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Synthesis, swelling behaviour, salt- and pH- sensitivity of crosslinked gellan gum-graft-poly (acrylamide-co-itaconic acid) hydrogels

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ABSTRACT

A new hydrogel of Gellan gum grafted Poly(acrylamide-co-itaconic acid) has been made through chemical cross-linking by graft copolymerization of acrylamide (AAm) and itaconic acid (IA) on to Gellan gum (GG) via free radical polymerization in presence of N, N'-methylene-bis-acrylamide (MBA) as crosslinking agent. Characterization of the gel has been made using FT-IR, TGA and SEM techniques. The influence of preparation conditions, pH and ionic strength on the swelling behaviour of the gels has been investigated. The results revealed that synthesized gels exhibit a reasonable sensitivity to pH and ionic strength.

Keywords: Gellan gum, Acrylamide, Itaconic acid, N,N'-methylene-bis-acrylamide, Swelling.

INTRODUCTION

Crosslinked hydrogel networks are widely investigated as therapeutic devices, scaffolds for tissue engineering and controlled/sustained drug release devices etc. taking advantage of their special properties such as high sorption capacity, hydrophilicity, low interfacial tension in contact with body fluids, minimal irritation to the surrounding tissue, [1-3] good biocompatibility and carrier properties, and high permeability or nutrients and metabolites [4-6].

Gellan gum is an anionic deacetylated exocellular polysaccharide gum with high molecular weight, produced as a fermentation product by pure culture of *Pseudomonas elodea* (aerobic, gram negative, non pathogenic bacterium). It has tetrasaccharide repeating units each consisting of one α-L-rhamnose, one β-D-glucouronic acid and two β-D-glucose residues [7]. Gellan gum is a potentially useful pH sensitive polymer that swells at high pH and collapses at low pH conditions and hence finds use as a controlled drug delivery device matrix material [8-10]. Triggered drug delivery occurs upon an increase in the pH of the gel environment.

Graft copolymerization of the hydrophilic monomers such as acrylamide, N-t-butyl acrylamide, itaconic acid, maleic acid etc. onto polysaccharides, namely Starch and Sodium alginate and subsequent gel formation have been reported [11-14]. Literature on grafting of acrylamide on Gellan gum is very limited [15]. Hence in the present investigation, grafting of copolymer of acrylamide, itaconic acid and MBA on Gellan gum has been attempted. The influence of gel structure, pH and salt conditions of swelling medium on the swelling behaviour of the synthesized gel has been investigated in detail.

MATERIALS AND METHODS

2.1. Materials:

Gellan gum (GG), N,N^1-methylene-bis-acrylamide (MBA) were obtained from Aldrich Chemical Company, India. Itaconic acid (IA), Acrylamide (AAm), Ammonium per sulphate (APS), Potassium chloride, Hydrochloric acid, Potassium hydrogen phthalate, Potassium dihydrogen phosphate, Disodium hydrogen phosphates were obtained from Merck India. All the chemicals and reagents were used as received. Double distilled water was used for polymerizations and swelling experiments. Aqueous solutions of MBA (1.5%), APS (1.5%) were prepared and used for the polymerization reactions.

Solutions containing 0.05M KCl and 0.085M HCl was made to obtain a buffer solution of pH-1.2; 0.2M potassium hydrogen phthalate and 0.022M HCl to obtain a buffer solution of pH-3.0; 0.16M disodium hydrogen phosphate and 0.084M potassium dihydrogen phosphate to obtain buffer solution of pH-6.8; 0.013M disodium hydrogen phosphate, 0.0014M potassium dihydrogen phosphate and 0.13M sodium chloride to obtain buffer solution of pH-7.4 and 0.011M disodium hydrogen phosphate was used to make buffer solution of pH-9.0.

2.2. Methods:

2.2.1. Synthesis of hydrogels of Gellan gum grafted Poly (acrylamide-co-itaconic acid) (GG-g-Poly (AAm-co-IA) :

0.1g of GG was dissolved in deionized water under constant stirring at 70 ℃ for 1h. After complete dissolution and formation of homogenous solution, certain amounts of AAm (0.1g), IA (0.1g), APS solution (0.66-1.66mL) and MBA solution (0.66-1.66mL) were simultaneously added to the reaction mixture. After 2h, the reaction mixture was allowed to cool to ambient temperature. Gel formation is observed. The gel was transferred to excess methanol (50mL) and retained in it for 24h to dewater. The dewatered gel was removed and washed with fresh methanol. Finally, the gel was dried in an oven at 50 ℃ for 24h.

2.2.2. IR spectroscopy:

The IR spectra of GG and representative sample GG-g-Poly (AAm-co-IA)-A1 were recorded in KBr pellet form using the FTIR spectrophotometer (Perkin-Elmer, USA).

2.2.3. Thermogravimetric analysis:

TGA of GG and representative sample GG-g-Poly (AAm-co-IA)-A1 were recorded on SDT Q600 V20.9 (Japan) thermogravimetric analyser. The samples were heated from zero to 800^0C, under nitrogen atmosphere, at a rate of 5^0C/min.

2.2.4. Scanning Electron Microscopic (SEM) analysis:

The micrographs of GG and representative sample GG-g-Poly (AAm-co-IA)-A1 were recorded on a JEOL-JSM5800LV scanning electron microscope. The micrographs were recorded with magnifications of 100 and 1000 under a voltage of 20 KV.

2.2.5. Swelling studies:

The swelling behaviour of the hydrogels under different pH conditions was investigated by carrying weight measurements in distilled water and in buffer media of pH-1.2 and 7.4. The weight measurements were made using an electronic balance (Shimadza AUX120, Japan) with an accuracy of ±0.1mg. Pre-weighed dry gels were immersed in excess of the buffer solution, maintained at 30 ℃ . After specific intervals of the time, the gels were removed from the medium, the surface adhered liquid drops were wiped with blotting paper and the increase in weight was measured. The measurements were continued till the weights of the swollen gels attained constant values. The swelling ratio (SR) was calculated using the following expression,

$$SR(g/g) = (W_t - W_o) / W_o \qquad (1)$$

Where W_o and W_t are the weights of the sample in the dry state and swollen state at time'*t*' respectively.

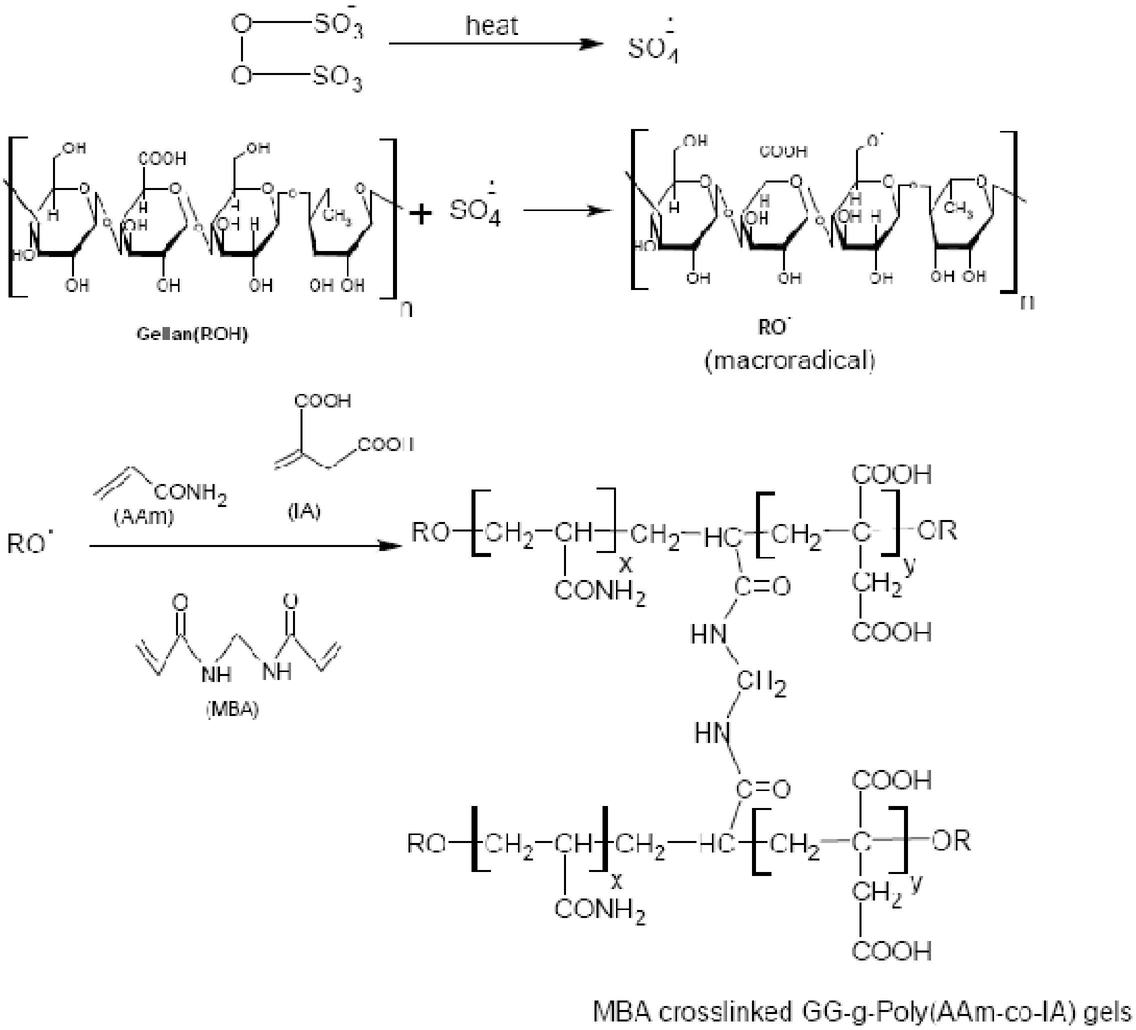
RESULTS AND DISCUSSION

Grafting and gel formation was carried out with different amounts of APS and MBA. The details of preparation and designation of gels is given in **Table 1**.

Table 1. Details of preparation and designation of GG-g-Poly (AAm-co-IA) gels.

Formulation code		GG(g)	AAm(g)	IA(g)	APS(mM)	MBA(mM)
GG-g-P(AAm- co-IA)	A1	0.1	0.1	0.1	0.066	0.096
	A2	0.1	0.1	0.1	0.043	0.096
	A3	0.1	0.1	0.1	0.088	0.096
	A4	0.1	0.1	0.1	0.109	0.096
	B1	0.1	0.1	0.1	0.066	0.064
	B2	0.1	0.1	0.1	0.066	0.128
	B3	0.1	0.1	0.1	0.066	0.160

Copolymerization of AAm, IA and MBA on the GG backbone leads to the formation of crosslinked GG structure. The mechanism of gel formation is shown in **Scheme 1**. The sulphate anion radical produced by the decomposition of APS abstracts hydrogen from primary hydroxyl groups at C_6 position of GG substrate (ROH) to form corresponding alkoxyradicals ($RO^{\cdot}$). These macroradicals initiate polymerization of monomers leading to the formation of graft copolymer. The presence of MBA in the grafted chain results in a three dimensional network [16].



Scheme 1. Proposed mechanistic pathway for preparation of GG-g-Poly (AAm-co-IA).

3.1. IR Spectroscopy:

The FT-IR spectroscopy of GG, GG-g- Poly(AAm-co-IA)-A1 are shown in **Fig. 1(a) & (b)** respectively. The IR spectra of GG **(Fig. 1(a)**) shows a broad absorption band at 3417 cm^{-1}, due to the stretching of the –OH bonds. The band at 2924 cm^{-1} is attributed to C–H stretching. Two strong peaks are observed at 1619 and 1420 cm^{-1} due to the asymmetrical and symmetrical stretching of $–COO^-$ groups.

In the IR spectra of GG-g-Poly (AAm-co-IA)-A1 **(Fig. 1(b)),** the peaks observed at 1435 and 1165cm^{-1} are due to the presence of –OH bending and -C-O-C stretching vibrations of GG respectively. The peak at 1309 cm^{-1} is for the -C-O stretching of primary $–CH_2OH$ groups of GG. In addition to the characteristic peaks of GG, few new bands appeared in IR spectra of GG-g-Poly (AAm-co-IA)-A1. The peak found at 3427 indicates the N–H stretching of AAm and the peak at 1658 indicates C=O stretching of the amide groups of AAm, MBA units. Moreover the band at 1705 $cm^{-1}$is attributed to carboxylic carbonyl group of IA. These peaks provide evidence for the formation of graft copolymer on GG backbone.

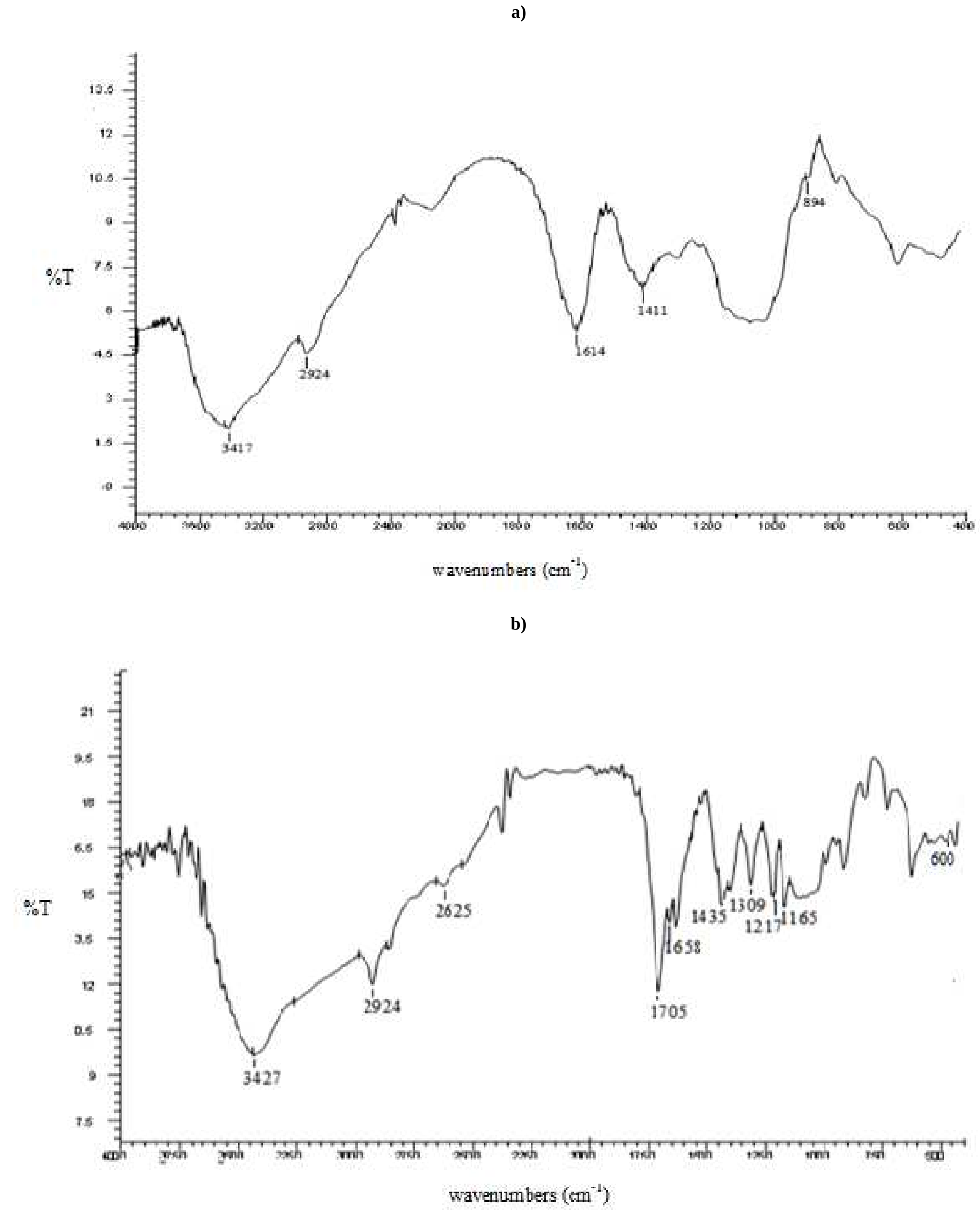


Fig 1. IR Spectra of a) GG and b) GG-g- Poly (AAm-co-IA) - A1.

3.2. Thermogravimetric analysis:

As shown in **Fig. 2 (a))** GG shows a three-step characteristic thermogram, wherein the first stage of weight loss of about 10% occurs in the temperature range of 50-100°C which is attributed to loss of moisture in the sample. The major weight loss (57 %) takes place in the second step within the temperature range of 236–540°C. Finally 10% weight loss occurs around 600°C.

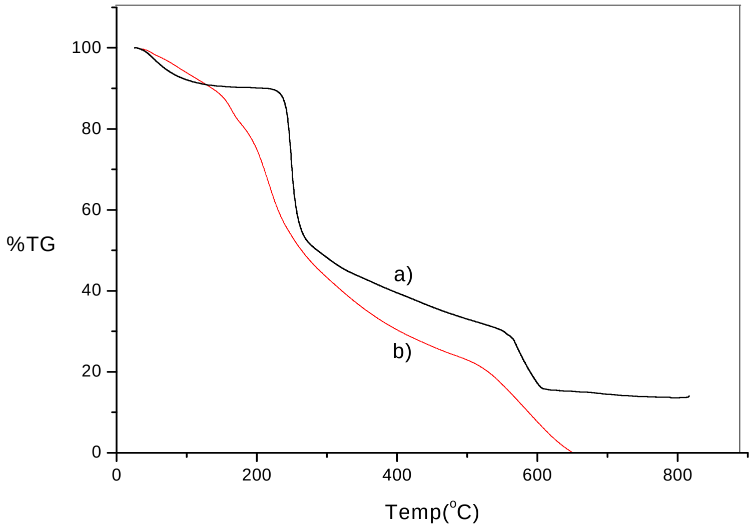


Fig 2. Thermograms of a) GG and b) GG-g-Poly (AAm-co-IA)-A1.

In case of GG-g-Poly (AAm-co-IA)-A1 (**Fig.2 (b)**), slight change in the degradation pattern were observed. The initial weight loss due to loss of water contained in gel occurred in the range, 25-130°C . At the second stage from 130 to 440°C, there is decomposition at the branches of the graft copolymer. At the third stage from 440 to 633°C, there is a complete degradation of GG in the graft copolymer; the weight loss is attributed to the degradation of the polysaccharide. The DTG curve (**Fig.3)** supports the above data and confirms the lower stability of grafted polymer when compared to GG.

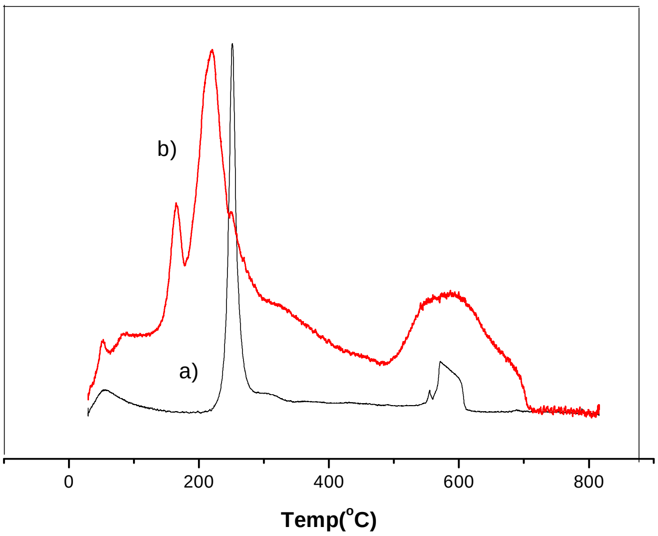


Fig 3. Derivatograms of a) GG and b) GG-g-Poly (AAm-co-IA)-A1.

From the TGA and DTG curves, it is concluded that the thermal stability of the polysaccharide decreases with the grafting of Poly(AAm-co-IA) chains onto the polysaccharide backbone. This may be attributed to the low thermal stability of itaconic acid. Similar phenomenon has also been reported by N. Isklan [17] where the thermal stability of the sodium alginate is reduced due to grafting of IA onto it.

3.3. SEM analysis:

Fig. 4 shows scanning electron micrographs of **a)** GG and **b)** GG-g-Poly (AAm-co-IA)-A1 sample. The SEM images reveal that a smooth, heterogeneous surface of GG changes on grafting and crosslinking. The GG-g-Poly (AAm-co-IA)-A1 sample shows crystalline and fibre like structure formation on the surface of GG. The morphology of GG-g-Poly (AAm-co-IA)-A1 is observed to be highly irregular and heterogeneous in nature.

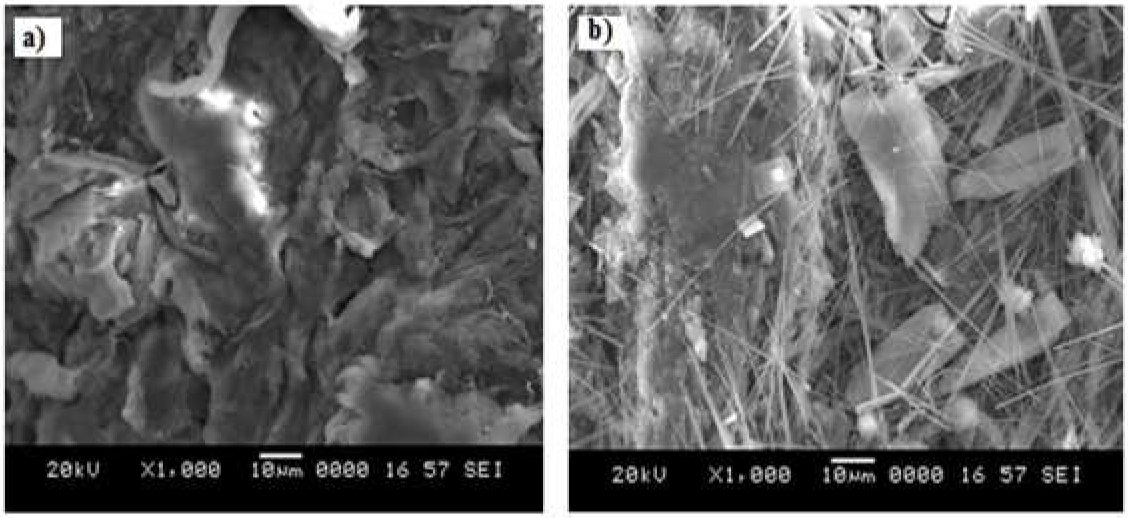


Fig 4. SEM images of a) GG and b) GG-g-Poly (AAm-co-IA)- A1.

3.4. Swelling behaviour of hydrogels:

3.4.1. Effect of nature of gel on swelling:

Swelling behaviour of gel depends on the hydrophilicity of polymer network, crosslinking density, elasticity of polymer network, etc. Hence, variation in gel structure directly influences its swelling ability. In the present investigation, GG-g-Poly (AAm-co-IA) samples with varying crosslink density and extent of grafting have been achieved by varying the composition of the reaction mixture; the concentration of initiator and the crosslinking agent. The effect of [MBA] and [APS] on the nature of gel is evident from the swelling behaviour as shown in **Fig 5** and **6** respectively.

Fig 5. shows the swelling data after 60 min of swelling for samples B1, A1, B2 and B3 made with increasing concentration of MBA. High swelling was observed for the gel B1 made with minimum concentration of MBA. Swelling drastically decreases for the gels in expected order B1>A1>B2 > B3. This is due to decrease in the length of the grafted chains as the crosslinker concentration is increased.

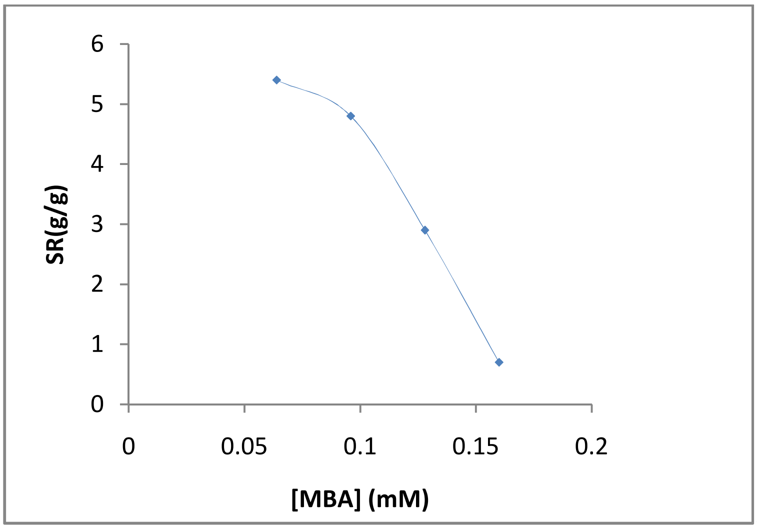


Fig 5. Effect of crosslinking on swelling behaviour of GG-g-Poly (AAm-co-IA) hydrogels -B1, A1, B2 and B3 at 60 min, in pH 7.4 medium

Fig.6. shows that the gels made with [APS] <0.05mM and [APS]>0.09mM exhibit low swelling compared to the gel sample A1 with [APS] =0.06mM. The number of active free radicals on the GG backbone is too low at concentrations lower than 0.06mM which, in turn, results in lower extent of grafting and consequently lower swelling capacity. Concentration of APS higher than 0.09mM may lead to copolymer formation without involvement of GG, reducing the extent of grafting. An intermediate concentration of [APS] =0.066mM appears to be ideal for copolymer grafting on GG.

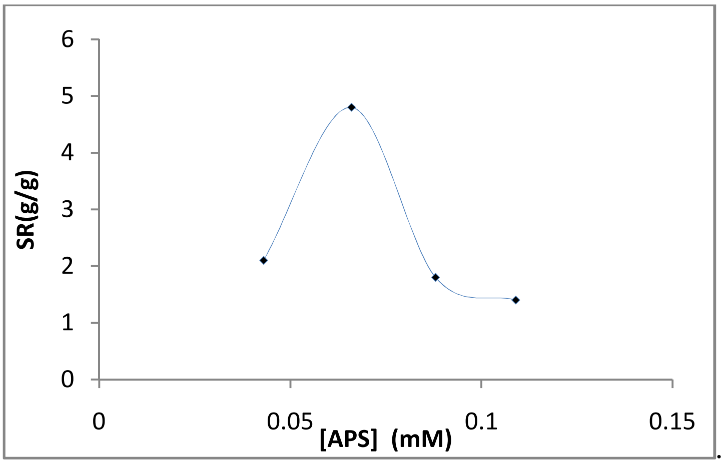


Fig 6. Effect of extent of grafting on swelling behaviour of GG-g-Poly (AAm-co-IA) hydrogels -A2, A1, A3 and A4, at 60 min, in pH 7.4 medium.

3.4.2. Effect of salt on swelling behaviour:

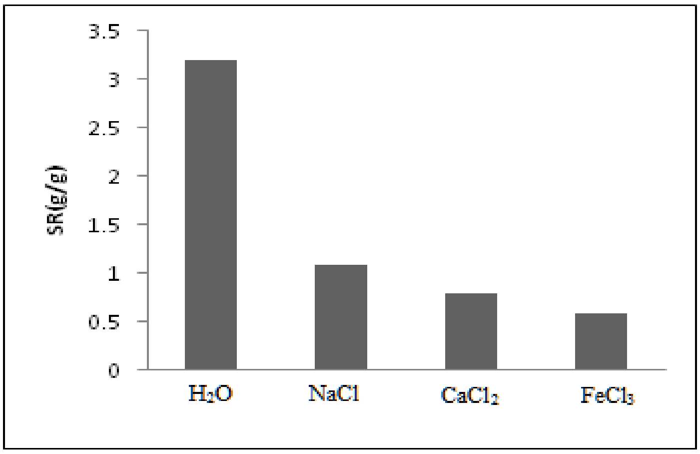


Fig 7. Effect of nature of salt on swelling behaviour of GG-g-Poly (AAm-co-IA) -A1.

The swelling behavior of the gel sample in water and 0.1M aqueous solutions of different salts is compared in **Fig.7.** It is evident that swelling is highly depended on the "type" and "concentration" of salt added to the swelling medium. The swelling of the gel in saline solution of 0.1M decreased about three times compared to water. This well-known phenomenon, commonly observed in the swelling of ionic hydrogels [18], is often attributed to a screening effect of the additional cations of the medium causing a non-perfect anion-anion electrostatic repulsion, leading to a decreased osmotic pressure difference between the gel network and the external solution. The swelling capacity of the gel further decreases in $CaCl_2$ and $FeCl_3$ solutions with increasing charge on the cation of the solution, the order being, $Na^+ > Ca^{2+} > Fe^{3+}$. It is explained by the enhanced crosslinking ability of the multivalent cations with carboxylate groups present in the gel. This ionic crosslinking mainly occurs at the surface of gel particles and gels turn hard in Ca^{2+} or Fe^{3+} solution.

3.4.3. Effect of pH on swelling behaviour:

Fig. 8 displays the pH dependence of swelling of the gel sample at ambient temperature, 30°C. Swelling increases with increase in pH of the medium up to a pH of 7.4 and drastically decreases thereafter. The swelling is influenced by the degree of ionization of the weakly ionizable groups namely, the carboxylic groups of glucouronic acid unit of GG, carboxylic groups of IA and amine groups of acrylamide and MBA. In swelling media of pH<4.0, the carboxylic groups get protonated and under pH>8.0, the amine groups are deprotonated reducing the number of ionic functionality in the gel system. But, at the intermediate pH of 7.4, maximum numbers of ionic groups exist in the gel resulting in highest swelling.

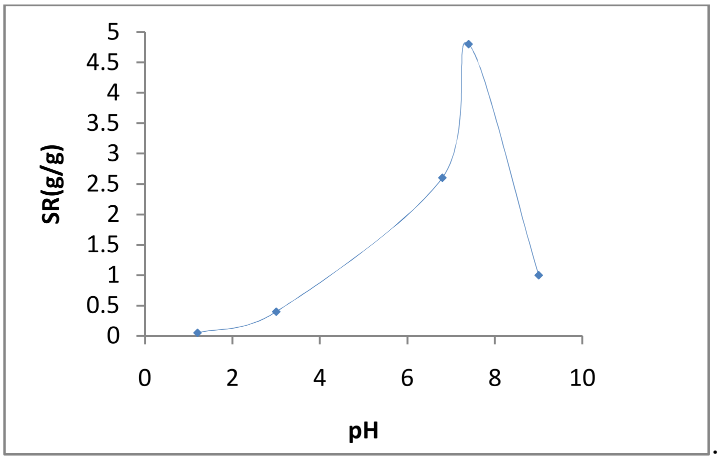


Fig 8. Effect of pH on swelling behaviour of GG-graft-Poly (AAm-co-IA) –A1.

CONCLUSION

A new 'salt'- and 'pH'-responsive GG based copolymer hydrogel has been synthesized through graft copolymerization of AAm and IA along with MBA on GG. The extent of grafting on GG and the crosslinking of gel network was varied by changing the gel preparation conditions. The conditions for obtaining highest swelling were optimised. The swelling studies revealed that hydrogels exhibited high sensitivity to pH, with maximum swelling at pH7.4 and considerably low swelling in highly acidic and basic media. Swelling measurements of the grafted gel in different salt solutions showed appreciable lowering in swelling capacity which increasing charge on the cation of the salt. Furthur, the lower swelling observed in salt solutions, in comparison with water, can be attributed to charge screening effect and ionic crosslinking by mono- and multi-valent cations, respectively.

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