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Synthesis & Characterization of Pectin - N-isopropyl acrylamide modified graft co-polymers for potential Applications

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ABSTRACT

Recently research is being carried out for the development of various copolymers, modified polymers for various potential applications like coatings, composite, paints, adhesives, drug delivery systems, hydrogel-based delivery devices can be used for oral, rectal, ocular, epidermal and subcutaneous application. tablet coatings, etc.[1] In this work we tried to improve the properties of the polysaccharide based polymers pectin, by graft copolymerization with N-isopropylacrylamide polymers using emulsion polymerization technique. The effect of various variables like initiator concentration, monomer concentration, temperature and time has been studied. The grafted copolymer was characterized by Fourier transform infrared spectroscopy (FT-IR), organic elemental analysis, differential scanning calorimetry (DSC) and X-ray diffraction (XRD). FT-IR studies indicated incorporation of amide group. Rheological behavior of pectin solution was compared with that of the grafted copolymer. The comparative rheological properties of pectin and grafted copolymer indicated change in the property of the product. Differential scanning calorimetry and XRD suggested formation of the grafted copolymer.

Keywords: Graft Copolymerization, Pectin, N-Isopropyl Acrylamide, Rheology, Differential Scanning Calorimetry.

I. INTRODUCTION

A graft copolymer is a system comprised of a backbone material to which a second polymer is attached at intervals along the chain to make them into synthesized modifications and synthesized polymer gel networks to form natural reforming different attractive properties like pH sensitivity, Thermo sensitivity, light sensitivity etc. This gives technological advances in various application and potential benefits. Pectin is one of the natural heterogeneous biocompatible, biodegradable, and abundant. polysaccharide containing linear chains of a-(1-4)-linked- D-galacturonic acid residues.[2]

In our study, we have grafted N-isopropyl acrylamide on pectin to improve its properties like gel strength, film forming ability, pH sensitive behavior, thermal sensitivity and to make it more shear stable so that it can finds its potent application. Pectin graft copolymer is modified natural polymeric materials that have a three-dimensional network structure and can swell considerably in aqueous medium without dissolution after crosslinking.[2]. Swelling behavior of hydrogel is control by cross-linking by varying concentration of cross-linking agent. The grafted copolymer(GP) was characterized by Fourier transform infrared spectroscopy (FT-IR), organic elemental analysis, differential scanning calorimetry (DSC) and X-ray diffraction (XRD). FT-IR studies indicated incorporation of amide group. Rheological behavior of pectin solution was compared with that of the grafted copolymer. The comparative rheological properties of pectin and grafted copolymer indicated change in the property of the product. Differential scanning calorimetry and XRD suggested formation of the grafted copolymer.

II. MATERIALS AND METHODS

A. MATERIALS

Pectin, methanol, and glutaraldehyde **(GA)** were obtained from Loba-Chemie Indoaustranal Co., Mumbai, India.

N-isopropyl acryl amide is obtained from SRL research laboratories, Mumbai, India. Hydrochloric acid 35%

pure was obtained from Merck Limited, Mumbai, India. Double distilled water was used throughout the study. Commercial pectin was purified and converted in to H-form by washing with 0.1mol/1 HCl dissolved in the ethanol water mixture (1:1 v/v). Pectin subsequently was washed several times with ethanol water mixture fallowed by 96% ethanol until the chloride reaction was negative, and finally dried at 60^0C.

B. METHODS

1. Synthesis of modified Pectinand Purification ofgraft-copolymer from homo-polymer(Poly-N-isopropylacrylamide)

The reaction has been done according to method of (Sinitsya et. al. 2000). The reaction was carried out in heterogeneous medium with methanol as a solvent. Pectin powder (3 gram) was weighed in a well equipped 250 ml three necked flask and it was suspended in 70 ml methanol. An amount of 10 ml N-isopropyl acrylamide was get dissolved in a 50 ml methanol and the solution was added gradually in the flask under stirring. The reaction has been carried out at 25^o C temperature until 48 hours of continuous stirring. After completion of the reaction the product was obtained in the powder form by simple filtration method. The product obtained is converted in to acid forms by washing with 0.1M HCl in an ethanol- water mixture (1:1,v/v) to convert free carboxylic group in to protonated form. Finally the sample were washed several times with 40% (v/v) ethanol until it shows the negative reaction to chloride, then treated with 80% (v/v) ethanol, filtered and dried at 60^0C. This Graft-copolymer (GP) is used for the preparation of hydrogel membranes using glutaraldehyde(GA) as crosslinking agent. Theprobable mechanismofthereaction isshowninFig.1.Theproduct waspurified byextracting thehomo-polymer of Poly-N-isopropyl acrylamide (that might be produced during the polymerization)from the crude product by washing with acetone-watermixture (30:70).The procedure was repeated10times.Thepuregraftpolymer(GP)soobtained wasfinallywashedwithpureacetone and wasallowedto dryinlaminar flowairdrierfor72hatroomtemperature.The percentage grafting (PG) and grafting efficiency (GE) were calculated according to following formula Table1.[3]

Table 1. Formula for calculation of percentage grafting (%PG) andgrafting efficiency (%GE)

$$GE\ \% = \frac{\text{Weight of the grafted side chain polymer}}{\text{Weight of the grafted side chain polymer} + \text{Homo-polymer}} \times 100$$

$$PG\ \% = \frac{\text{Weight of the grafted side co- polymer} - \text{Weight of pectin}}{\text{Weight of Pectin}} \times 100$$

2. Reaction Mechanism

Graft copolymers are synthesized by opening the monomer rings of polysaccharide backbone and grafting of N-isopropyl acrylamide onto the free radicals generated in emulsion. The opening imparts slight flexibility to the backbone. Moreover, the percentage of polysaccharide is small in comparison with the poly vinyl alcohol.The radical formation occurs by breaking of carbon- carbon bond and monomer polymerization reaction occurs.When the Ce^{+4} reacts with C_2 and C_3 carbon atom, it gives radical formation via breaking the C-C bond, immediately monomer adds to the radical and chain polymerization reaction occurs.[3] Probable reaction mechanism is given in Figure 1.[4]

3. Swelling behavior study of the GP hydrogels

Preparation of graft copolymer (GP) cross-linked with glutaraldehyde(GA) hydrogel and Swelling study

The GP/GA hydrogel cross-linked with different concentration of GA used in the study were fabricated using a casting/solvent evaporation technique.[5] A stock viscous solution of GP in water (10% w/v) was prepared by dissolving 2g of GP in 20ml of distilled water and stirring for 7hrs. at room temperature.To the dissolved GP solution 0.2, 0.4, 0.6, 0.8 ml of glutaraldehyde was added and each solution was acidified with a drop of HCL . Those solution was heated at 70^0C for 20 min to complete the crosslinking reaction.The thick cross-linked gel was finally obtained further sonicated to remove the trapped air bubbles and used for study.[6]

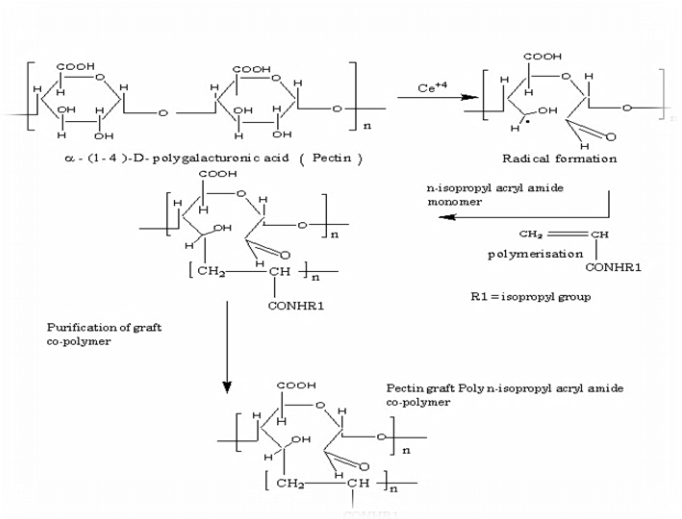

Figure 1. Probable Reaction Mechanism of polymerization Reaction

The film was made from prepared gel by pouring it into shallow dishes (with diameter 8.5 cm) and dried in laminar flow air chamber at room temperature for 3 days. Finally, the cross-linked GP films were thoroughly rinsed with distilled water to remove residual GA. After drying in air, the cross-linked GP films(0.15 mm thickness) were cut into small disks (with diameter of ~9 mm) and used for swelling study.The swelling characteristics of test hydrogels were determined by immersing dried test samples to swell in 5 ml of a phosphate buffer solutions at pH 1.4, 5.4, 7.4, simulating gastrointestinal tract conditions [7-9] and 9.4 solutions for 24 hours. At specific time intervals, the samples were removed from the swelling medium and were carefully blotted with a piece of paper towel to absorb excess water on the surfaces. The % swelling (% Sw) of test samples were calculated from the following expression.

$$\% Sw = (Ws - W_d) / W_d \times 100$$

Where `Ws' is the weight of the swollen test sample and `Wd' is the weight of the dried test sample. The sample, which had the best swelling characteristics, was subsequently selected for the salicylic acid release profile study.

4. Characterization

Characterization were done at outsourced laboratory. Pectin, N-isopropylacrylamide and GP were subjected to FTIR spectroscopy in the range of 4000-400 cm^{-1} as KBr pellets and the patches were subjected to Attenuated total reflectance (ATR) spectroscopy in the range of 4000-400 cm^{-1}. An FTIR spectrophotometer (NEXUS-870, Thermo Nicolet Corporation) was used for the study. The raw materials and the GP were subjected to X-ray diffraction (XRD-PW 1700, Philips, USA) using CuKα radiation generated at 40 kV and 40 mA; the range of diffraction angle was 10.00-100.00^0 2θ. A Netzsch DSC-200 PC Phox, Germany was used for studying the melting and crystallization behavior of the polymeric materials. The temperature and energy scales were calibrated with the standard procedures. The melting studies were performed in the temperature range of 25-200^{0}C at a heating rate of 10_C/min in N2 atmosphere. For the study of viscoelastic properties GP gel without cross-linked was subjected to Rheometer (Advance Rheometer AR 1000, TA Instrument, England.) with nip gap was 600μ.

III. RESULTS AND DISCUSSION

3.A. EffectofInitiator Concentration

It is evident from **Figure 2** that percentage-grafting increaseduptocertainlevelofinitiator concentrationand thendecreased.Themaximumpercentagegraftinghasbeen observedat[CAS]¼0.006molel^{-1}.Ithasbeenpresumed that up to the critical initiator concentration,the entire radicalproducedfromtheinitiatorareusedin producing growing monomer radicals as well as homo-polymer radicals.Afterthislimit,theradicalsaremostlyinvolved in recombinationandothertermination processesandhence decreaseinpercentagegraftingandgraftingefficiency.

3.B. EffectofReactionTime

The percentage grafting and grafting efficiencygraduallyincreasedwithtimeandthenleveledoff(Fig.3).This result may beattributedto the fact that the freeradical formed initially on the polymeric backbone contribute moreforgrafting reaction, whereaswithincreaseofsome of the free radicals and the macro radicals might be involvedinthehomo-polymerformation.**NAM**(N-isopropyl acrylamide).

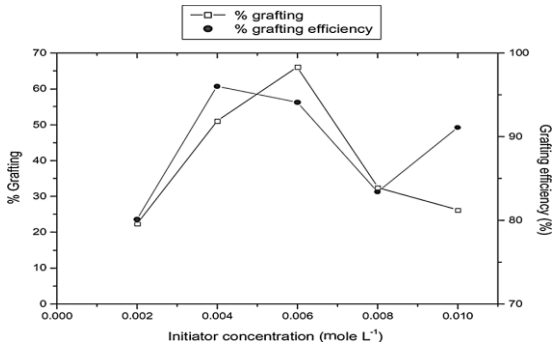

Figure 2. Effect of initiator concentration on percentage grafting of **NAM**(N-isopropyl acrylamide) on pectin; reaction conditions were pectin (2 g), acrylamide (0.440 mole l—1), time (150 min), and temperature (35oC).

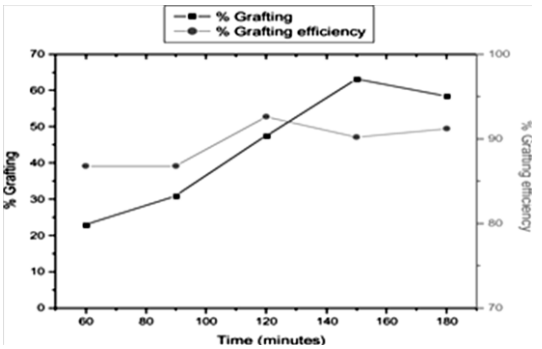

Figure 3.Effectofreaction timeonpercentage grafting ofNAMonpectin;reaction conditions werepectin (2g),acrylamide(0.440mole l^{-1}), CAS(0.006molel $^{-1}$),andtemperature(35^{o}C).

3.C. Effect of Monomer Concentration

Figure.5 shows that, with increase in monomer concentration the percentage grafting and grafting efficiency gradually increased and then decreased after a monomer concentration of 0.44 mole l—1. The decrease in grafting efficiency may be attributed to the participation of initiator radicals in graft copolymerization rather than homo polymerization. As the monomer concentration is increased, more monomer units are competing for the initiator radicals resulting in the increased rate of homo polymerization. Hence, there was decrease in percentage grafting and grafting efficiency.[9]

3.D. Effect of Reaction Temperature

It was observed that with increase in the temperature of grafting reaction, the percentage grafting and grafting efficiency increased up to certain level and then decreased (Fig. 6). It was established that more grafting sites would be created by frequent chain transfer of growing radicals to the backbone at higher temperature resulting in an increase in percent grafting and grafting efficiency. Further increase in temperature decreased the percentage grafting and grafting efficiency, this fact can be attributed to involvement of growing radicals in termination processes. Increase in temperature not only facilitated the chain transfer process, but also accelerated homo polymer formation thereby decreasing the grafting efficiency.[9-11]

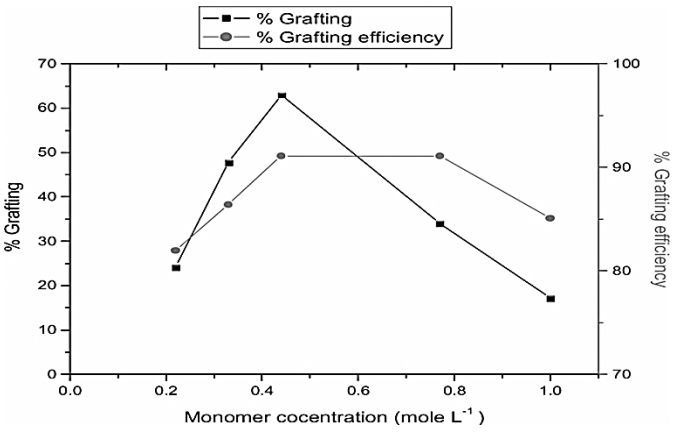

FIG. 6. Effect of reaction temperature on percentage grafting of NAM on pectin; reaction conditions were pectin (2 g), acrylamide (0.440 mole l^{-1}), CAS (0.006 mole l^{-1}), and time (150 min).

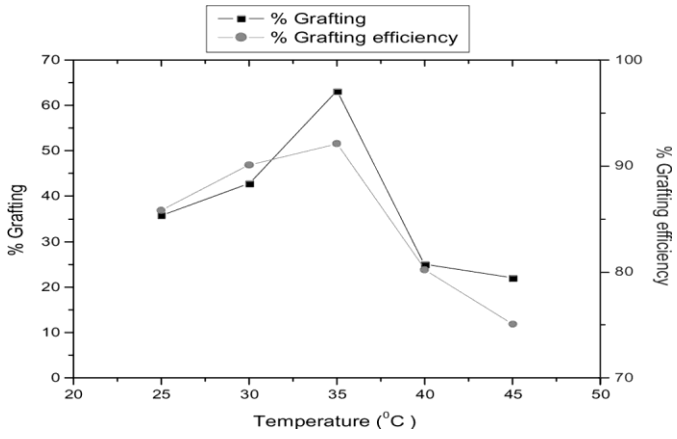

FIG. 5. Effect of monomer concentration on percentage grafting of NAM on pectin; reaction conditions were pectin (2 g), CAS (0.006 mole l^{-1}), Time (150 min) and temperature (35°C).

3.E. FTIR Characterization

The FTIR spectrum of GP and pectin were taken in the range of 4000 - 400 cm^{-1} as KBr pellet and Attenuated total reflectance (ATR) with the help of FTIR spectrophotometer (NEXUS - 870,Thermo Nicolet Corporation).

Figure 7.a. shown the FTIR spectra of the pure pectin. As the spectrum shown a broad band at 3415 cm^{-1} due to stretching frequency of the -OH groups. The band at 2913 cm^{-1} is due to -C-H stretching vibration. The presence of a strong absorption band at 1756 cm^{-1} due to >C=O stretching vibrations confirms the presence of -$COOCH_3$ group. The bands around 1441 cm^{-1} and 1342 cm^{-1} are assigned to -CH_2scissoring and -OH bending vibration, respectively. The band at 1023 cm^{-1} is due to -CH-O-CH- stretching. The broad band around 1150 cm^{-1} is due to characteristic peak of -CH-OH in aliphatic cyclic secondary alcohol C-O stretch.

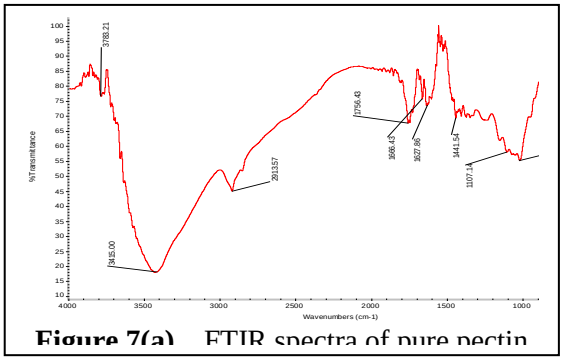

Figure 7(a) FTIR spectra of pure pectin

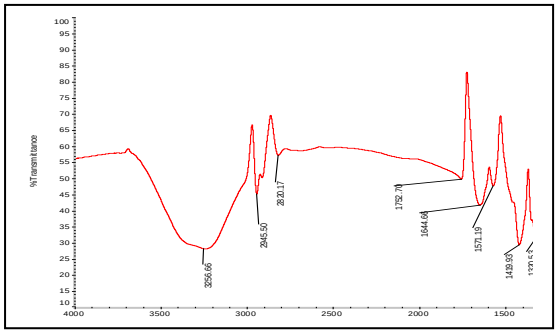

Figure 7b. shows FTIR spectra of graft copolymer (GP). presence of a broad absorption band around 3200 -3260 cm^{-1} is due to the overlap of -OH stretching band of pectin. The presence of a band at 1752 cm^{-1} is due to free acid groups (-COOH). The sharp band at 2937 cm^{-1} and 2910 cm^{-1} peaks observed due to C-H stretching frequency of -CH_2 groups. The band at 1419 cm^{-1} is due to -CH bend of -CH_2 group. The band at 1085 cm^{-1} shows -OH bending, 1041 cm^{-1} shown secondary alcohol(characteristic peak of -CH-OH in cyclic alcohol C-O stretch). The presence of all above bands in the graft copolymer gives strong evidence of grafting. Pectin FTIR shows1737 cm^{-1} C=O stretch of ester, acid or aldehyde, 1187 cm^{-1} C-O stretch ester, 2937 cm^{-1} -CH_2 - methylene stretch, 1449 cm^{-1} -CH_2 - methylene scissors deformation, 1085 cm^{-1} C-O stretch, and GP shows polymer backbone2829 cm^{-1} C-H stretch, 3200-3400 cm^{-1} OH stretch, 1409 cm^{-1}, 1571NH bends, 1321, 613 cm^{-1} NH deformation this spectra show formation of expected copolymer.

3.6. X-ray diffraction study (XRD)

The pectin sample was finely powdered and film sample of GP were subjected to X-ray diffraction (XRD-PW 1700, Philips, USA) using CuKα radiation generated at 40 kV and 40 mA; the range of diffraction angle was 10^o-100^o 2θ. The XRD patterns of pectin **(Figure 8a)** and GP **(Figure 8b)** revealed that the pectin peak was at ~20.10^o 2θ while that of pectin was ~13.58^o 2θ. The XRD patterns of the GP shown noisy with diffused peaks pattern revealed that the amorphous nature of graft copolymer and the crystalinity of the GP disrupt slightly because of the grafted backbone of poly n-isopropyl acryl amide. There had been a marked decrease in crystalinity of GP as compared to that of parent pectin.

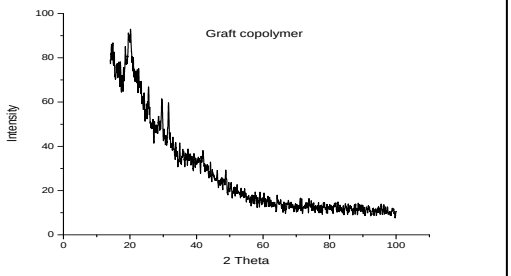

Fig.8a. Graft copolymer (GP)

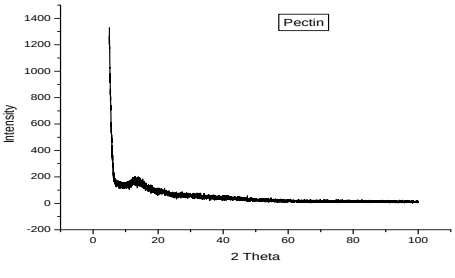

Fig.8b. Low methylated pure Pectin

3.G. Differential Scanning Calorimetry Characterizations (DSC)

In differential scanning calorimetry characterization. A 11.21 mg pectin and 7.13 mg GP sample was placed in a 100 µL aluminum pan, weighed on a microbalance and crimped with a lid containing a pinhole and placed in the DSC unit along with a similar pan as a reference. The sample was heated at a rate of 10 ◦C/min from -20 to 200^{0}C. Nitrogen was used as a purge gas with a flow rate of 50 mL/min.

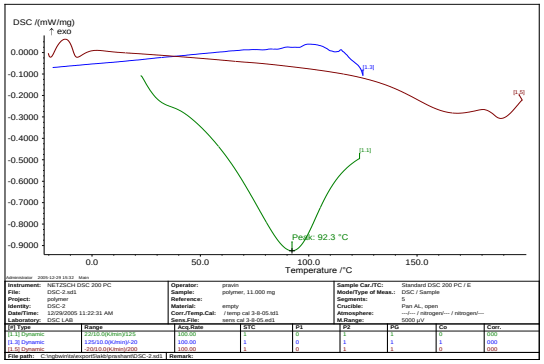

Figure 9(a). DSC of pectin

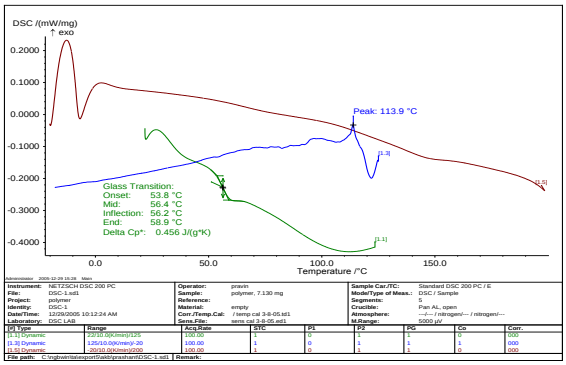

Figure 9 (b). DSC of GP

3.H. Swelling behavior of the GP cross-linked with 0.2, 0.4, 0.6, 0.8 ml of glutaraldehyde GA

The GP was water soluble co-polymer so it required cross-linking by cross-linking agent.[11-15] The GP hydrogel was cross-linked with different concentration of glutaraldehyde (GA),B, C, D, F (as shown in the **Fig.10.**) 0.2, 0.4, 0.6, 0.8 ml of GA cross-linking agent respectively (acidified GA with HCl, kept constant 0.05 ml) were allow to swell in 5 ml of phosphate buffer solutions having pH 1.4, 5.4, 7.4 and 9.4. The results indicates that GA cross-linked GP hydrogel swelled more significantly (swelling 190 %) at pH 7.4 due to a large swelling forces created by the electrostatic repulsion between the ionized acid groups -COO$^-$ as well as amide groups. Figure 11. showing effect of crosslinking density on swelling behavior of GP.

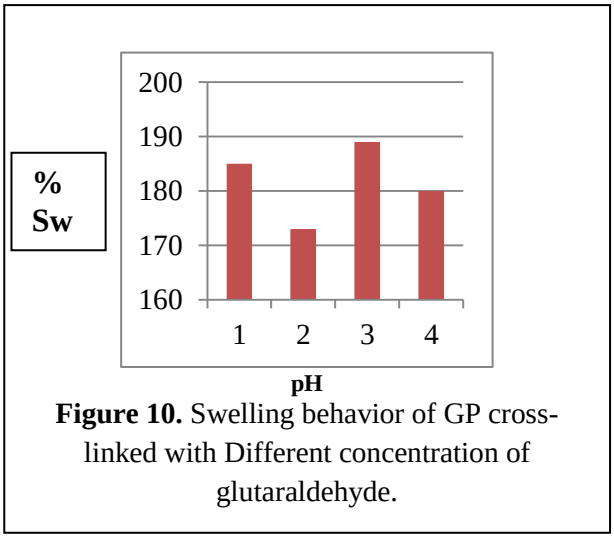

Figure 10. Swelling behavior of GP cross-linked with Different concentration of glutaraldehyde.

At pH 7.4, the carboxylic acid group on the GA cross-linked GP hydrogel became progressively ionized (-COO $^-$). By observing swelling behavior, it had shown that GP cross-linked hydrogel had pH dependent swelling behavior. The degree of swelling increased from pH 1.4 to 7.4 but in pH 9.4 swelling decreases again. The hydrogel swells maximum in the pH 7.4 .The swelling behavior also depends upon cross-linking density, as concentration of GA

increases % swelling decreases because greater extent of chemical cross-linking of the polymer chains that restricts the mobility of the polymer chains. Thus we can control % swelling by varying concentration of cross-linking agent.[11-15]

3. I. Rheological Study

Viscosities versus shear rate **(Figure 11(a).)** of the 5% polymer solution were plotted. The viscosity of the polymer solutions decreases with increase in shear rate. Similar results were are observed earlier[16-18] for carboxymethyl cellulose Rheological viscosity measurement study. Both the aqueous 5% solutions of GP and Pectin showed strong pseudo plastic behavior. It is evident from the viscosity versus shear rate curve **(Figure 11(a).)** that at shear rate of 11.56 the viscosity of the 5% GP solution is 45.93 poise, but with further increase in shear rate leads to regular decrease in viscosity of the GP solution, and the lowest viscosity of8.13 poise was observed at shear rate of 100. A similar pattern was observed in the case of 5% pectin solution **(Fig. 11b.)**, and the figure shows that at shear rate of11.56, the viscosity of pectin solution was 0.49 poise but as the shear rate increased up to 99.8 the viscosity of the solution decreased to 0.37 poise. Hence, it can be concluded from the results that at low and high shear rates the viscosity of the GP solution was higher than pectin solution and it can be suggested that grafted pectin solution was more shear stable than the native pectin. This is due to the grafting of longer polyacrylamide chain on pectin back- bone[19,20]. It can be attributed to the fact that, the graft copolymers having fewerbut longer branches are found to be more shear-stable than ungrafted polymers[21-22].

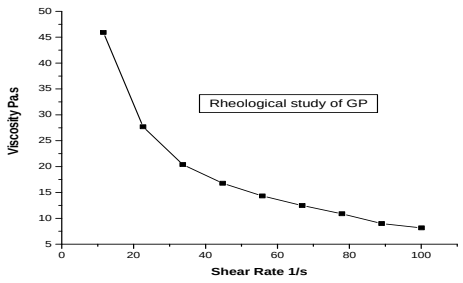

Figure 11(a). Viscosity versus shear rate curves of 5% GP solution.

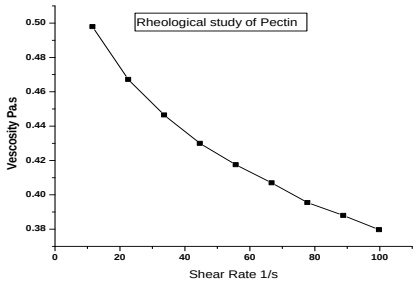

Figure 11(b). Viscosity versus shear rate curves of 5% pectin solution

3.J. Haemocompatibility Study

In the Haemocompatibility study we found Haemolysis of test sample is 36.36 % ; therefore we can assume that the GP had less haemolytic effect on the human red cell suspension. So that GP material is highly haemocompatible [25-27].

Table 2. Haemocompatibility observations and results.

OD of Negative control	OD of Positive control	OD of Test Sample	% Haemolysis and Result
0.04	**0.37**	**0.052**	**36.66 % Highly Haemocompatibility**

IV. CONCLUSIONS

The main objective in relation to this study was to graft - copolymer consist of N-isopropyl acrylamide on the pectin backbone by ceric ammonium sulphate as an initiator. . The impact of polymerization variables including initiator concentration, monomer concentration, reaction

time and temperature on grafting parameters was investigated. Study of FT-IR, elemental analysis, XRD, and DSC confirmed our view that the PAM side chain was grafted on the pectin backbone by graft copolymerization. Rheological study of the pectin and GP solution shows that the shear stability of pectin was improved after grafting. Further, it can be suggested that synthesized grafted pectin could be tried as a hydrogel for drug delivery systems and other various potent applications.

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