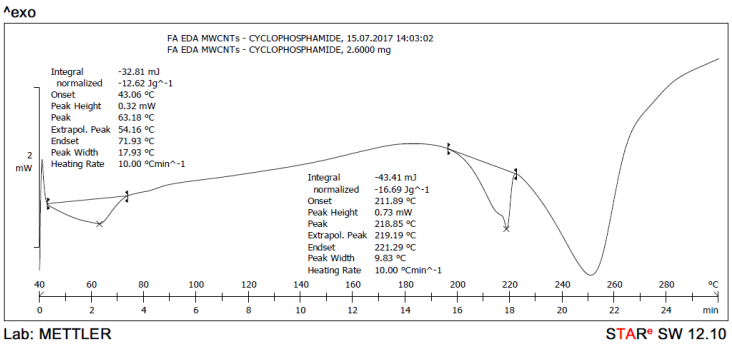


Figure 16. DSC of CNTs-Cyclophosphamide

The DSC graph of loaded MWCNTs cyclophosphamide shows that broad endothermic peak at 63.18°C was observed which is slight near to the M.P. of pure cyclophosphamide which indicates that small amount of drug is present in the (FA-EDA-MWCNTs/CP) conjugate as shown in Table as below. Two more peaks also present in the graph describe the M.P. of various elements present in the conjugate. The broad peak at 218.85^{0}C is due to conjugate that is presence of MWCNTs.

Table 4. Melting Point

Name	Melting point	
	Observed	Reported
Cyclophosphamide	51.88^{0}C	49-53 ^{0}C
Cyclophosphamide/MWCNTs	63.18°C	-

VIII. PARTICLE SIZE ANALYSIS

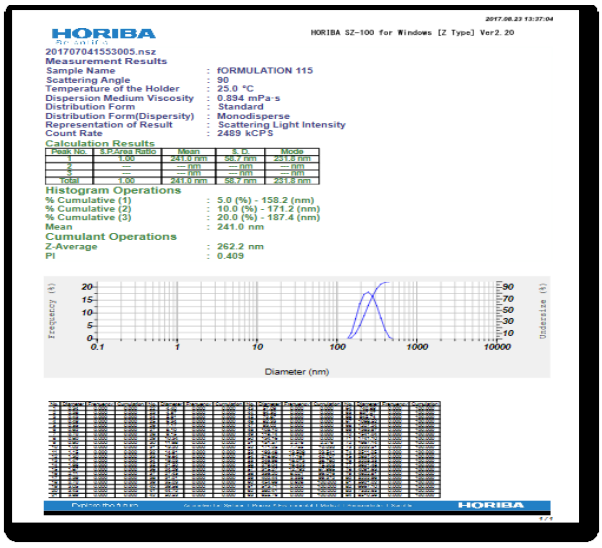


Figure 17. Particle size of Pure MWCNTs

The mean particle size of pure MWCNTs was found to be 262 nm (Figure No-17). Lesser the particle size, more is its solubility into the solution. Higher is the solubility, higher is the rate of dissolution. Due to small particle size, they are able to fit into the solvent pockets.

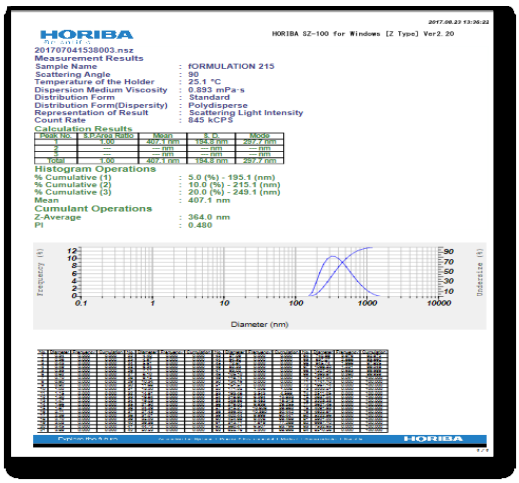


Figure 18. Particle Size of Cyclophosphamide loaded with F- CNTs

Particle size increases on loading of drug on the surface of F-MWCNTs. It was found that particle size was increased on loading of the drug which was found to be 407 nm and that of pure MWCNTs was to be 262 nm. Increase in particle size confirms drug has been loaded on the surface of pure MWCNTs.

IX. POLY DISPERSIVE INDEX (PDI)

It is measure of distribution of molecular mass in a given polymer sample which indicates the distribution of individual molecular masses in the polymeric solvent. Monodisperse sample have lower PDI value, where higher value of PDI indicates wider particle size distribution.

Table 5. Polydispersive Index

PDI	Type of dispersion
0-0.05	Monodispersion
0.05-0.08	Nearly monodisperse
0.08-0.7	Mid-range polydispersity
>0.7	Very polydispersity

PDI of pure MWCNTs and F.A.-EDA-MWCNTs/cyclo nano conjugates was found to be less than 1. Hence the particle size distribution was uniform. The PDI of pure MWCNTs was found to be 0.411 which is less than 0.7 indicating that sample is polydisperse. The PDI of F.A.-EDA-MWCNTs/cyclo nano conjugates was found to be 0.572 which is less than 0.7 indicates that particles are polydisperse [19,20]

Therefore PDI value of sample under investigation was well accepted.

X. IN-VITRO RELEASE STUDIES

The cumulative in-vitro release of Cyclo from the FA-EDA-MWCNTs/CP formulations was studied at the normal physiological and lysosomal pH for determining the overall pharmaceutical therapeutic efficacy in blood stream and at target site (Fig-19). The pH of the cytosol is neutral to mildly alkaline (7.4-7.8) while lysosomal pH is acidic (4.0-5.5). During the internalization of the FA-EDA-MWCNTs/CP into the target MCF-7 cells, initially the drug has to be released from the nanotube formulations in order to exert its overall therapeutic effect. The in-vitro release behavior of CP from the

surface engineered MWCNTs formulations exhibited bi-phasic pattern that was characterized by an initial faster followed by sustained release. As expected, the release of CP from FA-EDA-MWCNTs/CP nano-conjugate should be pH-dependent because of the pH-dependent π-π stacking interaction between CP and MWCNTs. The release profile of CP from the nano-conjugates was explored under two different pH conditions (pH= 5.3 and 7.4), which represent the acidic microenvironment and physiological environment respectively. It can be seen that at the same point, the CP release rate is faster at pH 5.3 than at pH 7.4. After 48 hr, the cumulative percent CP release was found to be 56.12% at the acidic pH (pH=5.3), while only 39.56 % Cyclo was released at the physiological pH (pH=7.4). The pH-responsive Cyclo release of the FA-EDA-MWCNTs/CP nano-conjugates is beneficial for treating tumor site with slightly acidic pH microenvironment. The initial fast release was attributed to the rapid swelling of CP associated with diffusion and then sustained release of CP was observed due to the limited solubility.

Table 6. Diffusion Profile of FA-EDA-MWCNTs/CP

Time (hr)	Sodium acetate buffer pH 5.3 (%)	Phosphate buffer pH 7.4 (%)
2	25.42	16.22
4	27.65	19.45
6	31.26	21.32
8	33.52	22.49
10	34.65	24.79
24	46.23	31.26
48	56.12	39.56
72	58.45	40.39
96	59.23	41.42

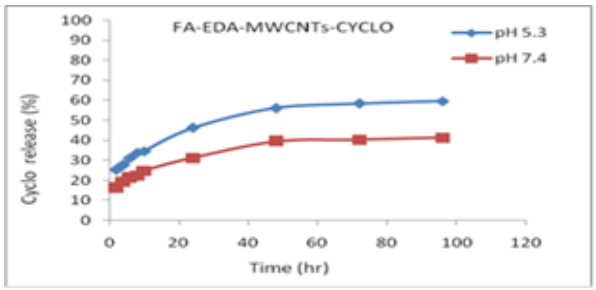


Figure 19. Cumulative Cyclo release (%) from FA-EDA-MWCNTs/CP nanoconjugates at 37±0.5°C in sodium acetate buffer pH 5.3 and phosphate buffer pH 7.4

XI. STABILITY STUDIES[44]

Stability study of the nano-conjugates (FA-EDA-MWCNTs/CP) were studied at different conditions of temperature (5±2°C, 25±2°C and 40±2°C), after keeping in dark (amber color bottle) and light (colorless glass vials) and evaluated every week up to 8 weeks. The developed nano-conjugates were found to be most stable in dark at 5±2°C. However, on storage in light at 25±2°C, slight turbidity was observed, which might be due to aggregation of nanotubes (Table No-7). At 40±2°C, the nano-conjugates show the higher turbidity that may be ascribed to the formation of larger aggregates and bundling of nanotubes. In terms of stability profile F-MWCNTs could possibly present themselves as a most stable system due to π-π stacking interaction in all temperature ranges and environment required for biological applications. Thus we conclude that the (FA-EDA-MWCNTs/CP) nano-conjugates is more stable in dark at 5±2°C than in other 25±2°C and 40±2°C temperature conditions, and suggesting that the developed nano-conjugate may be suitably stored in amber color bottle or vials at a cool place.[6,20,40,44]

Table 7. Accelerated stability study data for FA-EDA-MWCNTs/CP nano-conjugates:

Stability Parameter	FA-EDA-MWCNTs/CP after 8 weeks					
	Dark (°C)			Light (°C)		
	5 ± 2	25 ± 2	40 ± 2	5 ± 2	25 ± 2	40 ± 2
Turbidity	-	-	++	+	++	+++
Precipitation	-	-	+	-	+	++
Change in color	-	+	+	-	+	++
Crystallization	-	-	+	-	+	+
Change in consistency	-	+	++	-	+	++

Where, (-) no change; (+) small change; (++) considerable change; (+++) enough change.

XII. EX-VIVO STUDY: CELL CULTURE STUDY

METHYL THIAZOLE TETRAZOLIUM (MTT) ASSAY (Cytotoxicity Assay/ Cell Viability Assay) [43,,51,58]:

The MTT assay is a simple, non-radioactive, colorimetry based assay for determining the relative percent cell viability or cell cytotoxicity. The cytotoxocity of Cyclophosphamide conjugated with Functionalized Multi-Walled Carbon Nanotubes (F-MWCNTs) at different micromolar concentrations against MCF-7 (human breast cancer) cells after 24 hr was determined by using MTT cytotoxicity assay. MTT assay clearly revealed that upon increasing the concentration from 1 to 100 µM of FA-EDA-MWCNTs/CP nano-conjugates the relative percent cell viability of the cancerous cells was decreased following initial 24 hr treatment due to apoptosis by intercalating CPwith DNA. The increased cytotoxic response may possibly be due to caveolae-mediated endocytosis, and specific uptake by cancerous cells causing dose-dependent cytotoxic response. Folate receptors (FRs) are common tumor marker highly over-expressed on the cancerous cells surface that facilitates cellular internalization. Thus, FA-EDA-MWCNTs/CP formulation could efficiently deliver CP to the nucleus of the cell possibly by nanoneedle -transporter or receptor-mediated endocytosis (RME) mechanism.

Non-apoptotic cell death (i.e, mitotic catastrophe) is a major response to Cyclophoshamide in human cancer cell lines at the doses and frequencies used in this study. This action of Cyclophoshamide may be partly responsible for their efficacy in treating breast cancers, which are normally resistant to apoptosis. In MCF-7 cells, the drug conjugate FA-EDA-MWCNTs/CP shows the cytotoxicity of 06.46% and 33.88% at 1µg/ml and 100 µg/ml respectively (Figure No-26). Reduced apoptosis was observed for drug conjugate on MCF-7 cells, which may be due to the fact that the anti-apoptotic factor Bcl-2 was over expressed and caspase-3 is a major component of the effector phase of the majority of apoptotic signaling pathways, was not expressed. Therefore, the drug conjugate demonstrated less toxicity on MCF-7 cells.

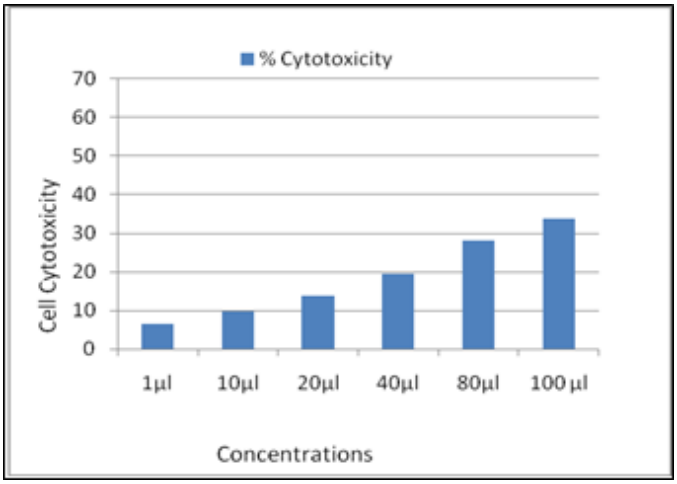
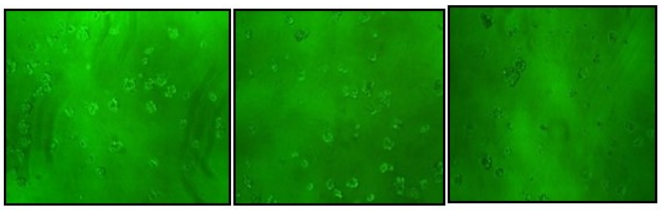


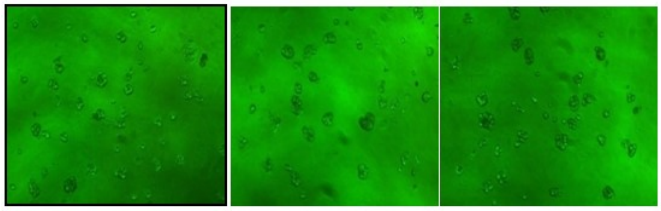
Figure 20. Cytotoxicity Study

Table 8. % Cell Viability and % Cell Cytotoxicity at different (1 to 100 µg/ml) concentrations on MCF-7 cell line.

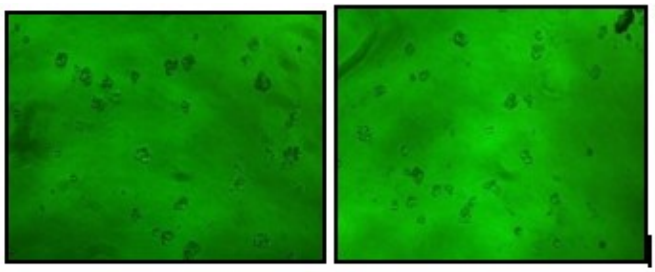
Group	O.D at 492 nm			Mean	% Viability	% Cytotoxicity
Vehicle Control	**0.055**	**0.031**	**0.077**	**0.054**		
Control	0.141	0.124	0.107	**0.124**		
1µl	0.119	0.107	0.122	**0.116**	93.54	06.46
10µl	0.103	0.125	0.109	**0.112**	90.32	09.68
20µl	0.128	0.100	0.093	**0.107**	86.29	13.71
40µl	0.104	0.100	0.097	**0.100**	80.64	19.36
80µl	0.093	0.085	0.089	**0.089**	71.77	28.23
100 µl	0.088	0.079	0.083	**0.082**	66.12	33.88



(a) **(b)** **(c)**

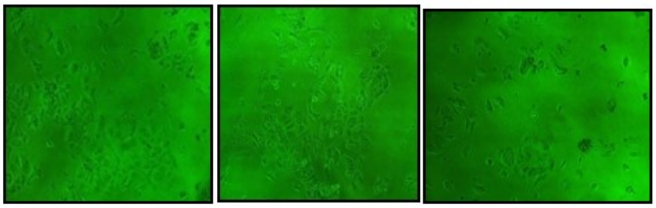


(d) **(e)** **(f)**

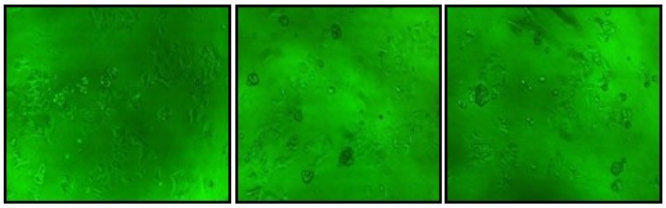


(g) (h)

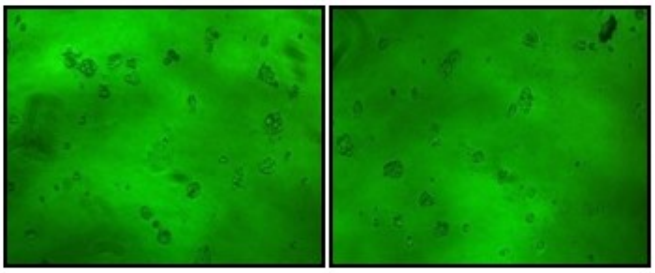
Figure 21. Inverted Microscopic Images of MCF-7 Cells at 0 Days (A) Control-1, (B) Control-2, (C) 1 µg/ml, (D) 10 µg/ml (E) 20 µg/ml, (F) 40 µg/ml, (G) 80 µg/ml, (H) 100 µg/ml



(a) (b) (c)

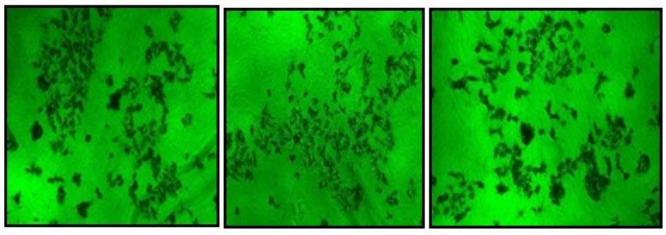


(d) (e) (f)

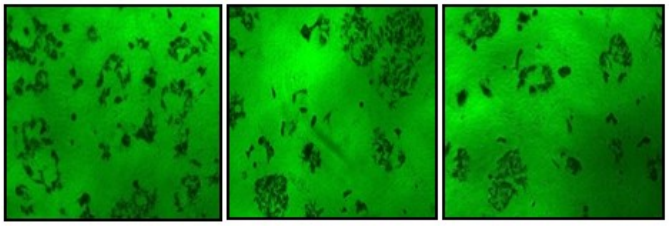


(g) (h)

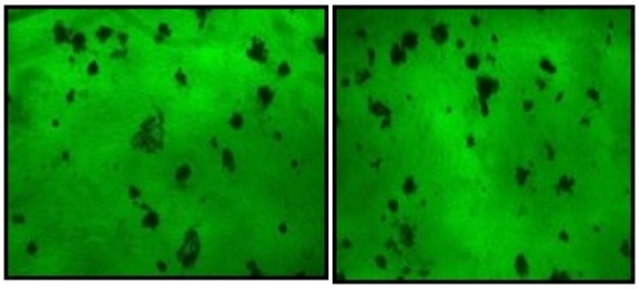
Figure 22. Inverted Microscopic Images of MCF-7 Cells treated with CYC/FA-EDA-MWCNTs conjugate after 24 hr
(A) Control-1, (B) Control-2, (C) 1 µg/ml, (D) 10 µg/ml (E) 20 µg/ml, (F) 40 µg/ml, (G) 80 µg/ml, (H) 100 µg/ml



(a) (b) (c)



(d) (e) (f)



(g) (h)

Figure 23. Inverted Microscopic Images of MCF-7 Cells after addition of MTT
(A) Control-1, (B) Control-2, (C) 1 µg/ml, (D) 10 µg/ml (E) 20 µg/ml, (F) 40 µg/ml, (G) 80 µg/ml, (H) 100 µg/ml

XIII. CONCLUSION

Efficacy of Cyclophosphamide is lowered due to its significant toxicity, including infusion-related events, such as chills, fever, headache, nausea, vomiting, dose limiting nephrotoxicity. By using CNTs (Loaded with Cyclophosphamide), efficacy of Cyclophosphamide is increased. CNTs are used as carrier for the Delivery of Cyclophosphamide, it acts as needle like work on fungal cell membrane & easily Target the Cancerous cell membrane.

UV-Visible Spectroscopy, NMR & MTT Assay studies shows that Cyclophosphamide successfully loaded to the amide-functionalized Carbon nanotubes.

Results of MTT Assay, clearly shows that the efficacy and Target delivery of Cyclophosphamide is increased which results in less side effects of the drug along with normal cells being unaffected . The covalent linkage of Cyclophosphamide to the CNTs is an approach that may be used to modulate the therapeutic action of the Cyclophosphamide.

From the outcomes of our present research studies, it can be concluded that the CP loaded on surface of amide f-MWCNTs shows the better in-vitro, ex-vivo efficacy as compared to other nano-carriers and sustained release profile especially at acidic micro-environments corresponding to conditions existing at cancerous tissues or sites. In-vitro release studies about 59.23% of CP in FA-EDA-MWCNTs/CP conjugate was release at pH 5.3 suggested that CP release pattern exhibited linear release profile characterized by relatively initial faster release followed by sustained or slower release in the later period.

XIV. REFERENCES

[1]. Mehra N.K, and Jain N.K,"Development, characterization and cancer targeting potential of surface engineered carbon nanotubes", (Journal of Drug Targeting, 3 July 2013, 745-758)

[2]. Vaibhav Rastogi, Pragya Yadav , Shiv Shankar Bhattacharya, Arun Kumar Mishra, Navneet Verma and jayanta Kumar Pandit,"Carbon Nanotubes: An Emerging Drug Carrier for Targeting Cancer Cells",(Journal of drug delivery Volume 20141-23)

[3]. H.Qui,J.Yang," Structure and Properties of Carbon Nanotubes",(Elsevier Publication,2017,47-66)

[4]. Swatantra Kumar Singh Kushwaha,Saurav Ghoshal,Awani Kumar Rai,Satyawan Singh,"Carbon Nanotubes As A Novel Drug Delivery System For Anticancer therapy: A Review"(Brazalian journal Of Pharmaceutical Sciences,4 Oct,2013,630-642)

[5]. Rajashree Hirlekar,ManohYamagar,HarshalGarse, Mohit Vij,Vilasrao kadam, Carbon Nanotubes And It's Application: A Review (2009); 17-27

[6]. Neelesh Kumar Mehra and N.K.Jain,"Cancer targeting propensity of folate conjugated surface engineered Multi-Walled Carbon Nanotubes", (Colloids and Surfaces B: Biointerfaces, 27 April 2015)44

[7]. Maurizio Prato, Kostas Kostarelos and Alberto Bianco, "Functionalized Carbon Nanotubes in Drug Design and Discovery"(Accounts Of Chemical Research,Jan,2008,60-68)

[8]. Mehra N.k , and Jain N.K," Development , Characterization and Cancer Targeting Potential of Surface Engineered CNTs"(Journal Of Drug Targeting ,3 July 2013,745-758)

[9]. "Folic Acid". Drugs.com. American Society of Health-System Pharmacists. 1 January 2010. Retrieved 1 September 2016.

[10]. Florey Klaus, Abdullah A. Al-Badr, Wozniak Timothy J,"Analytical Profile of Drug Substances", Volume-9, Academic Press An Inprint of Elsevier, 24-28 Oval Road, London 2005.

[11]. Monagle, J. J. (1962). "Carbodiimides. III. Conversion of Isocyanates to Carbodiimides. Catalyst Studies". J. Org. Chem. 27 (11): 3851-3855.

[12]. Sheehan, John; Cruickshank, Philip; Boshart, Gregory, "A Convenient Synthesis of Water-Soluble Carbodiimides", (1961); J. Org. Chem. 26 (7): 2525.

[13]. Good, Norman E.; Winget, G. Douglas; Winter, Wilhelmina; Connolly, Thomas N.; Izawa, Seikichi; Singh, Raizada M. M. "Hydrogen Ion Buffers for Biological Research", (1966); Biochemistry 5 (2): 467-477.

[14]. "N-Hydroxysuccinimide" (http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/56480). Sigma-Aldrich. http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/56480. Retrieved 2007-07-03.

[15]. Hans-Jurgen Arpe,"Industrielle Organische Chemie-6", Auflage (2007); Seite 245, Wiley VCH.

[16]. Nabil Fakhre, Alaadin Naqisshbandi,"Spectrophotometric derivation of Cyclophosphamide", (African Journal of Biotechnology,Nov 2013,6531-6537).

[17]. "Indian Pharmacopeia" Edition 2010, Volume-II, Published by Indian Pharmacopeia Commission, Ghaziabad.

[18]. Klaus Florey, Jerome I. Bodin, Hans-Georg Leeman,"Analytical Profile of Drug Substances", Volume-9, Academic Press An Inprint of Elsevier, 24-28 Oval Road, London 2005.

[19]. Neelesh Kumar Mehra and N.K.Jain,"Cancer targeting propensity of folate conjugated surface engineered Multi-Walled Carbon Nanotubes", (Colloids and Surfaces B: Biointerfaces, 27 April 2015)44

[20]. Neelesh Kumar Mehra, and Narendra Kumar Jain,"One platform comparison of Estrone and Folic acid anchored surface engineered MWCNTs for Doxorubicin delivery", (Molecular Pharmaceutics, ACS Publications Washington DC, 17 Dec 2014)43

[21]. Sharma Sonam, Neelesh Kumar Mehra, Keerti Jain, and Narendra Kumar Jain, "Effect of functionalization on drug delivery potential of carbon nanotubes" Artificial cells, Nanomedicine, and Biotechnology 44, no. 8 (2016): 1851-1860.
[22]. S.K. Smart, A.I. Cassady, G.Q. Lu, D.J. Martin,"The biocompatibility of carbon nanotubes", S.K. Smart et al. / Carbon 44 (2006); 1034-1047
[23]. T. Ramanathan, Frank T. Fisher, Rodney S. Ruoff, and L."Catherine Brinson, Apparent Enhanced Solubility of Single-Wall Carbon Nanotubes in a DeuteratedAcidMixture" Research Letters in Nanotechnology Volume 2008; 4 pages
[24]. VukovicGoran, AleksandarMarinkovic, Maja Obradovic, Velimir Radmilovic, MiodragČolic, Radoslav Aleksic, and Petar S. Uskokovic, "Synthesis, characterization and cytotoxicity of surface amino-functionalized water-dispersible multi-walled carbon nanotubes" Applied Surface Science 255, no. 18 (2009): 8067-8075.
[25]. Joseph Goldstein (2003),"Scanning Electron Microscopy and X-Ray Microanalysis" Springer. ISBN 978-0-306-47292-3. Retrieved 26 May 2012.
[26]. Huang H, Yuan Q, Shah J.S, Misra R.D.K,"A new family of folate-decorated and carbon nanotubes-mediated drug delivery system: synthesis and drug delivery response" Adv Drug Deliv. Rev. 2011, 63, 1332-1339.
[27]. VukovicGoran, AleksandarMarinkovic, Maja Obradovic, Velimir Radmilovic, MiodragČolic, Radoslav Aleksic, and Petar S. Uskokovic, "Synthesis, characterization and cytotoxicity of surface amino-functionalized water-dispersible multi-walled carbon nanotubes" Applied Surface Science 255, no. 18 (2009): 8067-8075.
[28]. Shen M, Wang S.H, Shi X, Chen X, Huang Q, Petersen E.J, Pinto R.A, Baker J.R, Weber W.J,"Polyethyleneimine-Mediated Functionalization of Multiwalled Carbon Nanotubes: Synthesis,Characterization, and In Vitro Toxicity Assay", J. Phys. Chem. C, 2009, 113, 3150-3156.
[29]. Zumdahl, Steven S."Chemical Principles", (2009); 6th Ed. Houghton Mifflin Company. p. A23.
[30]. "British Pharmacopeia" Edition 2011, Volume-I, Published by British Pharmacopeia Commission, Queen's Road Teddington Middlesex.
[31]. Good, Norman E.; Winget, G. Douglas; Winter, Wilhelmina; Connolly, Thomas N.; Izawa, Seikichi; Singh, Raizada M. M. "Hydrogen Ion Buffers for Biological Research", (1966); Biochemistry 5 (2): 467-477.
[32]. Son K.H, Hong J.H, Lee J.W,"Carbon nanotubes as cancer therapeutic carriers and mediators" International Journal of Nanomedicine 2016, Vol-11, 5163-5185
[33]. Martel, R. et al. "Ambipolar Electrical Transport in Semiconducting Single-Wall Carbon Nanotubes", (2001); Physical Review Letters 87 (25): 256805.
[34]. ManouchehrVossoughi, Shiva Gojgini, A. Kazemi, Iran Alemzadeh and MajidZeinali,"Conjugation of Amphotericin B to Carbon Nanotubes via Amide-Functionalization for Drug Delivery Applications", (Advance online publication: 19 November 2009)
[35]. V. Datsyuk, M. Kalyva, K. Papagelis, J. Parthenios, D. Tasis, A. Siokou, I. Kallitsis, C. Galiotis,"Chemical oxidation of multiwalled carbon nanotubes", CARBON 46(2008); 833-840
[36]. T. Ramanathan, Frank T. Fisher, Rodney S. Ruoff, and L."Catherine Brinson, Apparent Enhanced Solubility of Single-Wall Carbon Nanotubes in a Deuterated Acid Mixture" Research Letters in Nanotechnology Volume 2008; 4 pages
[37]. VukovicGoran, AleksandarMarinkovic, Maja Obradovic, Velimir Radmilovic, MiodragČolic, Radoslav Aleksic, and Petar S. Uskokovic, "Synthesis, characterization and cytotoxicity of surface amino-functionalized water-dispersible multi-walled carbon nanotubes" Applied Surface Science 255, no. 18 (2009): 8067-8075.
[38]. Uttekar. P.S, Kulkarni.A.M, Sable.P.N, Chaudhari.P.D,"Surface modifications of carbon nanotubes with Nystatin for drug delivery applications", (IJPER: Vol-50, Issue-3, Jul-Sept 2016).
[39]. Xueyan Cao, Lei Tao, Shihui Wen, WenxiuHou, Xiangyang Shi,"Hyaluronic acid-modified multi-walled carbon nanotubes for targeted delivery of Doxorubicin into cancer cells", (Carbohydrate Research, 27 June 2014)48
[40]. NeerajLodhi, Neelesh Kumar Mehra, and Narendra Kumar Jain,"Development and characterization of Dexamethasone Mesylate anchored on Multi-Walled Carbon Nanotubes", (Journal of Drug Targeting, 08 Sept 2012, 21(1):67-76)46
[41]. Huang H, Yuan Q, Shah J.S, Misra R.D.K,"A new family of folate-decorated and carbon nanotubes-mediated drug delivery system: synthesis and drug delivery response" Adv Drug Deliv. Rev. 2011, 63, 1332-1339.
[42]. Sharma Sonam, Neelesh Kumar Mehra, Keerti Jain, and Narendra Kumar Jain, "Effect of functionalization on drug delivery potential of

carbon nanotubes" Artificial cells, Nanomedicine, and Biotechnology 44, no. 8 (2016): 1851-1860.
[43]. Anbarasan B, S. VigneshBabu, K. Elango, B. Shriya, and S. Ramaprabhu, "pH responsive release of doxorubicin to the cancer cells by functionalized multi-walled carbon nanotubes" Journal of nanoscience and nanotechnology 15, no. 7 (2015): 4799-4805.
[44]. ICH Topic Q1A Stability testing Guidelines: stability testing of new drug substances and products, the European agency for the evaluation of medicinal products Human medicines evaluation unit, CPMP/ICH/380/95.
[45]. Iijima, Sumio; Ichihashi, Toshinari, "Single-shell carbon nanotubes of 1-nm diameter", (1993); Nature 363 (6430): 603-605
[46]. H.Qui, J. Yang, Structure and properties Of Carbon Nanotubes (2017):47-65
[47]. Flahaut, E.; Bacsa, R; Peigney, A; Laurent, C "Gram-Scale CCVD Synthesis of Double-Walled Carbon Nanotubes". Chemical Communications (2003); 1442-1443
[48]. Rajashree Hirlekar,ManohYamagar,HarshalGarse, Mohit Vij,Vilasrao kadam, Carbon Nanotubes And It's Application: A Review (2009); 17-27
[49]. Kinghong Kwok, Wilson K.S. Chiu."Growth of carbon nanotubes by open-air laser-induced chemical vapor deposition" Carbon 43 (2005); 437-446
[50]. K. Jurewicz, K. Babeł, R. Pietrzak, S. Delpeux, H. Wachowska,"Capacitance properties of multi-walled carbon nanotubes modified by activation and ammoxidation" Carbon 44 (2006); 2368-2375
[51]. Harini Kantamneni,Akhila Gollakota,"carbon Nanotubes Based Systems for targeted drug delivery: A Review"(International Journal of Engineering Research & Technology , Feb,2013,1-9)
[52]. Alberto Bianco, Kostas Kostarelos and Maurizio Prato,"Applications of carbon nanotubes in drug delivery, Current Opinion in Chemical Biology" (2005); 9:674-679)
[53]. 9.Wolfgang K. Maser, Ana M. Benito, M. Teresa Martinez, Production of carbon nanotubes: the light approach, Carbon 40 (2002); 1685-1695
[54]. S.K. Smart, A.I. Cassady, G.Q. Lu, D.J. Martin, The biocompatibility of carbon nanotubes, Carbon 44 (2006); 1034-1047
[55]. Jonathan N. Coleman, Umar Khan, Werner J. Blau, Yurii K. Gun'ko,"Small but strong: A review of the mechanical properties of carbon nanotube-polymer composites", Carbon 44 (2006); 1624-1652 [52]
[56]. V. Datsyuka, M. Kalyvaa, K. Papagelisb, J. Partheniosa, D. Tasisb, A. Siokoua, I. Kallitsisa,c, C. Galiotis,"Chemical oxidation of multiwalled carbon nanotubes", CARBON 46 (2008); 833 - 840

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Synthesis and Characterization of Related Substances Observed in Macitentan, an Endothelien Receptor Antagonist

Kunal M. Jagtap[1,2], Raghunath B. Toche[2], Vijayavitthal T. Mathad[1]

[1]Department of Process Research and Development, Megafine Pharma (P) Ltd., Lakhmapur, Dindori, Nashik, Maharashtra, India
[2]Organic Chemistry Research Center, Department of Chemistry, K. T.H.M College, Nashik, Maharashtra, India

ABSTRACT

During the process development of Macitentan (1), four related substance (impurities) were detected by high performance liquid chromatography (HPLC) in the final crude material ranging from 0.20 to 0.50% and these are identified by Liquid chromatography-mass spectrometry (LC–MS). All impurities were subsequently synthesized, characterized by spectroscopic techniques and further analyzed and confirmed by chromatographic techniques by spiking and purging study and this impurities are namely 2, 2'-{[5-(4-bromophenyl) pyrimidine-4,6-diyl]bis(oxy)}diethanol (8), 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]- pyrimidine (9), N-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl] -N'-propylsulfamide (10) and 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl]amino} pyrimidin-4-yl]oxy}ethylacetate (11).

Keywords: Macitentan, Antagonist, Impurities, Synthesis.

I. INTRODUCTION

The Macitentan (OPSUMIT®), an orally active endothelin receptor antagonist (ERA), is approved for treatment of pulmonary arterial hypertension (PAH), and is chemically also known as *N*-[5-(4-bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-4-pyrimidinyl]-*N*'-propylsulfamide [1]. Macitentan is a dual ERA, i.e. acts as an antagonist of two endothelin (ET) receptor subtype, ETA and ETB [1]. However, Macitentan has 50-fold increased selectivity for the ET_A subtype compared to the ETB subtype [2]. Macitentan blocks the ET1-dependent rise in intracellular calcium by inhibiting the binding of ET-1 to ET receptors. Blocking of the ET_A receptor subtype seems to be of more importance in the treatment of PAH than blocking of ET_B, likely because there are higher numbers of ET_A receptors than ET_B receptors in pulmonary arterial smooth muscle cells [3-7].

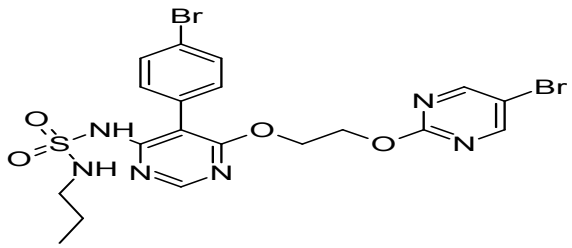
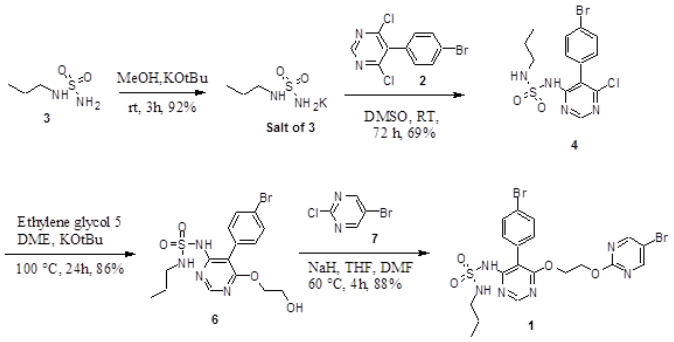


Figure 1. Structure of Macitentan **(1)**

The presence of impurities in drug substance can have a significant impact on the quality and safety of the drug product. The acceptance criteria of these impurities are stringent i.e. acceptable level for all impurities present in the drug should be less than 0.10% based on the guideline laid down by the international conference on harmonization (ICH) and pharmacopeia [8]. In order to meet these impurities present in the drug substance greater than above mentioned value must be identified and characterized. It is equally important to have impurity in pure form required for analytical development such as specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), and relative retention factor. Thus; it is essential and important to establish the facile and robust synthesis for the related substances and their characterization during drug development activity. During the laboratory development of Macitentan various related substances were observed in crude samples, based on LC-MS study and its fragmentation patterns, probable structures were predicted, synthesized the predicted compounds and further confirmed by spiking & purging study by HPLC. Present work describes identification, synthesis, and characterization of four related impurities (**8, 9, 10,** and **11**) during development of Macitentan (**1**).

II. RESULT AND DISCUSSION

Macitentan (**1**) was synthesized by known literature synthetic methods [9, 10] (Scheme-1). It involves the reaction of *N*-propylsulfamide (**3**) with potassium tertiary butoxide (KOtBu) in presence methanol to provide potassium salt of **3**, which is then condensed with 5-(4-bromophenyl)-4,6-dichloropyrimidine (**2**) in dimethylsulfoxide (DMSO) to provide 6-chloro compound (**4**). Compound **4** was then reacted with ethylene glycol (**5**) in presence of potassium tertiary butoxide in dimethoxy ethane (DME) to provide 2-hydroxyethoxy compound (**6**). Finally, nucleophilic substitution of **6** with 5-bromo-2-chloropyrimidine (**7**) in presence of sodium hydride (NaH) in tetrahydrofuran (THF) and dimethylformamide (DMF) resulted in desired Macitentan (**1**).



Scheme 1. Reported synthetic scheme for Macitentan **(1)**

During the development we observed that crude sample of Macitentan containing 4 unknown peaks, further our purification process for **1** is capable to remove these impurities but resulted in less yield of **1**. Hence, for identification of unknown peaks appeared at RRT 0.10, 1.50 and 1.60 and 0.50 and further avoiding their formation in reaction the crude sample of Macitentan (**1**) was subjected for LC-MS analysis. The relative molecular mass of these of unknown peaks appeared at RRT 0.10, 1.50 and 1.60 and 0.50 are 357 (**8**), 475 (**9**), 671 (**10**) and 699 (**11**) respectively by LC-MS based on the molecular mass of compound predicted the probable structures for these impurities and they are namely 2,2'-{[5-(4-bromophenyl)pyrimidine-4,6-diyl]bis(oxy)}diethanol (**8**), 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]-pyrimidine **(9),** *N*-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl] -*N*'-propylsulfamide (**10),** 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl] amino} pyrimidin-4-yl] oxy}ethylacetate (**11**) and further theire chemical structures are captured in Figure 2.

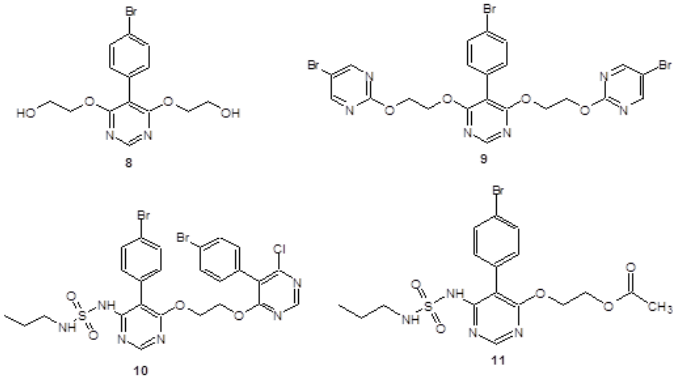
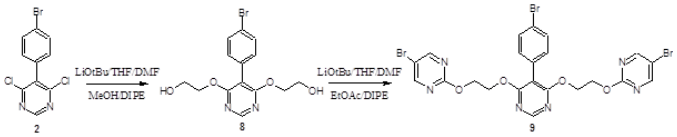


Figure 2. Structure of Macitentan related substances.

Further to confirm these probable impurities **8, 9, 10** and **11** we synthesized these impurities by possible synthetic path and were further confirmed by spectroscopic and chromatographic data followed by spiking and purging study by HPLC. The detailed synthesis of impurities **8, 9, 10** & **11** and subsequently their formation in reaction mass of **1** are as follows,

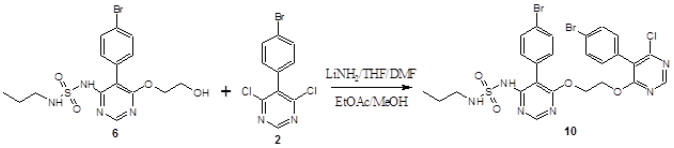
Synthesis of impurity 8: The nucleophilic substitution of unreacted 5-(4-bromophenyl)-4,6-dichloropyrimidine (**2**) with two molecules of ethylene glycol (**5**) in presence of potassium tert. butoxide in DMSO leads to formation of impurity **8**. It is further synthesized by condensing **2** with **5** in presence of lithium *tert*. butoxide in DMSO at elevated temperature to provide white solid powder of **8** (Scheme 2)**.**

Synthesis of impurity 9: The impurity **9** was formed due to traces amount of **8** was present in **6**, which was condensed with two molecules of **7** in the presence of KOtBu in DMF and THF. The impurity **9** was synthesized by condensing **8** with **7** in presence of lithium tert. butoxide in THF and DMF at elevated temperature (Scheme 2).



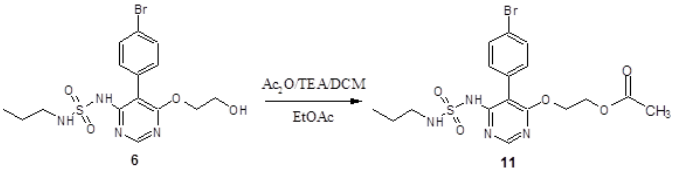
Scheme 2. Synthesis of impurity **8**, and **9**

Synthesis of impurity 10: The impurity **10** was formed during the synthesis of **6;** compound **6** was condensed with readily available **2** in presence of KOtBu in DMSO. Impurity **10** was synthesized by condensation of **6** with **2** in the presence of lithium tert. butoxide in THF and DMF at elevated temperature to give compound **10** (scheme 3).



Scheme 3. Synthesis of impurity **10**

Synthesis of impurity 11: Impurity **11** was formed due to side reaction of **6** with ethyl acetate which is used as a solvent for the extraction, leads to the formation of acetate compound **11**. This impurity was synthesized by the reaction of **9** with acetic anhydride in the presence of lithium tert. Butoxide to provide impurity **11** (Scheme 4).



Scheme 4. Synthesis of impurity **11**

III. CONCLUSIONS

In conclusion, we have successfully identified, four process related impurities of Macitentan namely, **8**, **9**, **10,** and **11** by LC-MS data and which is further confirmed by spiking and purging study after synthesizing and characterization of these impurities.

IV. EXPERIMENTAL SECTION

Melting points were determined on Analab melting point apparatus, in open capillary tubes and are uncorrected. The 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane as internal standard and are given in δ units. The solvents used for NMR spectra were deuterated chloroform and deuterated dimethylsulfoxide unless otherwise stated. Infrared spectra were taken on Perkin Elmer Spectrum 100 in potassium bromide pallets unless otherwise stated. Elemental analyses were performed on a Hosli CH-Analyzer and the results were within ±0.3 % of the calculated values. High-resolution mass spectra were obtained with a Shimadzu GC–MS QP mass spectrometer with an ionization potential of 70 eV. All the reaction were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60F254 (Merck) plates using UV light (254 and 366 nm) or High performance liquid chromatography (HPLC) on Agilent Technologies 1200 series for detection. Gas chromatography on Agilent Technologies 7683B with head space was used for analyzing the residual solvents. Common reagent–grade chemicals are either commercially available and were used without further purification or were prepared by standard literature procedures.

Synthesis of 2,2'-{[5-(4-bromophenyl)pyrimidine-4,6-diyl]bis(oxy)}diethanol (8)

To a solution of lithium tert. butoxide (17.0 g, 0.21 mol) in DMSO (100 ml) and ethylene glycol **5** (250 ml), added compound **2** (25.0 g, 0.08 mol) at 25-30°C. The reaction mass was heated to 55-60 °C and maintained for 2-6 hrs. After completion of reaction monitored by HPLC, reaction mass was cooled to 25-30 °C. Quenched the reaction mass by 5% citric acid solution in water (250 ml), and product were extracted with ethyl acetate (250 x 2 ml), organic layer was washed with 5% brine solution (250 ml). The organic layer was concentrated under reduced pressure at below 55 °C to obtain syrup. The resulting syrup was dissolved in methanol (75 ml) and heated to 60-65 °C, added DIPE (200 ml) at 60-65 °C. The precipitated solid was cooled to 25-30 °C and maintained for 45-60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50°C for 2-4 h to provide title compound **8**. Yield: 16.5 g (72.0 %). IR (cm^{-1}): 3383.75, 2950, 1577, 1438, 1314, 1100, 827. MS *m/z* (%): 357.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 8.72 (s, 1H), 7.48-7.51 (d, 2H), 7.40-7.42 (d, 2H), 4.25-4.28 (t, 4H), 3.91-3.95 (t, 4H), 2.93-2.95 (t, 2H). ^{13}C NMR (DMSO, 300 MHz): δ 172.21, 152.34, 142.23, 134.62, 129.2, 122.21, 114.43, 78.34, 63.32. Chemical purity by HPLC: 97.15%

Synthesis of 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]-pyrimidine (9)

To a stirred solution of **8** (10 g, 0.028 mol) in a mixture of DMF (100 ml) and THF (100 ml) at 25-30°C, were added lithium tert. butoxide (7.0 g, 2.5 mol) followed by **7** (12.0 g, 0.0 mol). The reaction mass was heated to 55-60 °C and maintained for 4-6 h. After completion of

reaction monitored by HPLC, cooled the reaction mass to 25-30°C. The reaction mass was quenched with water (500.0 ml), and adjusted pH 5-6 using acetic acid. The product was extracted with ethyl acetate, and organic layer was washed with 5 % brine solution in water (250 ml). The organic layer were concentrated under vacuum at below 50 °C to obtain syrup. The syrup was diluted in DIPE (100 ml) and heated to 55-60 °C. Precipitated solid was cooled to 25-30 °C and maintained for 60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50°C for 3 h to offer title compound **9**. Yield: 7.6 g, (40 %). Purity by HPLC: 96.21%. IR (cm^{-1}): 3445, 2957, 1569, 1430, 1308, 1120, 1064, 822. MS *m/z* (%): 670.9 [M^+ + 1]. ^{1}HNMR (DMSO, 300 MHz): δ 8.71 (s, 4H), 8.46 (s, 1H), 7.40-7.42 (d, 2H), 7.25-7.28 (d, 2H), 4.60-4.69 (t, 8H). ^{13}C NMR (DMSO, 300 MHz): δ 166.16, 163.11, 159.72, 155.70, 132.33, 130.49, 129.12, 120.65, 111.88, 103.87, 65.50, 64.91.

Synthesis of *N*-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl]-*N*'-propylsulfamide (10)

To a stirred solution of compound (**6**) (25.0 g, 0.057 mol) in a mixture of THF (100 ml) and DMF (100 ml) at 25-30 °C, added lithium amide (), followed by **2** (18.0 g, 0.059 mol). Heated the reaction mass to 55-60 °C and maintained for 2-4 h. After completion of reaction monitered by HPLC, cooled the reaction mass to 25-30°C and quenched with 5 % citric acid solution in water (500 ml). The product was extracted with ethyl acetate (500 X 2 ml), washed with water (500 x 2 ml), and concentrated organic layer under vacuum to obtain syrup. The syrup was diluted in methanol (100 ml) and heated to 60-65 °C. Cooled the reaction mass to 25-30 °C and maintained for 60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50 °C for 2h to provide title compound 10. Yield: 35.0 g, (86.40%). IR (cm^{-1}): 3314, 2953, 1571, 1434, 1305, 1173, 1045, 826. MS *m/z* (%): 699.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 9.23 (s, 1H), 8.12 (s, 1H), 7.52-7.49 (dd, 4H), 7.24-7.29 (dd, 4H), 4.46-4.48 (t, 4H), 3.2 (t, 2H), 2.1 (m, 3H), 0.98-1.01 (t, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 172.09, 170.01, 169.36, 163.24, 154.21, 152,21, 140.83, 139.84, 132.21, 128.06, 128.26, 127.66, 127.76, 127.41, 127.48, 114.96, 41.1, 23.54, 11.10. Chemical purity by HPLC: 98.57%.

Synthesis of 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl]amino}pyrimidin-4-yl]oxy}ethyl acetate (11)

To a stirred solution of compound (**6**) (10.0 g, 0.23 mol) in DCM (50 ml) at 25-30 °C, added triethyl amine (25 ml, 0.24 mol) followed by acetic anhydride (3.6 g, 0.035 mol), and maintained for 25-30 °C for 2-4 h. After completion reaction monitored by HPLC, quenched reaction mass with water (100 ml) and product was extracted in DCM (100 ml). The organic layer was washed with water (50 ml), and concentrated under vacuum below 45 °C to provide title compound **11** as an oil. Yield: 8.0 g, (73.0 %). IR (cm^{-1}): 3282, 2968, 1738, 1658, 1574, 1436, 1390, 1171, 1087, 829. MS *m/z* (%): 475.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 8.47 (s, 1H), 7.58-7.64 (dd, 2H), 7.17-7.27 (dd, 2H), 4.54-4.57 (t, 2H), 4.29-4.32 (t, 2H), 2.96 (t, 2H), 2.00 (s, 3H), 1.55-1.62 (m, 2H), 0.91-1.05 (m, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 170.54, 165.95, 157.68-156.18, 151.68, 132.38-136.46, 131.57-131.77, 127.89-12859, 122.78-123.09, 104.48, 65.29, 64.42-64.59, 61.83-61.92, 53.51, 46.39, 22.29, 20.60-20.90, 10.87-11.18. Chemical purity by HPLC: 98.65%.

V. ACKNOWLEDGEMENT

Authors thank the management of Megafine Pharma (P) Ltd. For the permission to publish this work. Authors also thank colleagues of the Analytical Research and Development team for their valuable contribution to this work

VI. REFERENCES

[1]. Hong, I.S., Coe, H.V., & Catanzaro, L.M. (2014). Macitentan for the treatment of pulmonary arterial hypertension. The Annals of Pharmacotherapy, 48, 1-10.

[2]. Iglarz, M., Qiu, C., Fischli, W., Morrison, K., Binkert, C., Clozel, M., Hess, P., Capeleto, B., Buchmann, S., Boss, C., Bolli, M.H., Weller, T., Treiber, A., & Gatfield, J. (2008). Pharmacology of macitentan, an orally active tissue-targeting dual endothelin receptor antagonist. Journal of Pharmacology and Experimental Therapeutics, 327(3), 736-745

[3]. Gatfield, J., Grandjean, C. M., Sasse, T., Clozel, M., & Nayler, O. (2012). Slow receptor dissociation kinetics differentiate macitentan from

other endothelin receptor antagonists in pulmonary arterial smooth muscle cells. PLoS ONE, 7(10), e47662.
[4]. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Goto K, Masaki T (1988) Nature 332:411
[5]. Battistini B, Botting R, Endothelins (1995) Drug News Persp 8:365
[6]. Kohan DE (2008) Curr Hypertens Rep 10:65
[7]. Thorin E, Clozel M (2010) Adv Pharmacol 60:1
[8]. International Conference on Harmonization guidelines Q3A (R) Impurities in New Drug Substances February 2002 (this guideline provides guidance for registration application on the content and qualification of impurities in new drug substances produced by the chemical synthesis) 18 October 2016.
[9]. Bolli MH, Boss C, Binkert C, Clozel M, Fischli W, Weller T (2006) Sulfamide and their use as endothelin receptor antagonist. US Patent 7,094,781B2, Aug 22, 2006; (2006).
[10]. Bolli MH, Boss C, Binkert C, Buchmann S, Bur D, Hess P, Iglarz M, Meyer S, Rein J, Rey M, Treiber A, Clozel M, Fischli W, Weller T (2012) J Med Chem 55:7849

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Green Synthesis of CuO Nanoparticles using Ziziphus Mauritiana L. Extract and Its Characterizations

Shreyas Pansambal[1*], Sonali Gawande[1], Suresh Ghotekar[2], Rajeshwari Oza[1], Keshav Deshmukh[1]

[1]Department of Chemistry, S.N. Arts, D.J.M. Commerce and B.N.S. Science College, Sangamner, Savitribai Phule Pune University, Maharashtra, India

[2]Department of Applied Science & Humanities, G. M. Vedak Institute of Technology, Tala, University of Mumbai, Maharashtra, India

ABSTRACT

Copper oxide nanoparticles (CuONPs) synthesized by a completely environmentally safe and facile process using Ziziphus mauritiana L. aqueous plant extract as an effective stabilizing agent. Phytochemical screening of the fresh aqueous leaves extract showed the presence of coumarins, tannins, saponins, flavonoids, and glycosides. The synthesized CuO nanomaterial was characterized by XRD, SEM, EDX, TEM and surface area. This green synthesis route indicates that, it is efficient method than chemical method.

Keywords: Nanotechnology, Ziziphus mauritiana L., CuONPs, Phytochemical screening.

I. INTRODUCTION

Nowadays CuONPs have generated a great deal of interest, especially in the field of gas sensing [1], catalysis [2] and biomedical [3] because of their remarkable applications. These CuONPs fabricated using different synthetic routes such as ionic liquid assisted, microwave irradiation, sonochemical, electrochemical, sol-gel technique and thermal treatment [4-9] have been reported. Nevertheless, these routes have some drawbacks like the use of toxic chemical, use of acrimonious synthesis conditions like high temperature and requirement of external additives during the reaction time. Hence there is limited scope for the development of efficient, environmentally safe and additive free synthesis of CuONPs. Currently plant extract mediated nonmaterial synthesis is getting lot of attention with several advantages offered by chemical methods [10-12]. The scrutiny of the literature revealed some notable plant extract used for facile synthesis of CuO-NPs. For example, Rauvolfia serpentina [13], Leucaena leucocephala [14], Calotropis gigantean [15], Aloe barbadensis [16], Ficus religiosa [17], Albizia lebbeck [18] and Acanthospermum hispidum [19] have been reported.

Ziziphus mauritiana L. (Figure 1) belongs to family Rhamnaceae is an annual plant which grows in tropical and sub-tropical regions of world. Different parts of this plant have been used in the traditional medicine for the treatment of different ailments. In this study, we report the use of Ziziphus mauritiana L. as stabilizing and reducing agent in the ecofriendly synthesis of CuONPs.

Figure 1. Leaves of *Ziziphus mauritiana* L.

II. MATERIALS AND METHODS

2. 1. Materials

Copper acetate monohydrate was used as a precursor. The fresh leaves of Ziziphus mauritiana L. were sourced from Sangamner college campus, Maharashtra, India. The collected leaves were washed with deionized water, cut into small pieces. All glassware's are washed with distilled water and acetone and dried in oven before use.

2.2. Biogenic synthesis of CuONPs

5g chopped leaves of Ziziphus mauritiana L. were transferred into 500 mL beaker containing 100 mL deionized water. The mixture were refluxed at 80 ^{o}C for 30 minutes and cooled at room temperature and resultant filtrate was filtered through Whatmann No. 1. The filtered extract is stored in refrigerator at 5 ^{o}C and used for synthesis of CuONPs. The aqueous solution of 1 mM copper sulphate monohydrate was prepared in deionized water. Ziziphus mauritiana L. leaf extract was mixed to 1 mM aqueous copper acetate solution in 1:9 ratios in a 250 ml beaker with constant stirring on magnetic stirrer. After time of period the color of solution turns to dark yellow. The mixture was kept in a muffle furnace at 250 ^{o}C. A fine black colored material was obtained and this was carefully collected and packed for characterization purposes.

2. 3. Characterization techniques

The morphology and composition of the synthesized CuONPs were examined by field emission scanning electron microscopy (FESEM, FEI, Nova Nano SEM 450), FESEM coupled energy-dispersive X-ray spectroscopy (EDX, Bruker, XFlash 6I30). Find the exact morphological structures and size of the CuONPs using transmission electron microscopic (TEM) analysis is done by using a PHILIPS-CM200. The crystallinity and crystal phases were characterized by X-ray diffraction (XRD, Brukar, D8-Advanced Diffractometer) pattern measured with Cu- Kα Radiation (λ= 1.5406 Å) in the range of 5–90^{o}.

2. 4. Phytochemical Screening

The fresh aqueous extract of Ziziphus mauritiana L. leaves were investigated for the presence of phytochemicals viz. coumarins, saponins, tannin, flavonoids, and glycosides by standard biochemical method [20].

III. RESULTS AND DISCUSSION

3.1. Structural & crystallographic analysis

The CuONPs fabricated from Ziziphus mauritiana L. leaves extract were confirmed by the characteristic peaks observed in the XRD patterns, as shown in Fig. 2. XRD analysis evinced prominent diffraction peaks at 32.59º, 35.47º, 38.69º, 53.41º, 61.45º and 75.19º corresponding to (110), (002), (111), (020), (113) and (222) of face-centered-cubic structure of copper oxide nanoparticles with a monoclinic phase (JCPDS No. 45-0937). The XRD pattern exposed that synthesized copper oxide nanoparticles are crystalline in nature [30].

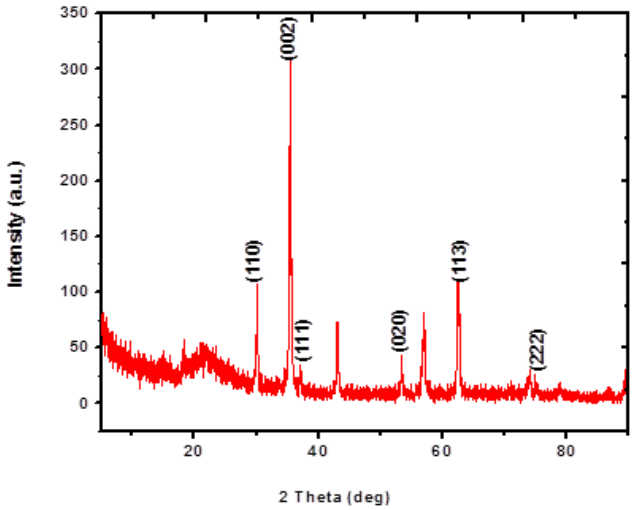


Figure 2. X-Ray diffraction pattern of synthesized CuO-NPs at room temperature

3. 2. FE-SEM microphotographs

From the FESEM image as shown in Fig. 3 the synthesized CuONPs present uniform and define spherical morphology. Each CuONPs possesses the average particles size of 20-45 nm. It is noticed that green synthesis of CuONPs produces the small and uniform size of spherical particles.

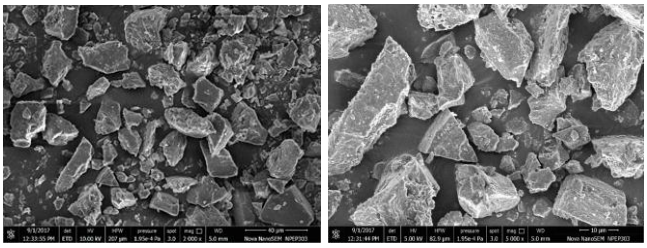


Figure 3. FE-SEM microphotographs of CuO-NPs deposited on a carbon strip.

3. 3. TEM images

Figure 4 shows the TEM images of synthesized CuONPs. The low magnification TEM image reveals almost similar spherical morphology of CuONPs as seen in FESEM image. From TEM images, the average particle size is estimated to be 20-45 nm spherical particles, which is consistent with the FESEM results.. Therefore, the morphological characterizations confirm the

spherical morphology of CuONPs biosynthesized from the leaves of Ziziphus mauritiana L. plant.

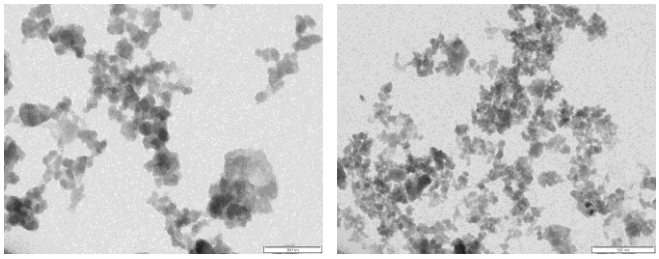


Figure 4. TEM images indicating the presence of spherical CuO-NPs recorded at various magnifications.

3. 4. EDS studies

The composition of synthesized CuONPs has been analyzed by investigating the energy-dispersive X-ray spectroscopy (EDS), as shown in Fig. 5. EDS spectrum displays the Cu and O peaks. The quantitative data confirms the formation of CuO instead of other copper oxide in the synthesized materials by green synthesis of *Ziziphus mauritiana* L. plant.

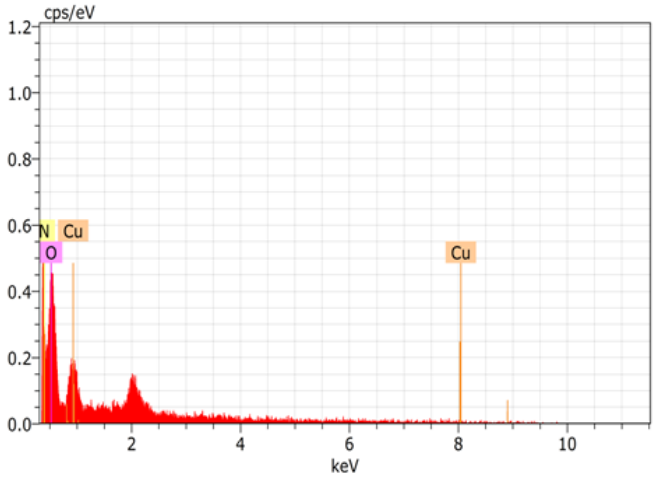


Figure 5. EDS spectrum of synthesized CuO-NPs.

3. 5. Specific surface area and porosity studies

The considerable parameters such as particle size, shape and density are related to the specific surface area measurements ($m^2 \cdot g^{-1}$) Fig. 6 exhibit BET plots of CuONPs. The specific surface area of CuONPs calculated using the multipoint BET-equation is 14.98 m^2/g. Assuming that the particles have solid, spherical shape with smooth surface.

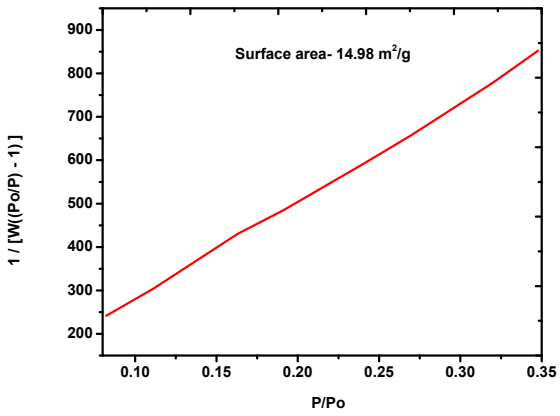


Figure 6. BET plots of CuONPs.

Figure 7 shows the typical BJH desorption pore size distribution curves of CuONPs. From the curves, we can see that most of the micropores with a size smaller than 36.19 nm, the pore size of which estimated from the peak position are about 3.711 nm possesses a relatively narrow pore size distribution. Therefore, these particles are actually grain clusters and small polycrystalline in nature.

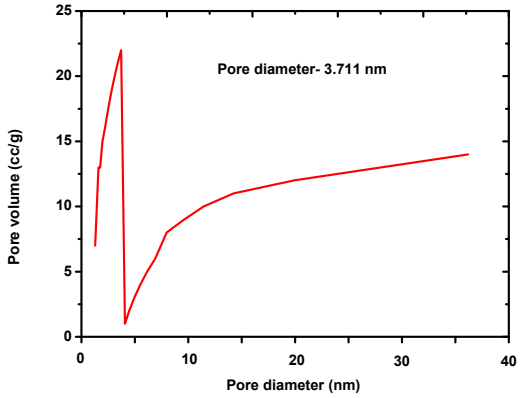


Figure 7. BJH desorption pore size distribution curves of CuONPs

3. 6. Phytochemical screening

The results of qualitative phytoconstituents analysis of aqueous leaf extract of *Ziziphus mauritiana* L. are shown in table 1. Phytochemical analysis of *Ziziphus mauritiana* L. leaves revealed and highlighted the presence of tannins, saponins, coumarins, flavonoids, and glycosides which may be responsible for the efficient stabilizing agent of nanoparticles.

Table 1. Phytochemical screening of aqueous leaves extract of *Ziziphus mauritiana* L.

Phytoconstituents	Test	Phytoconstituents	Test
Tannin	+	Saponins	+
Coumarins	+	Emodins	-
Proteins	-	Flavonoid	+
Glycosides	+	Anthraquinone	-
Anthocyanosides	-	Phytoterols	-

IV. CONCLUSION

In present work a new green protocol for the synthesis of CuONPs using aques extract of Ziziphus mauritiana L. leaves is developed, which is ecofriendly method and a promising, low cost & without using any toxic chemicals. Average size of synthesized CuONPs is 5-40 nm.

V. ACKNOWLEDGEMENT

We are thankful to CIF Savitribai Phule Pune University and SAIF IIT Powai for providing the technical and instrumental activities supports. Authors are thankful to DST for providing FIST grant to the college and BCUD SPPU is kindly acknowledged for sponsoring research project [Proposal No: 15SCI001364].

VI. DISCLOSURE

The authors declare no conflicts of interest in this work.

VII. REFERENCES

[1]. Frietsch M, Zudock F, Goschnick J, Bruns M. CuO catalytic membrane as selectivity trimmer for metal oxide gas sensors. Sens. Actuators B. 2000; 65(1): 379-381.

[2]. Gawande M B, Goswami A, Felpin F-X, Asefa T, Huang X, Silva R, Zou X, Zboril R, Varma R S. Cu and Cu-based nanoparticles: synthesis and application in catalysis. Chem. Rev. 2016; 116: 3722-3811.

[3]. Rehana D, Mahediran D, Senthil Kumar R, Kalilur Rahiman A. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed. & Pharmaco. 2017; 89: 1067-1077.

[4]. Kessler M T, Robke S, Sahler S, Prechtl M H G. Ligand-Free copper (I) oxide nanoparticle-catalysed amination of aryl halides in ionic liquids. Catal. Sci. Technol. 2014; 4: 102−108.

[5]. Wang H, Xu J Z , Zhu J J, Chen H Y. Preparation of CuO nanoparticles by microwave irradiation. J. Cryst. Growth. 2002; 244: 88-94.

[6]. Kumar R V, Diamant Y, Gedanken A. Sonochemical synthesisand characterization of nanometer-size transition metal oxids from metal acetates. Chem. Mater. 2002; 12: 2301-2305.

[7]. Borgohain k, Singh J B, Rama Rao M V, Shripathi T, Mahamuni S. Quantum size effects in CuO nanoparticles. Phys. Rev. 2000; 61: 11093-11096.

[8]. Eliseev A A, Lukashin A V, Vertegel A A, Heifets L I, Zhirov A I, Tretyakov Y D. Complexes of Cu (II) with polyvinyl alcohol asprecursor for the preparation of CuO/SiO2 nanocomposites. Mater. Res. Innov. 2000; 3: 308-312.

[9]. Salavati-Niasari M, Davar F. Synthesis of copper and copper (I) oxide nanoparticles by thermal decomposition of a new precursor. Mater. Lett. 2009; 63: 441−443.

[10]. Savale A, Ghotekar S, Pansambal S, Pardeshi O. Green synthesis of fluorescent CdO nanoparticles using Leucaena leucocephala L. extract and their biological activities. J Bacteriol Mycol open acess. 2017; 5: 00148.

[11]. Ghotekar S K, Pande S N, Pansambal S S, Sanap D S, Mahale K M, Sonawane B. Biosynthesis of silver nanoparticles by using Annona reticulata L. And its characterization. Wor. Jou. Pha. & Pha. Sci. 2015; 11: 1304-1312.

[12]. Pande S N, Bharati K T, Wakchure S K, Ghotekar S K, Gujrathi D B, Phatangare N D. Green synthesis of silver nanoparticles by carallua fimbriata L. and its characterization. Ind. Jou. App. Res. 2015; 5: 749-750.

[13]. Lingaraju K, Raja Naika K, Manjunath K, Nagaraju G, Suresh D, Nahabhushna H. Rauvolfia serpentine-mediated green synthesis of CuO nanoparticles and its multidisciplinary studies. Acta Metall. Sin. 2015; 18: 1134-1140.

[14]. Aher Y B, Jain G H, Patil G E, Savale A R, Ghotekar S K, Pore D M, Pansambal S S, Deshmukh K K. Biosynthesis of copper oxide nanoparticles using leaves extract of Leucaena leucocephala L. and their promising upshot against the selected human pathogen. Int. Jou. Mol. Clin. Micro. 2017; 7: 776-786.

[15]. Sharma J, Akhtar J K, Ameen M S, Srivastava S, Singh P G. Green synthesis of CuO nanoparticles with leaf extract of Calotropis gigantea and its dye-sensitized solar cells applications. J. Alloys Compd. 2015; 632: 321.
[16]. Gunalan S, Sivaraj R, Venckatesh R. Aloe barbadensis Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: optical properties. Spectrochim. Acta A. 2012; 97: 1140.
[17]. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari K S, Ravikumar V. Anticancer activity of Ficus religiosa engineered copper oxide nanoparticles. Spectrochim. Acta A. 2014; 121: 746.
[18]. Jayakumarai G, Gokulpriya C, Sudhapriya R, Sharmila G, Muthukumaran C. Phytofabrication and characterization of monodisperse copper oxide nanoparticles using Albizia lebbeck leaf extract. Appl. Nanosci. 2015; 5: 1017-1021.
[19]. Pansambal S, Deshmukh K, Savale A, Ghotekar S, Pardeshi O, Jain G, Aher Y, Pore D. Phytosynthesis and biological activities of fluorescent CuO nanoparticles using Acanthospermum hispidum L. extract. J. Nanostruct. 2017; 7: 165-174.
[20]. Fransworth N R. Biological and phytochemical screening of plants. Jou. of Pharma. Sci. 1996; 55: 225-227.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Transition metal (II) Complexes with Schiff base of 1, 2, 4-Triazole-4-amine & 4-dimethylamino-benzaldehyde and their Antimicrobial Activity

Bharati K.T*, Gujarathi D. B, Tryambake P. T, Hase G. J, Gagare N. B, Bhor J. B
S. N. Arts, D. J. M. Commerce & B.N.S Science College, Sangamner, Ahmednagar, Maharashtra, India

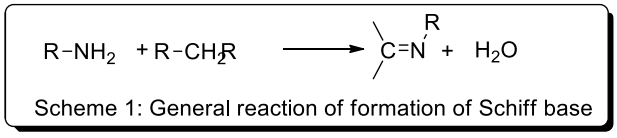
ABSTRACT

This study summarizes the synthesis of new Schiff bases derivatives of 1, 2, 4-triazole-4-amine with 4-dimethylamino-benzaldehyde. The co-ordination compounds of transition metal ions like Cu (II) and Zn (II) with the Schiff bases were synthesized and characterized by UV-Visible Spectroscopy, Infra-Red Spectroscopy, NMR, Elemental Analysis and Magnetic Susceptibility Measurement. These co-ordination compounds show moderate biological activity against bacterial strains.

Keywords: 1, 2, 4- triazole-4-amine, Schiff base and Co-ordination compounds.

I. INTRODUCTION

1,2,4-triazole heterocyclic compounds play a very vital role in our lives through their biological activities including anti-inflammatory, antiviral, antimicrobial, analgesic, antitubercular and anticancer properties[1-3]. Schiff bases are the condensation products (following Scheme 1)[1] of primary amines and carbonyl compounds, named after Hugo Schiff, who discovered them in 1864[4].People have synthesized some new 1, 2, 4-triazole[5-8] and 1, 3, 4-triazole[9]derivatives with their antimicrobial activity[10, 11].



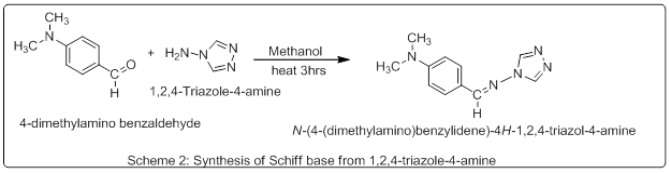
Scheme 1: General reaction of formation of Schiff base

The stability of metal complexes with Schiff bases and their applications as antifungal, antibacterial, anticancer, antiviral and herbicidal etc.[12] prompted us to synthesize new Schiff base of 1, 2, 4-triazole-4-amine with 4-dimethylamino-benzaldehyde and its metal complexes with Cu(II) and Zn(II), their characterization and investigation of their antimicrobial activity.

II. EXPERIMENTAL

2.1 Preparation of ligand

4-dimethylamino benzaldehyde (2.38 mmole) and 1, 2, 4-triazole-4-amine (2.38 mmole) were mixed in 5 mL methanol and the mixture was refluxed for 3 hrs on water bath. The faint yellowish color product was obtained after the addition of 10 mL of ice water. The synthesized Schiff base was filtered and washed with ice water and dried at room temperature. It shows practical yield of 89.78% and M.P. is 202 ^{0}C. Following reaction takes place (Scheme 2) and it was characterized by Spectrophotometer (Chemito UV-2100), Infra-Red Spectrophotometer (Bruker Optics, Model Alpha T), Bruker Advance II 400 NMR Spectrometer and Perkin-Elmer 2400 CHN Analyzer. The physical parameters and results of elemental analysis were shown in Table 1.



Scheme 2: Synthesis of Schiff base from 1,2,4-triazole-4-amine

Table 1. Characterization of ligand

Sr. No.	Name & molecular formula of the ligand	Color	M.P.	% Yield	Elemental analysis Calculated (Found) %		
					C	H	N
1	N-(1-(4-(dimethylamino)phenyl) ehtylidene)-4H-1,2,4,-triazol -4-amine	Faint Yellowish white	202 ^{0}C	89.78	61.39 (61.10)	6.04 (5.95)	32.55 (32.25)

2.3 Synthesis of metal complexes

The solid complexes were prepared by mixing of aqueous solution of Copper chloride (45 mg) and Zinc chloride (40 mg) with 5 mL of 2 % methanolic solution of ligand (100 mg) and refluxed for 2hrs. Blue and white

colored complexes were obtained for Copper (II) and Zinc (II) respectively (Figure 1).

2.4 Analysis of synthesized metal complexes

The characterization of metal complexes were interpreted by UV Spectrophotometer (Chemito UV-2100), Infra-Red Spectrophotometer (Bruker Optics, Model Alpha T) and elemental analysis by Perkin-Elmer 2400 CHN Analyzer. The physical parameters and result of elemental analysis were shown in Table 2.

Table 2. Characterization of metal complexes

Sr. No.	Name & molecular formula of the complex	Color	M.P.	% Yield	Elemental analysis Calculated (Found) %			
					C	H	N	M
1	$(C_{11}H_{13}N_5)_2.CuCl_2$	Blue	96 ^{0}C	30 %	46.76 (46.65)	4.60 (4.52)	24.79 (24.69)	11.25 (11.15)
2	$(C_{11}H_{13}N_5)_2.ZnCl_2$	White	72 ^{0}C	34.42%	46.61 (46.59)	4.59 (4.55)	24.71 (24.68)	11.54 (11.45)

2.5 Antimicrobial activity

The antimicrobial activity of the metal complexes was evaluated with the help of ATCC cultures including gram positive (S.aureus) and gram negative (E.coli & P. aeruginosa) using Gentamicin as standard and antifungal activity was tested against Candida sp. using Nystatin as standard and adopting standard protocols[13]. Saturated solutions of complexes in DMSO were used for the antimicrobial studies.

III. RESULT AND DISCUSSION

The prepared complexes were found to be solids, insoluble in water but soluble in DMSO.

3.1 Electronic spectra

The characteristic peaks in electronic spectra of solutions of Schiff base in methanol and its complexes in DMSO are summarized in Table 3.The UV-Visible spectrum of Schiff base showed shoulder peaks and maximum absorbance at 336 nm (Figure 1). These shoulder peaks are observed due to π-π* transition.

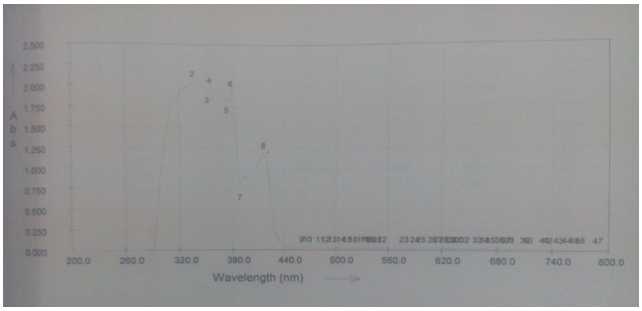


Figure 1. UV Spectra of Synthesized Ligand

The UV-Visible spectrum of Cu (II) complex assigned to d-d transition. The spectrum of Cu (II) complex shows band at 338 nm which is attributed to the electronic transition $^2E_g \rightarrow {}^2T_{2g}$ (Figure 2).

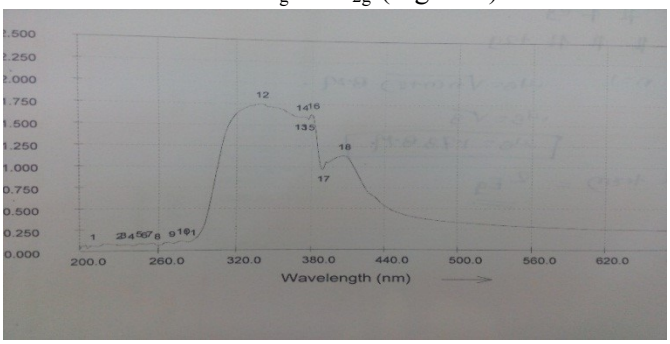


Figure 2. UV Spectra CuL_2 Complex

The magnetic susceptibility measurements provide data to characterize the structure of complex. The magnetic

moment for Cu (II) complex was approximately 1.73 BM. The Zn (II) complex was diamagnetic as expected for d^{10} ions so that no d-d transition can be expected in the Zn (II) complex (Figure 3). Ligand had no magnetic moment[14].

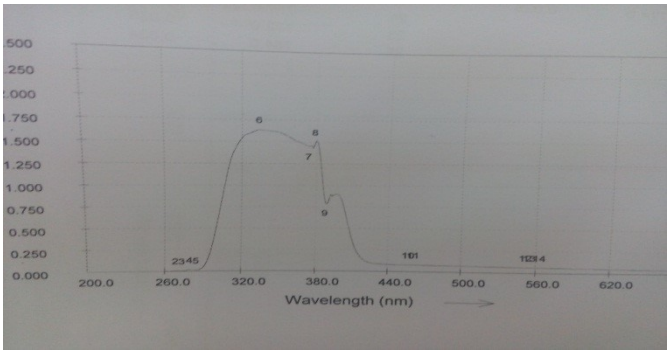


Figure 3. UV Spectra ZnL_2 Complex

Table 3. Important Peaks from UV Spectra of free Ligand and its complexes

Product	λ_{max} (nm)	Abs	Wavenumber cm^{-1}	Transition	BM
L	336	2.037	29,761	π- π*,	-
CuL_2Cl_2	338	1.748	29,585	π- π*, $^2E_g \rightarrow {}^2T_{2g}$	1.73
ZnL_2Cl_2	334	1.636	29,940	π- π*	-

3.1 FTIR spectra

The functional groups information of prepared ligand and complexes were confirmed by Infrared Spectroscopy (Figure 2-4). The wavenumber (cm^{-1}) of characteristic bands in the IR spectra of ligand and complexes were reported in Table 4.

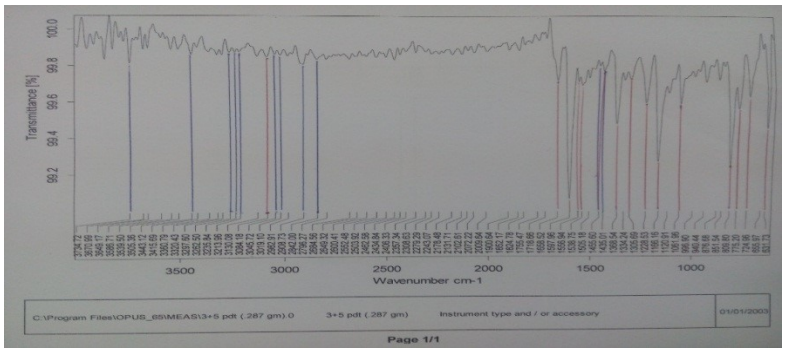


Figure 2. FTIR Spectra of Synthesized Ligand

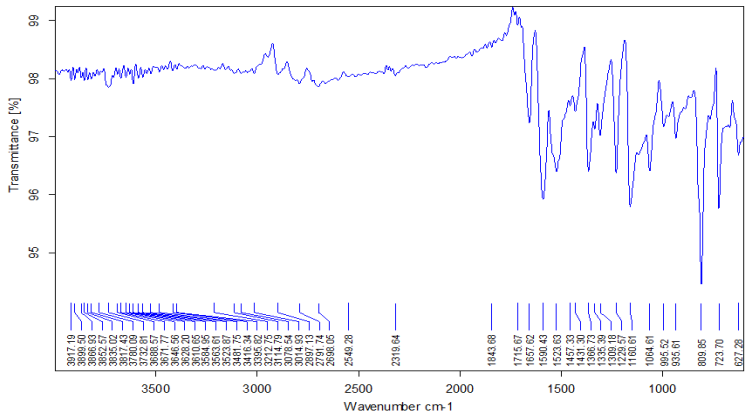


Figure 3. FTIR of CuL_2 Complex

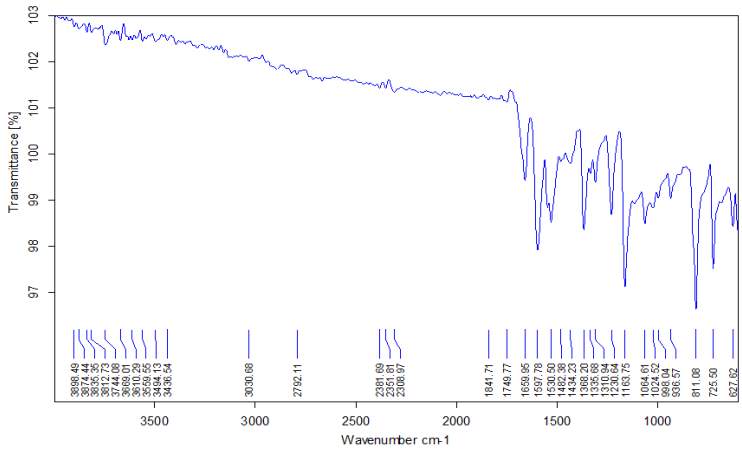


Figure 4. FTIR of ZnL_2 Complex

Table 4. Important Peaks from IR spectra of free ligand and its complexes

Product	Azomethine νC=N	Triazole νN-N	νM-N
L	1597.96	1305.69	-
CuL_2Cl_2	1590.43	1309.18	627.78
ZnL_2Cl_2	1597.78	1310.43	627.62

3.2 Nuclear Magnetic Resonance

The ^1H NMR spectra of ligand was recorded in DMSO. The ^1H NMR spectrum show sharp signals at δ 8.00 for one proton which could be attributed to the CH=N groups, aromatic proton in the range of δ 6.72-7.69 and two triazole proton shows a sharp signal at δ 8.76-8.87 (Figure 5).

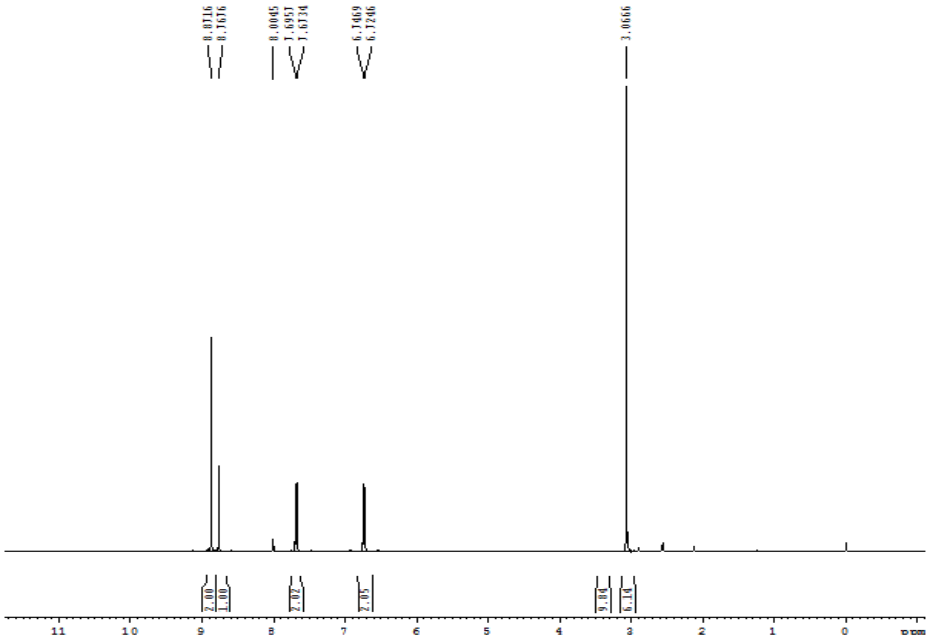


Figure 5. NMR of Synthesized Ligand

3.3 Antimicrobial activity

The synthesized metal complexes exhibited moderate biological activity against a Gram positive, two Gram negative as shown in Table 5.

Table 5. Effect of complexes on antimicrobial bacteria (Note- (-) indicates no activity.)

Compound	E.coli ATCC 25922	P.aeruginosa ATCC 27853	S.aureus ATCC 25923	Candida sp.
CuL_2Cl_2	08mm	08 mm	07 mm	(-)
ZnL_2Cl_2	08 mm	09 mm	07 mm	(-)
Gentamicin(Standard)	22 mm	27 mm	31 mm	(-)
Nystatin (Standard)	(-)	(-)	(-)	22 mm

IV. CONCLUSION

The complexes of Cu (II) and Zn (II) were synthesized by reaction of the synthesized ligand with the respective metal salts in 1:2 (M: L) ratio. The synthesized ligand and its complexes were characterized by UV-Visible, IR spectroscopic technique, NMR, magnetic susceptibility measurement, elemental analysis and their antimicrobial activity. The elemental and other spectral studies confirm the binding of Schiff base and metal ions and show the octahedral geometry of Cu (II) and Zn (II) complexes.

V. ACKNOWLEDGMENT

We are thankful to Principal Dr K.K.Deshmukh for providing the facilities and motivation.

VI. REFERENCES

[1]. Neslihan, D.; Ahmet, D.; Sengul, A and Sancak, K; "Synthesis and antimicrobial activities of some new 1, 2, 4-triazole derivatives", Eur.J.Med. Chem. (2004), 39:793-804.

[2]. Neslihan, D.; Ahmet, D.; Sengul, A and Elif. C; "Synthesis and antimicrobial activities of some new [1, 2, 4] triazole [3,4b] [1, 3, 4] thiadiazines" ARKIVOC (i). (2005), 75-91.

[3]. Aiyalu, R. and Kalasalingam A.R, "Synthesis of some novel triazole derivatives as anti-nociceptive and anti-inflammatory agents", 2009, 59: 355-364.

[4]. T. Mahmud, "Synthesis characterization and study of antibacterial activity of Enaminones complexes with Zinc and Iron", Arab. J. Chem; 2010, 3(4), 219-224.

[5]. Suresh Kumar G. V., Rajendraprasad Y, Malikarjuna B. P. Chandrasekhar S. M. and Kistayya C, "Synthesis of some novel 2-substituted-5-[isopropylthiazole] clubbed 1, 2, 4-triazole and 1, 3, 4-oxadiazoles as potential antimicrobial and antitubercular agents", Eur. J. Med Chem., 2010, 45, 2063-74.

[6]. Chohan Z. H. and Supran C. T., "In-vitro antibacterial and cytotoxic activity of Cobalt (II), Nickel (II) and Zinc (II) complexes of the antibiotic drug Cephalothin (Keflin)",J. Enz. Inhib. Med. Chem., 2005, 20, 463-468.

[7]. Singh K, Kumar Y, Pundir R. K., "Synthesis and characterization of biologically active organosilicon (IV) complexes with Schiff bases derived from o-aminothiophenol", Synth. React. Inorg. Met. Org. Chem., 2010, 40, 836-842.

[8]. Altundas A, Nursen S, Colak N, Ogutchi H., "Synthesis and biological activity of new cycloalkylthiophene Schiff bases and their Cr (III) and Zn (II) complexes", Med. Chem. Res., 2010, 19, 576-588.

[9]. Nurhan G.; Mevlut S.; Elif C.; Ali S.; and Neslishan D, "Synthesis and antimicrobial activity of some new 1, 2, 4-triazole derivatives," Turk. J. Chem., 2007, 31, 335-348.

[10]. Akbar Mobinkhaledi, Naser Foroughifar, Mansooreh Khanpour and Sattar Ebrahimi, "Synthesis of some novel Schiff bases containing 1, 2, 4-triazole ring", Eur. J. of Chem., 2010, 1(1), 33-36.

[11]. Katarzyna Wajda-Hermanowicz, Damian Pieniążczak, Aleksandra Zatajska, Robert Wróbel, Krzysztof Drabent and Zbigniew Ciunik; "A Study on the Condensation Reaction of 4-Amino-3,5-dimethyl-1,2,4-triazole with Benzaldehydes: Structure and Spectroscopic Properties of Some New Stable Hemiaminals", Molecules, 2015, 20(9), 17109-17131.
[12]. Bharati K.T; Gujarathi D. B; Tryambake P. T; Hase G. J; Gaikwad R. K. and Khatal M. B, "Preparation of Schiff base of 1, 2, 4-Triazole-4-amine with 3-nitrobenzaldehyde, its Complexation with Cu (II) and Zn (II) and Antimicrobial Activity of Complexes", Der Chemica Sinica, 8(2), 233-228, 2017.
[13]. Dr. M. K. Lalitha, "Manual of antimicrobial susceptibility testing", Indian Journal of Medical Microbiologist, Christian Medical College and Hospital, Vellore, India.
[14]. Song L, Xie Y, Wang H, "Synthesis and antibacterial activity of benzimidazole Schiff base," 2000, 10, 92-94.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Solution Combustion Synthesis of Nickel Oxide and Reduction to Ni Nano Particles for the Synthesis of Graphitic Carbon

Sandip V. Wakchaure[1] and Vilas R. Khairnarb[*2]

[1]Department of Chemistry, SGASc & GPC College, Shivlem Maharashtra, India

[*2]Department of Chemistry, Birla College of Arts Science and Commerce, Kalyan, Maharashtra, India.

ABSTRACT

Synthesis of nano carbon and it's different form like CNT, graphite, graphene DLC is popular topic of research now a days due to their applications in various field such as catalysis, supercpacitors, gas sensors, bio-sensors etc. For the synthesis of graphite carbon; synthesis of Nickel naoparticles is an important step, which is used as catalyst. So in the present study our efforts were to synthesize NiO nanoparticles using solution combustion synthesis method and its reduction to Ni nanoparticles. NiO particles were prepared by using urea thermal decomposition technique. Aqueous solution of Ni (NO3)2.6H2O and urea with different molar ratio was heated to 400oC temperature gave NiO particles; which when reduced in H2 atmosphere at 600oC temperature for 2 hour produced Ni nano particles. Percentage yield of Ni nanoparticles was 8-10%. Graphitic carbon is synthesized by CVD at 800oC by using oil as precursor and Ni nanoparticle as a catalyst. The surface morphology and nature of graphitic carbon obtained was confirmed by its SEM and XRD study respectively.

Keywords: CVD, Solution combustion, Nickel oxide, Nano particles

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

A Robust Synthesis of 4-substituted 1, 5- Benzodiazepines With the aid of Silica Supported-P2O5 as a Recyclable Catalyst in Solvent-free Conditions

Popat M. Jadhav[1], Vijay P. Pagore[1], Shivaji B. Munde[1], Gautum B. Salve[1], Vivekanand B. Jadhav[1*]

[1]Department of Chemistry, Shri Muktanand College, Gangapur, Gangapur, Aurangabad, Maharashtra, India

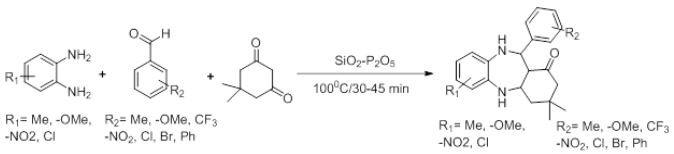
ABSTRACT

The possibility of using direct current electric conductivity measurement to study the solid state reactions involved in the preparation zinc oxide from zinc (II) maleate hydrated have been analyzed respectively. The study has been carried out in normal atmosphere. The steps corresponding to dehydration are well resolved in region from room temperature to 260 oC. The final product of decomposition in normal atmosphere was found to be ZnO from zinc maleate. The conductivity measurement was supplemented with the data obtained by chemical analysis; thermal analysis (TGA and DTG) and IR spectroscopy analysis.

Keywords : Thermal Decomposition; Electric Conductivity; Solid State; Maleate

Benzodiazepines have gathered name and fame in the market as they are one of the major constituents of drugs that are utilized for psychotropic, Anti-anxiety, Schizophrenia, Platelet-activating factor inhibitor and as Muscarinic receptor-M1 antagonist drugs. Benzodiazepine and their derivatives are one of the highly sold psychoactive drugs across the globe. In addition to this 1,5-benzodiazepines based fused ring system containing heterocyclic compounds has widened their medicinal utility as an antidepressant, anti-inflammatory, hypnotic, anticoagulant, antibacterial, analgesic and antiepileptic agents. All these pharmaceutical importance has the triggered notable attention from the researchers worldwide & a careful literature survey reveals numerous approaches for the synthesis of benzodiazepine but the majority of routes curtle down, their novelty due to use of a toxic catalyst, a toxic solvent, tedious workup procedure, a costlier catalyst with a longer reaction time and violating the environment.

Thus we thought of developing a facile route that can push back all these drawbacks and also meet with the concept of green chemistry also. Hence we have utilized a recyclable Silica supported-P_2O_5 as a novel catalyst for the three component reaction of dimedone, 1, 2-diamine and Aromatic Aldehyde in solvent-free conditions at 100^0C giving the product in an excellent amount of yield within 30-45 minutes of reaction time. Thus we have introduced an efficient, clean, solvent-free reaction protocol that uses a cheaper, environmentally benign, recyclable catalyst that can deliver the product with the excellent amount of yield within shorter reaction time period.



Scheme 1. Synthesis of 4-substituted 1, 5-Benzodiazepines

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Medicinal Chemistry

Prof. Farhana Ataurrahman
Ashoka College of Education, Nashik, Maharashtra, India

ABSTRACT

Medicinal chemistry in its most common practice focusing on small organic molecules encompasses synthetic organic chemistry and aspects of natural products and computational chemistry in close combination with chemical biology, enzymology and structural biology, together aiming at the discovery and development of new therapeutic agents. Medicinal chemistry is a specialised science that has evolved to encompass a broad range of disciplines concerned with the identification, synthesis and development of drug-like compounds for therapeutic use.

Keywords : Medicinal Chemistry, Structural Biology, ADME, Drug Design, Drug Discovery, Drug Targeting, Enzyme Inhibitor, Ligand Efficiency, Mechanism Of Action, Pharmacophore, Pharmacology, Pharmacokinetics

I. INTRODUCTION

Chemistry is the base for the branches of medicinal chemistry, biological chemistry and pharmaceutical chemistry. Chemistry is a basic broad science, embracing the concepts of creation of molecules and the manipulation of atoms. The role of chemistry in present day life is vast. It helps in the science of medicine. Chemical research goes hand in hand with medical research. Chemistry is an aid to agriculture. It covers interactions with plants, animals and humans through agriculture, biology and medicine and with the physical world through electronics, new building materials and new sources of energy.

Medicinal chemistry deals with the design, optimization and development of chemical compounds for use as drugs. It is inherently a multidisciplinary topic beginning with the synthesis of potential drugs followed by studies investigating their interactions with biological targets to understand the medicinal effects of the drug, its metabolism and side-effects. It needs a wide range of expertise, developed through years of training, dedication and learning from best practices in order to produce drugs that are good enough to enter clinical trials in patients.

- **Definition of medicinal chemistry:**

"Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the interest of the medicinal chemist is not restricted to drugs but include bioactive compounds in general. Medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of these drugs and related
compounds."

- **Areas covered under medicinal chemistry:**

- ✓ ADME("absorption, distribution, metabolism, and excretion,)
- ✓ Bioavailability
- ✓ Chemogenomics
- ✓ Drug class
- ✓ Drug delivery
- ✓ Drug design
- ✓ Drug discovery
- ✓ Drug targeting
- ✓ Enzyme inhibitor
- ✓ Ligand efficiency
- ✓ Mechanism of action
- ✓ Mode of action
- ✓ New chemical entity
- ✓ Pharmacodynamics
- ✓ Pharmacokinetics

- ✓ Pharmacology
- ✓ Pharmacophore
- ✓ Quantitative structure-activity relationship.

Medicinal chemists have an ever changing role in modern drug discovery. No longer are the days of simple synthesis; instead, complex synthetic methods and technologies such as combinatorial chemistry (combichem), microwave assisted organic synthesis (MAOS) and high-throughput (HTS) biological screening methods have evolved the daily life of a chemist. These new technologies are helping him to attain his goal much faster in the discovery process. Drugs must be designed, synthesized and purified successfully in order to aide in the first step of development. Medicinal chemist combines comprehensive knowledge of the synthetic chemistry, medicinal chemistry, and biology literature with the ability to drive the project forward. They are scientifically broad innovators who propose and work on all available drug targets.

- ➢ **Advances in Medicinal chemistry**:

Medicinal chemistry is by nature an interdisciplinary science, and practitioners have a strong background in organic chemistry, which must eventually be coupled with a broad understanding of biological concepts related to cellular drug target.In recent decades are increasing enormously due to pathogenesis of diseases. [1] Medicinal Chemistry helps in collaboration with scientific people in researching and developing new drugs. As a result drug discovery and drug research became predominant in role of controlling diseases with the discovery of powerful medicines like cytotoxic drugs, hypertensive drugs, antipyretic, anti-analgesic, anti HIV drugs, etc. Synthetic organic chemistry especially is involved in design, synthesis and development of drugs which are bio-active molecules. Synthesis and development is only the part of drug discovery, but the efficacy of that drug and target to the particular site is important for the treatment of diseases like cancer, Cardiovascular, pulmonary diseases, and these involve many factors.

[2] The world is getting better due to pharmaceutical industry. Today's life expectancy is the highest in human history. The quality of life is better than it was hundred years ago. In western countries, people no longer die of measles at the age of five, nor do we run of succumbing to pneumonia at age of 28. All these came along as a result of tremendous progress in medical sciences and pharmacology. Medicinal chemistry is the interdisciplinary science, with one having strong background in Organic chemistry coupled with the knowledge of biological concepts to deliver the required cellular targets. Medicinal chemistry in combating the diseases starts from preclinical studies, i.e., identification, drug design and development till the appropriate drug formulation is obtained. Then comes to clinical studies tested for efficacy of the compound and then comes into the treatment for combating the particular disease. Phytochemicals which are natural bioactive compounds found in various parts of plant vegetables, fruits possess wide range of potential chemical entities such as flavonoids, organo-sulphurs, polyphenols, catechin, isoflavones, carotenoids.

[3] Some of them were proved to reduce the risk of cardiovascular disease and considered as the lead structures for the cardiovascular drug design. Drugs were classified based on their antagonist properties of that particular disease. Serendipitous discovery sometimes led to the discovery of various prophylactic and therapeutic agents to combat diseases.

- ➢ **Applications**:

Chemical sciences have contributed significantly to the advancement of human civilization. With a growing understanding and ability to manipulate chemical molecules, the chemist is considered a societal problem solver. They play a significant role in the eradication of deadly diseases by developing life-saving pharmaceuticals and chemical pesticides. The chemical industry has been a vital sector of the modern industrialized economy and for the development in the Medicinal chemistry. Development of chemistry has revolutionized the basis of modern medicine. Application of chemistry from dyes to therapeutics has brought about incalculable benefits to humanity. Chemist is the magician who can turn waste products into things both beautiful and useful. Waste products like saw dust can be made to yield oil and acids. Marvelous indeed are the achievements of modern chemistry.

Medicinal chemists, especially in academia, are now increasingly involved in drug discovery and development process; they have become tremendously important in the innovation and discovery of drugs. Therefore, many industries are realizing this potentiality from academia and they are approaching for possible collaboration. In medical research the industry-academia cooperation often pair university (medicinal chemistry research) with industry (resources) for technology transfer to bring new medicines from bench to bedside. Such collaborations have and will continue to improve human health by facilitating novel and innovative drug molecules and treatments.

II. CONCLUSION

From all the above, Medicinal chemistry is concerned with the chemistry together the design, discovery and development of new pharmaceuticals. The discovery of new drugs is one of the most exciting and rapidly developing fields in science, and there is a growing need for safer, more effective pharmaceuticals against old diseases (like cancer), new ones (such as HIV/AIDS) and diseases that are becoming more widespread (such as malaria and tuberculosis). Medicinal chemistry looks at how to find drugs to combat these diseases, and how to make those drugs.

III. REFERENCES

[1]. Hughes, Jp; Rees, S; Kalindjian, Sb; Philpott, Kl (2011-03-01). "Principles of early drug discovery". British Journal of Pharmacology. 162 (6): 1239–1249. doi:10.1111/j.1476-5381.2010.01127.x. ISSN 1476-5381. PMC 3058157 . PMID 21091654.

[2]. Johnston, Kelly L.; Ford, Louise; Umareddy, Indira; Townson, Simon; Specht, Sabine; Pfarr, Kenneth; Hoerauf, Achim; Altmeyer, Ralf; Taylor, Mark J. (2014-12-01). "Repurposing of approved drugs from the human pharmacopoeia to target Wolbachia endosymbionts of onchocerciasis and lymphatic filariasis". International Journal for Parasitology: Drugs and Drug Resistance. Includes articles from two meetings: "Anthelmintics: From Discovery to Resistance", pp. 218--315, and "Global Challenges for New Drug Discovery Against Tropical Parasitic Diseases", pp. 316--357. 4 (3): 278–286. doi:10.1016/j.ijpddr.2014.09.001. PMC 4266796 . PMID 25516838.

[3]. Harvey, Alan L. (2008-10-01). "Natural products in drug discovery". Drug Discovery Today. 13 (19–20): 894–901. doi:10.1016/j.drudis.2008.07.004. PMID 18691670.

[4]. Cragg, Gordon M.; Newman, David J. (2013-06-01). "Natural products: A continuing source of novel drug leads". Biochimica et Biophysica Acta (BBA) - General Subjects. 1830 (6): 3670–3695. doi:10.1016/j.bbagen.2013.02.008. PMC 3672862 . PMID 23428572.

[5]. "Careers for 2003 and Beyond: Medicinal Chemistry". Chemical & Engineering News. 81 (25): 53–54, 56.

[6]. Bishop T, Sham P (2000) Analysis of Multifactorial Diseases. Academic Press, New York. pp: 1-320.

[7]. Vasanthi HR, ShrishriMal N, Das DK (2012) Phytochemicals from plants to combat cardiovascular disease. Current Med Chem 19: 2242-2251.

[8]. Joshi SD, Aminabhavi TM (2016) QSAR and 3D-QSAR in Drug Design Anti- Tubercular Drug Discovery Studies. J Pharma Care Health Sys 3: 1-2.

[9]. Yadav JS, Reddy BVS, Parimala G (2002) Indium tribromide catalyzed highly region-selective ring opening of epoxides and aziridines with pyrrole.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Natural Product

Prof. Madhuri Santu Shete
Ashoka College of Education, Nashik, Maharashtra, India

ABSTRACT

Natural products are small molecules produced naturally by any organism including primary and secondary metabolites. They include very small molecules, such as urea, and complex structures, such as Taxol. As they may only be isolable in small quantities, have interesting biological activity and chemical structures, natural product synthesis poses an interesting challenge in organic chemistry.

Keywords : Taxol, Chemical Structures, Organic Chemistry, Natural Product , Terpenoids, Alkaloids, Acyclic Monoterpenes, Monocyclic Monoterpenes, Bicyclic Monoterpenes, Acyclic Monoterpenes

I. INTRODUCTION

A **natural product** is a chemical compound or substance produced by a living organism that is, found in nature. In the broadest sense, natural products include any substance produced by life. Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. Natural products containing no additives or chemicals can easily be integrated into our beauty routine. Natural products sometimes have therapeutic benefit as traditional medicines for treating diseases, yielding knowledge to derive active components as lead compounds for drug discovery.

Classification of Natural Product:

- ✓ Terpenoids
- ✓ Alkaloids

Terpenoids

Definition: Any of a large class of organic compounds including terpenes, diterpenes, and sesquiterpenes. They have unsaturated molecules composed of linked isoprene units, generally having the formula (C_5H_8) *n*.

The **terpenoids**, sometimes called **isoprenoids**, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways.

Classification:

- ✓ Acyclic Monoterpenes
- ✓ Monocyclic Monoterpenes
- ✓ Bicyclic Monoterpenes
- ✓ Acyclic Monoterpenes

Biosynthetically, isopentenyl pyrophosphate and dimethylallyl pyrophosphate are combined to form geranyl pyrophosphate. Elimination of the pyrophosphate group leads to the formation of acyclic monoterpenes such as ocimene and the myrcenes. Hydrolysis of the phosphate groups leads to the prototypical acyclic monoterpenoid geraniol.

- **Citral :** Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is either a pair, or a mixture of terpenoids with the molecular formula $C_{10}H_{16}O$. The two compounds are double bond isomers. The *E*-isomer is known as geranial or citral A. The *Z*-isomer is known as neral or citral B.
- **Occurrence** : Citral is present in the oils of several plants, including lemon myrtle (90–98%), *Litsea citrata* (90%), *Litsea cubeba* (70–85%), lemongrass (65–85%), lemon tea-tree (70–80%), *Ocimum gratissimum* (66.5%), *Lindera citriodora* (about 65%), *Calypranthes parriculata* (about 62%), petitgrain (36%), lemon verbena (30–35%), lemon ironbark (26%), lemon balm (11%), lime (6–9%), lemon (2–5%), and orange.
- **Uses** : Geranial has a strong lemon (citrus) odor. Neral's lemon odor is less intense, but sweeter.

Citral is therefore an aroma compound used in perfumery for its citrus effect. Citral is also used as a flavor and for fortifying lemon oil. It also has strong antimicrobial qualities, and pheromonal effects in insects.

- **Health and safety information:** Two studies have shown 1–1.7% of people to be allergic to citral, and allergies are frequently reported. Citral on its own is strongly sensitizing to allergies; the International Fragrance Association recommends that citral only be used in association with substances that prevent a sensitizing effect. Citral has been extensively tested and has no known genotoxicity, and no known carcinogenic effect, but animal tests show dose-dependent effects on the kidneys.

➢ ***Monocyclic Monoterpenes:***

In addition to linear attachments, the isoprene units can make connections to form rings. The most common ring size in monoterpenes is a six-membered ring. A classic example is the cyclization of geranyl pyrophosphate to form limonene. The terpinenes, phellandrenes, and terpinolene are formed similarly. Hydroxylation of any of these compounds followed by dehydration can lead to the aromatic p-cymene. Important terpenoids derived from monocyclic terpenes are menthol, thymol, carvacrol and many others.

- Terpineol : Terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics, and flavors. α-Terpineol is one of the two most abundant aroma constituents of lapsang souchong tea; the α-terpineol originates in the pine smoke used to dry the tea. (+)-α-Terpineol is a chemical constituent of skullcap.
- **Uses:** Terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics, and flavors. α-Terpineol is one of the two most abundant aroma constituents of lapsang souchong tea; the α-terpineol originates in the pine smoke used to dry the tea
- **Benefits:** Terpineol is antibacterial and antiviral, an immune system stimulant, a good general tonic. In Essential Oils it is warming and uplifting.

➢ ***Bicyclic Monoterpenes***

Geranyl pyrophosphate can also undergo two sequential cyclization reactions to form bicyclic monoterpenes, such as pinene which is the primary constituent of pine resin.Camphor, borneol and eucalyptol are examples of bicyclic monoterpenoids containing ketone, alcohol, and ether functional groups, respectively.

❖ **Camphor** : Camphor is a waxy, flammable, white or transparent solid with a strong aroma. It is a terpenoids with the chemical formula $C_{10}H_{16}O$. It is found in the wood of the **camphor laurel** (*Cinnamomum camphora*),

- **Physical uses:**

II. Explosives

Camphor is used as a plasticizer for nitrocellulose, an ingredient for fireworks and explosive munitions. During the late 19th Century, as Western manufacturers developed machine guns and other rapid fire ordnance, it became imperative to reduce the smoke that obscured battlefields and revealed hidden gun emplacements.

III. Pest deterrent and preservative

Camphor is believed to be toxic to insects and is thus sometimes used as a repellent. Camphor is used as an alternative to mothballs. Camphor crystals are sometimes used to prevent damage to insect collections by other small insects. It is kept in clothes used on special occasions and festivals, and also in cupboard corners as a cockroach repellent.

- ***Medicinal uses:***

Camphor is readily absorbed through the skin, where it selectively stimulates nerve endings sensitive to cold, producing a warm sensation when vigorously applied, or a cool sensation when applied gently. This effect also induces a slight local anaesthesia, and has an antimicrobial secondary effect. These effects are particularly noticeable in the lungs and airways if camphor is inhaled as an aerosol.

- ***Traditional uses:***

Camphor has been used in traditional medicine from time immemorial in countries where it was native. It was probably the odour of the substance and its decongestant effect that led to its use in medicine.

Camphor was used in ancient Sumatra to treat sprains, swellings, and inflammation. It has long been used as a medical substance in ancient India, where it generally goes by the name Karpūra. It has been described in the 7th-century Āyurvedic work Mādhavacikitsā as being an effective drug used for the treatment of fever. The plant has also been named Hima and has been identified with the plant Cinnamomum camphora. According to the Vaidyaka-śabda-sindhu, it is one of the "five flavours" used in betel-chewing, where it is also referred to as Candrabhasma ('moon powder').

- ***Modern uses:***

Camphor was a component of paregoric, an opium/camphor tincture developed in the 18th century. Paregoric was used in various formulations for hundreds of years. It was a household remedy in the 18th and 19th centuries when it was widely used to control diarrhea in adults and children, as an expectorant and cough medicine, to calm fretful children, and to rub on the gums to counteract the pain from teething. Today the main use of camphor is as a cough suppressant and as a decongestant. It is an active ingredient (along with menthol) in vapour-steam decongestant products, such as Vicks VapoRub.

- **Alkaloids**:

Alkaloids are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids.

More recent classifications are based on similarity of the carbon skeleton (*e.g.*, indole-, isoquinoline-, and pyridine-like) or biochemical precursor (ornithine, lysine, tyrosine, tryptophan, etc.)

Alkaloids are often divided into the following major groups:

1. "True alkaloids" contain nitrogen in the heterocycle and originate from amino acids. Their characteristic examples are atropine, nicotine, and morphine.
2. "Protoalkaloids", which contain nitrogen and also originate from amino acids. Examples include mescaline, adrenaline and ephedrine.
3. Polyamine alkaloids – derivatives of putrescine, spermidine, and spermine.
4. Peptide and cyclopeptide alkaloids.
5. Pseudoalkaloids – alkaloid-like compounds that do not originate from amino acids. This group includes terpene-like and steroid-like alkaloids, as well as purine-like alkaloids such as caffeine, theobromine, theacrine and theophylline.

IV. CONCLUSION

The proposed payment system combines the Iris recognition with the visual cryptography by which customer data privacy can be obtained and prevents theft through phishing attack [8]. This method provides best for legitimate user identification. This method can also be implemented in computers using external iris recognition devices.

V. REFERENCES

[1]. Wikipedia, https://en.wikipedia.org/wiki/Terpineol
[2]. Beneforce, http://beneforce.com/Constituent/Constituent-pages/terpineol.htm
[3]. Record of alpha-Pinen in the GESTIS Substance Database of the Institute for Occupational Safety and Health, accessed on 07-January-2016
[4]. https://en.wikipedia.org/wiki/Alkaloid.
[5]. https://en.wikipedia.org/wiki/Terpene.
[6]. Record of beta-Pinen in the GESTIS Substance Database of the Institute for Occupational Safety and Health, accessed on 07-January-2016
[7]. Lincoln, D.E., B.M. Lawrence. 1984. The volatile constituents of camphorweed, Heterotheca subaxillar
[8]. https://sensiseeds.com/en/blog/medicinal-properties-terpenes-terpenoids.

© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Green Chemistry

Prof. Vinayak Ikade
Ashoka College of Education, Nashik, Maharashtra, India

ABSTRACT

Green chemistry is the new and rapid emerging branch of chemistry for the environment. It is really a philosophy and way of thinking that can help chemistry in research and production to develop more eco-friendly solutions. Green chemistry is a science based non-regulatory and economically driven approach to achieve the goals of environmental protection and sustainable development. All chemical wastes should be disposed of in the best possible manner without causing any damage to the environment and living beings. Another way to save the environment through sustainable chemistry is to make use of renewable food stocks. This article presents selected examples of implementation of green chemistry principles in everyday life, in industry and in domestic purpose.

Keywords : Green Chemistry. Eco-Friendly Solutions. Environmental Protection Agency, Geothermal Energy, PERC

I. INTRODUCTION

The idea of green chemistry was initially developed as a response to the Pollution Prevention Act of 1990, which declared that U.S. national policy should eliminate pollution by improved design (including cost-effective changes in products, processes, use of raw materials, and recycling) instead of treatment and disposal. Although the U.S. Environmental Protection Agency (EPA implemented green chemistry program. Paul T Anastas for the first time in 1991 coined term green chemistry**.**

The mid-to-late 1990s saw an increase in the number of international meetings devoted to green chemistry, such as the Gordon Research Conferences on Green Chemistry, and green chemistry networks developed in the United States, the United Kingdom, Spain, and Italy.

The 12 Principles of Green Chemistry were published in 1998, providing the new field with a clear set of guidelines for further development (1). In 1999, the Royal Society of Chemistry launched its journal Green Chemistry.

In 2005 Nobel Prize for Chemistry awarded to Chauvin, Grubbs, and Schrock, which commended their work as "a great step forward for Green Chemistry is the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances.

The green chemistry approach seeks to redesign the materials that make up the basis of our society and our economy including the materials that generate, store, and transport our energy—in ways that are benign for humans and the environment and possess intrinsic sustainability.

The concepts and practice of Green Chemistry have developed over nearly 20 years into a globe-spanning endeavor aimed at meeting the ''triple bottom line'' sustainability in economic, social, and environmental performance.

- **Definition:**

"Green chemistry, also called **sustainable chemistry**, is an area of chemistry and chemical engineering focused on the designing of products and processes that minimize the use and generation of hazardous substances."

- **Principle of green chemistry:**

1. Prevention: Prioritize the prevention of waste over clean-up and treatment once it's Created

2. Atom Economy: Synthesis methods are designed to maximize the incorporation of all Materials used in the process into the final product
3. Less Hazardous Chemical Synthesis: Design chemical reactions and synthetic routes to be as safe as possible for Human health and the environment
4. Designing Safer Chemicals: Chemical products should be designed to affect their desired function While minimizing their toxicity
5. Safer Solvents and Auxiliaries: The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary whenever possible and innocuous when used
6. Design for Energy Efficiency: Choose the least energy-intensive chemical route. Avoid heating/cooling and Pressurized/vacuum conditions (i.e. ambient temperature & pressure are Optimal)
7. Use of Renewable Feedstock's: Use chemicals which are made from renewable (i.e. plant-based) sources rather than other, equivalent chemicals originating from petrochemical sources.
8. Reduce Derivatives: Minimize the use of temporary derivatives such as protecting groups. Avoid derivatives to reduce reaction steps, resources required, and waste created
9. Catalysis: Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Design for Degradation: Products should be designed so that at the end of their function they break down into innocuous products and do not persist in the environment
11. Real Time Pollution Prevention: Monitor chemical reactions in real-time as they occur to prevent the formation and release of any potentially hazardous and polluting substances
12. Inherently Safer Chemistry for Accident Prevention: Substances, and the form of a substance used in a chemical process, should be chosen to minimize the potential for chemical accidents including releases, explosions, and fires.

- **Application of green chemistry:**

Agriculture application:

Some industries co-operate to make better use of energy. For example, the production of ammonia generates both waste heat and carbon dioxide, both derived from fossil fuel. One UK manufacturer pipes these to large commercial tomato greenhouses, greatly extending the season during which the plants may be grown economically.

Geothermal Energy

In geothermal power stations super-heated steam generated deep underground when water comes into contact with heated rock or magma from the earth's mantle is extracted through a series of boreholes and piped into a turbine, where the steam is used to generate electricity.

Small amounts of carbon dioxide and other gases such as hydrogen sulphide are emitted from the geothermal areas. In one area in Iceland, the gases from a power plant are piped to an adjacent installation where carbon dioxide is separated from other non-condensable gases and used as an input to a process, where hydrogen and carbon dioxide are passed over a solid catalyst under high pressure to produce renewable methanol. The hydrogen is made by electrolysis of water using electricity from hydro and geothermal power sources. This green methanol can be blended directly with standard petrol or can be used in esterification of vegetable oil or animal fats to produce biodiesel (Fatty Acid Methyl Ester).

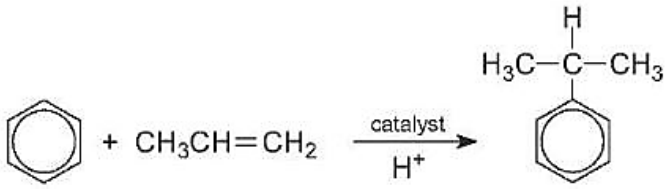
Green chemistry in day to day life:

Green Dry Cleaning of Clothes Perchloroethylene (PERC), Cl2C═CCl2 is commonly being used as a solvent for dry cleaning. It is now known that PERC contaminates ground water and is a suspected carcinogen. A technology, known as Micell technology developed by Joseph De Simons, Timothy Romark, and James McClain made use of liquid CO2 and a surfactant for dry cleaning clothes, thereby replacing PERC. Dry cleaning machines have now been developed using this technique. Micell Technology8has also evolved a metal cleaning system that uses CO2 and a surfactant thereby eliminating the need of halogenated solvents.

Catalysis

Aluminium chloride was used for many years in the production of alkylbenzene sulfonates, an active surfactant in many detergents. The aluminium chloride

was needed to effect the reaction between benzene and a long chain alkene. The aluminium chloride could not be recycled and became waste as aluminium hydroxide and oxide. Now a solid zeolite catalyst with acid groups is used and can be reused time and time again with no waste products. The zeolite is more environmentally friendly as the effluent is much cleaner and lower temperatures and pressures can be used.



II. CONCLUSION

Green Chemistry is new philosophical approach that through application and extension of the principles of green chemistry can contribute to sustainable development. Presently it is easy to find in the literature many interesting examples of the use of green chemistry rules. Great efforts are still undertaken to design an ideal process that start from non-polluting materials. It is clear that the challenge for the future chemical industry is based on safer products and processes designed by utilizing new ideas in fundamental research. Furthermore, the success of green chemistry depends on the training and education of a new generation of chemists. Students at all levels have to be introduced to the philosophy and practice of green chemistry.

III. REFERENCES

[1]. Principles of Green Chemistry
[2]. https://www.acs.org/content/acs/en/greenchemistry/what--is--green--chemistry/principles/12--
[3]. principles--of--green--chemistry.html
[4]. The Twelve Principles of Green Chemistry: What it is, & Why it Matters
[5]. http://www.compoundchem.com/2015/09/24/green--chemistry/
[6]. The original list of hazardous air pollutants (HAP)
[7]. https://www3.epa.gov/airtoxics/188polls.html
[8]. List of controlled ozone depleting substances (ODSs)
[9]. https://ftp.dot.state.tx.us/pub/txdot-info/gsd/pdf/listofco.pdf
[10]. Candidate list of substances of very high concern for authorization
[11]. https://echa.europa.eu
[12]. Volatile organic compounds
[13]. https://www.atsdr.cdc.gov/substances/toxchemicallisting.asp?sysid=7
[14]. Green Chemistry & Commerce Council's report
[15]. http://asbcouncil.org/sustainability--reports#.WV53L7pFw2w

Organised by

Department of Chemistry
AGASTI ARTS, COMMERCE AND DADASAHEB
RUPWATE SCIENCE COLLEGE
Akole District, Ahmednagar-422601, Maharashtra, India

Publisher

Technoscience Academy

Website : www.technoscienceacademy.com
Email: info@technoscienceacademy.com