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Swelling Studies Of pH And Salt Responsive Ethylene Glycol Dimethacrylate Crosslinked Gellan Gum And Sodium Alginate Gels

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Abstract: The study reports the preparation of gels containing Gellan gum and Sodium Alginate. Network formation has been achieved by chemical crosslinking using Ethylene glycol dimethacrylate. The detailed swelling study of the gels has been carried out to understand the influence of crosslinker concentration, pH and ionic strength conditions on swelling. The results indicate pH and salt sensitive swelling. Higher swelling was observed under neutral and basic pH and low ionic strength conditions. Also, the increasing charge of the metal ion in swelling medium decreases swelling. The swelling capacity can be controlled by changing the crosslinker concentration in the preparation mixture. The kinetic analysis of swelling data indicates second order swelling. The swelling exponent 'n' is observed to be < 0.5 indicating fickian diffusion to be operative in these gels.
Key Words: Gellan gum, Ethylene glycol dimethacrylate, Swelling, pH responsiveness, Salt sensitivity.

INTRODUCTION

Polymer compatibility can be improved through the establishment of physical and/or chemical interactions between components. One approach to do this is to combine polymers in interpenetrating network (IPN) form. Crosslinking, mutual chain entanglement and eventual internetwork grafting[1] produce finer dispersion of one polymer in the other. IPNs could help to improve the mechanical strength and resiliency of the polymers due to physical entanglements and network interactions when compared to individual crosslinked networks[2–5]. Polymeric hydrogels in the form of IPNs are able to absorb large amounts of water without dissolving. They have been proposed for many biomedical applications due to their good biocompatibility and water permeation properties[6]. Polymeric hydrogels are promising candidates for tissue engineering matrices due to the fact that the hydrophilic nature and mechanical properties of these materials resemble those of soft tissues.

Two of the natural polymers that have evoked great interest of researchers in biomedical field are Gellan gum (GL) and Sodium alginate (Na-Alg). GL has been used in ophthalmic drug delivery, oral sustained delivery[7-10], controlled-release hydrogel with scleroglucan and floating in situ gelling[11,12]. It 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 a tetrasaccharide repeat unit consisting of two glucose residues, one glucuronic acid residue, and one rhamnose residue. Na-Alg is an anionic natural macromolecule, which is composed of β-1, 4-D-mannuronic acid (M units) and α-1, 4-L-glucuronic acid (G units) in varying proportions. Na-Alg can be extracted from marine algae or produced by bacteria. It is an abundant, renewable, non-toxic, water-soluble, biodegradable and biocompatible polymer. In addition, Na-Alg can be easily modified through various chemical or physical methods such as

grafting with hydrophilic vinyl monomers[13], polymer blending [14] and compounding with other functional components[15]. By virtue of these advantages, Na-Alg has received considerable attention in industrial and medical fields [16, 17].

In the present work, we have reported the preparation of network of GL and Na-Alg in which Ethylene glycol dimethacrylate (EGDMA) has been used as a crosslinker. Ammonium persulfate(APS) has been used for creating radical sites on polysaccharides chains. The effect of presence of Na-Alg and extent of crosslinking on gel property has been investigated. Detailed study of swelling of the gels under different pH and salts condition has been studied.

MATERIALS AND METHODS

Materials

Gellan gum(GL) was obtained from Sigma-Aldrich, USA. Sodium alginate(Na-Alg) was obtained from s.d.fine chemicals, Mumbai, India. Ethylene glycol dimethacrylate(EGDMA) and ammonium persulfate(APS) were obtained from Merck, Mumbai, India. All other reagents were of analytical grade and used as received. Double distilled water was used in the preparation of hydrogels and for swelling studies.

Preparation of EGDMA crosslinked GL / Na-Alg hydrogels (GL-Alg)

EGDMA crosslinked GL/Na-Alg hydrogels were prepared by free radical polymerization process. Na-Alg (0.1 g) and GL (0.2g) were dissolved in 12 mL water by vigorous stirring for 5 h. On getting a homogeneous solution, APS and EGDMA were added and the mixture was maintained at 70 ^{o}C and for 2 h in order to achieve complete gelation. In one batch, gelation was achieved under similar conditions with 0.2 g of GL but without addition of Na-Alg & gel is coded as GL-1. The gels obtained were cut into small pieces, purified by repeated washing with methanol and dried at 50-60 ^{o}C under vacuum overnight and used for further studies.

The amount of crosslinker EGDMA in the polymer mixture was varied as given in Table 1.

Instrumental analysis

Spectral and thermal characterization was carried out for a representative gel sample, GL-Alg-1 and compared with that of pure component GL.

Fourier transform infra-red spectrophotometry (FTIR)

The FTIR spectra of GL and GL-Alg-1 gel sample were recorded as KBr pellets on a FTIR spectrophotometer (Perkin-Elmer, USA).

Thermal Analysis

TGA of GL and GL-Alg-1 gel sample was recorded on SDT Q600 V20.9 (Japan) thermogravimetric analyser. The samples were heated from zero to 700 ^{o}C, under nitrogen atmosphere, at a rate of 5 ^{o}C / min.

DSC of GL and GL-Alg-1 gel was recorded on Q20 V24.4 Build 116 (USA) calorimeter. The samples were heated under nitrogen atmosphere from 0 to 250 ^{o}C at a rate of 10 ^{o}C / min.

SWELLING STUDIES

Swelling in Buffer solutions

The swelling behaviour of the GL-Alg gel samples under different pH conditions was investigated by carrying out swelling measurements in aqueous buffer media of pH-1.2, 6.8 and 9.0 using standard buffer solutions, at 30 ^{o}C. The weight measurements were made using electronic balance (Shimadza AUX120, Japan) with an accuracy of ±0.1mg. Pre-weighed dry hydrogels were immersed in excess of the buffer solution, maintained at 30 ^{o}C. 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 (S) and the amount of water the gel can hold at equilibrium (percent equilibrium water content, %EWC) were calculated using standard equations [18].

$$S = \frac{\text{Weight of the swollen gel} - \text{Weight of the dry gel}}{\text{weight of the dry gel}} \quad (1)$$

$$\%\ EWC = \frac{\text{Weight of the swollen gel} - \text{Weight of the dry gel}}{\text{Weight of the swollen gel}} \times 100 \quad (2)$$

Swelling in salt solutions

The swelling behaviour of the GL-Alg samples in different salt solutions was investigated by carrying out swelling measurements in solutions of NaCl(0.1, 0.3 & 0.6M), $CaCl_2$(0.1M) & $FeCl_3$(0.1M). The swelling ratio (S) was evaluated.

Table 1 Composition of reaction mixture (12 ml) for preparation of GL-Alg samples

Sample Code	GL (g)	Na-Alg (g)	EGDMA (g)	APS (g)
GL-1	0.2	0.0	0.0148	0.00115
GL-Alg -1	0.2	0.1	0.0148	0.00115
GL- Alg-2	0.2	0.1	0.0633	0.00115
GL- Alg-3	0.2	0.1	0.1080	0.00115

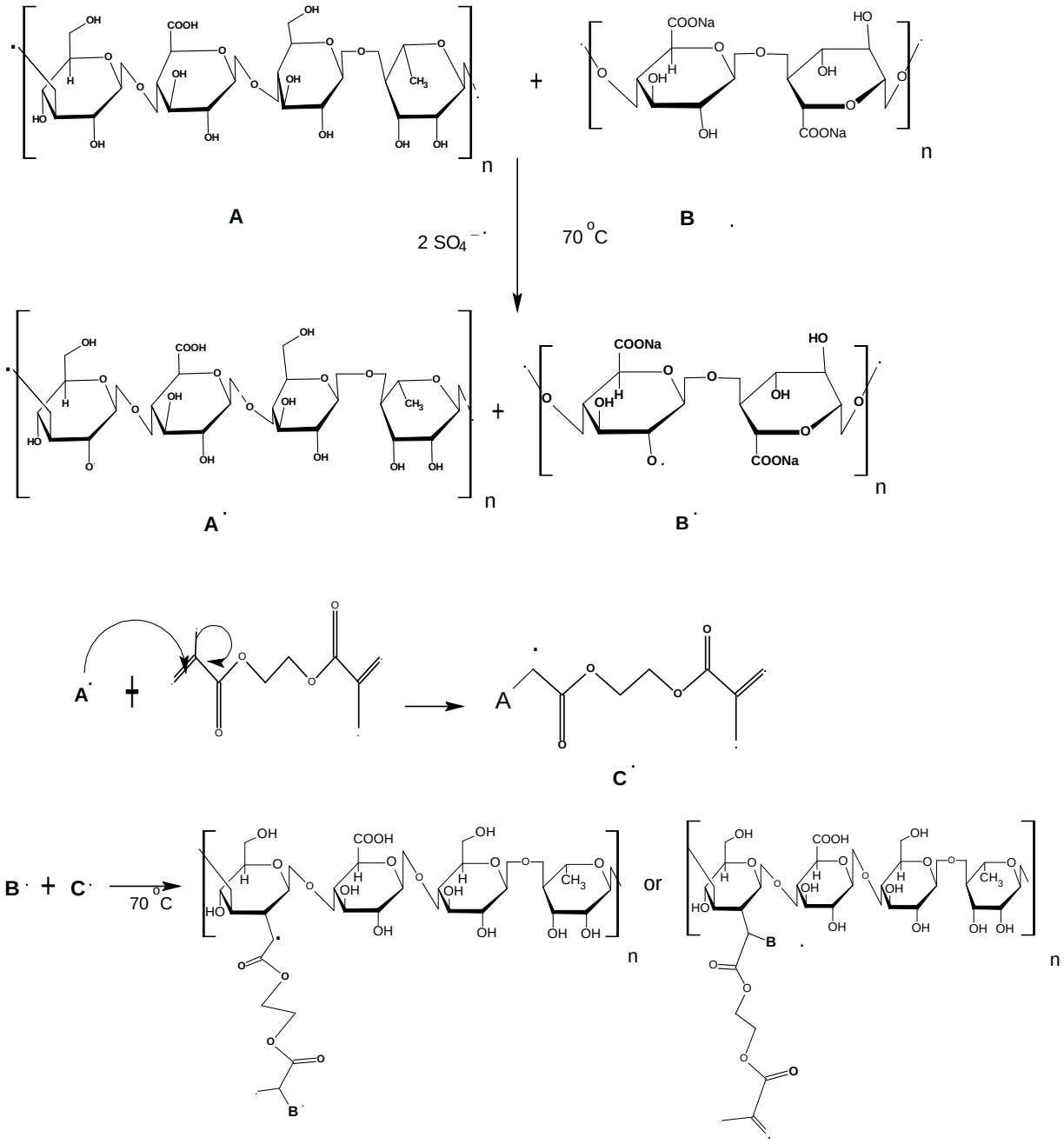
Table 2 Comparison of Swelling data in water and metal salt solutions (0.1 mol/L) and salt sensitivity factor ' f '

Swelling medium	carrageenan-g-PAAgel [25]		Na-Alg /CMC gel [26]		GL-Alg -1	
	S R (g/g)	f	S R (g/g)	f	S R (g/g)	f
H_2O	374	_	194	_	28	_
NaCl	37	0.89	53	0.72	9.5	0.67
$CaCl_2$	13	0.96	28	0.85	2.9	0.89
$FeCl_3$	4	0.99	11	0.94	1.1	0.96

RESULTS AND DISCUSSION

Synthesis of GL-Alg gels

GL and Na-Alg were simultaneously crosslinked using APS as radical initiator and EGDMA as crosslinking agent. Scheme 1 shows the mechanism of crosslinking of GL and Na-Alg in the presence of EGDMA. The sulphate anion radical produced by the thermal decomposition of APS abstracts hydrogen from the primary hydroxyl groups of the polysaccharide substrates to form corresponding alkoxy radicals on the substrates. The alkoxy radicals on the GL and Na-Alg backbones form the active centers, initiating free radical reactions with EGDMA to form the gel network [19].



Scheme 1 Formation of GL-ALG network structure

Characterization of GL-Alg samples

Chemical structure

Infrared spectra of GL and GL-Alg-1 gel sample are shown in Figure 1 (a) and (b), respectively. GL showed characteristics peaks at 3533 cm^{-1} for -OH stretching, 2924 cm^{-1} for C-H stretching, 1660 cm^{-1} for C=O stretching, 1406 cm^{-1} for methyl C-H bending and 891.11 cm^{-1} for C-O stretching of alkyl ether.

The spectra of GL-Alg-1gel shows additional peaks at 2850 and 1720 cm^{-1} due to C-H stretching and C =O stretching of acrylate of EGDMA respectively indicating the formation of crosslinked structure.

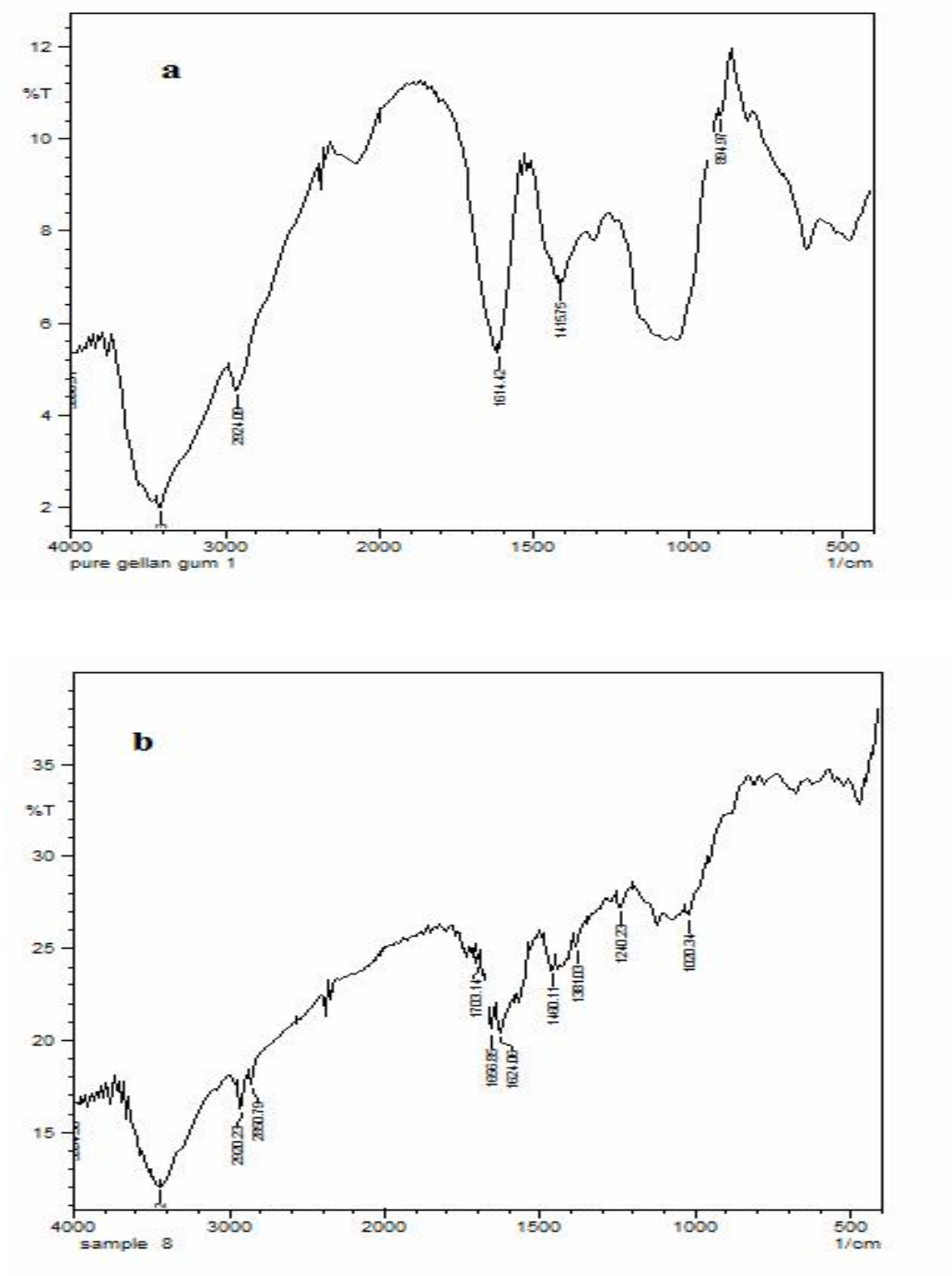


Figure1. FTIR of (a) GL and (b) GL-Alg-1

Thermal behaviour

Figure 2 depicts the TGA thermograms of the GL and crosslinked sample. In Gellan gum, the first weight loss occurs in the temperature range 90–190 ^{o}C accounting to 14.7% which is due to loss of free and bound water in the polymer. Above 190 to 360 ^{o}C mass loss(45.3%) occurs due to the depolymerisation with formation of H_2O, CO and CH_4. The residual mass decomposes gradually in the temperature range 300-600 ^{o}C [20, 21]. In GL-Alg-1 gel sample, the pattern appears to be different in the third region, with a sharp temperature change around 450 ^{o}C which clearly indicates the presence of crosslinker units in the gel sample.

The DTG curve (Figure. 2b) shows a single decomposition stage with a maximum at 240 ^{o}C, hence 10 ^{o}C lesser than the first decomposition stage of the pure GL (Figure. 2a). The slight decrease in the thermal stability is a consequence of the chemical bond between the two polymers. The explanation could be advanced that during degradation some reactions would develop between the decomposition products of the

polysaccharide and of the synthetic polymer leading to new products of lower thermal stability, which decompose below 500 ^{o}C. where as GL decompose at 600 ^{o}C.

The DSC thermograms of GL-Alg-1sample shows an additional endotherm at 200 ^{o}C compared to the pure GL sample (Figure 3) which could be due to the presence of crosslinks.

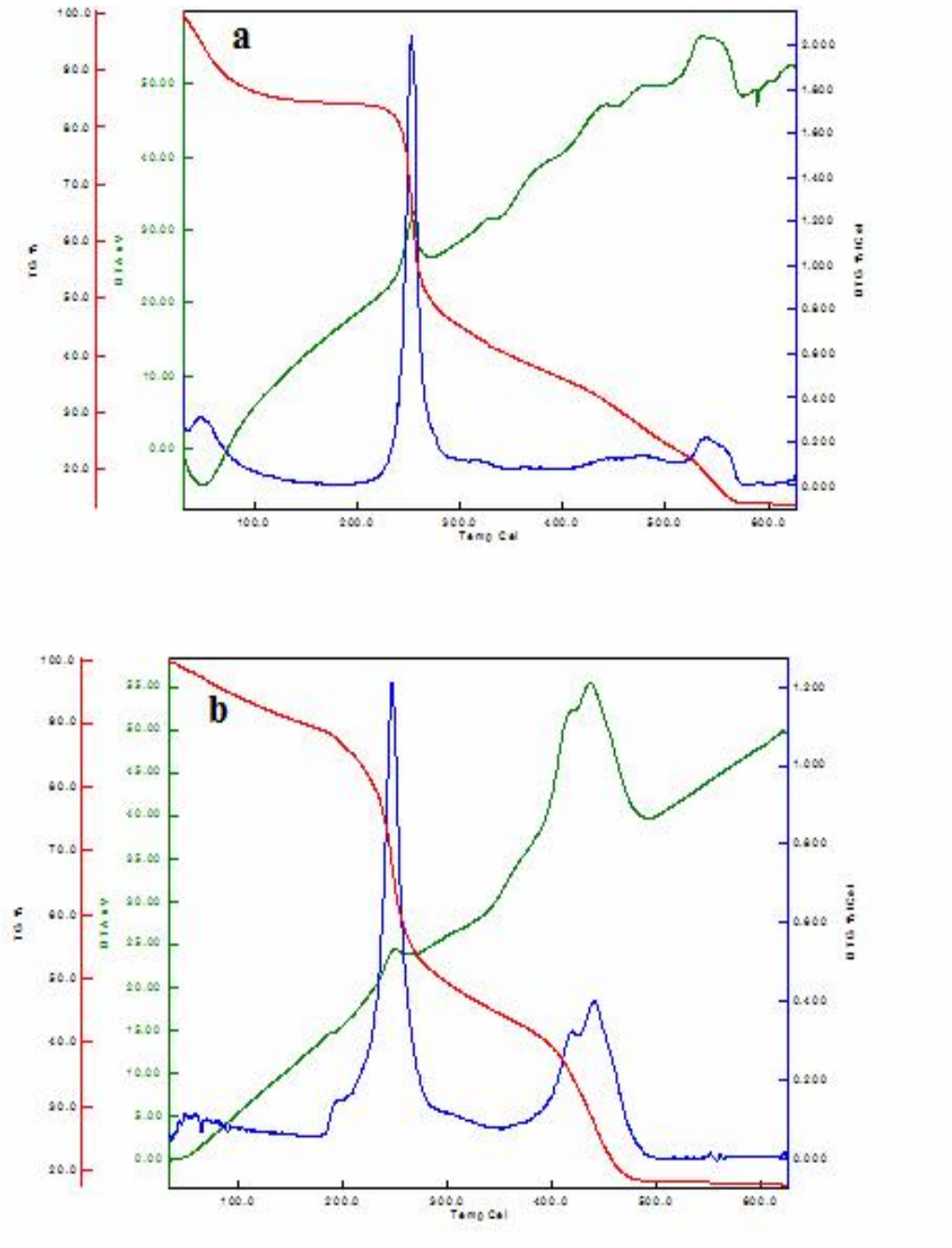


Figure 2. TGA thermograms (a) GL and (b) GL-Alg-1

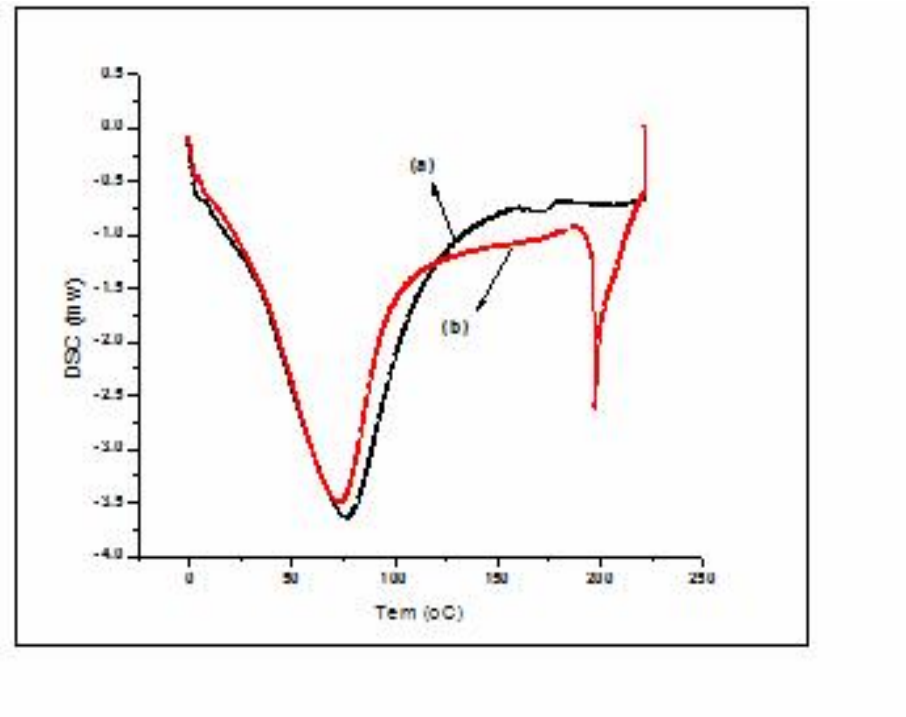


Figure 3. DSC thermograms of (a) GL and (b) GL-Alg-1

The Swelling behaviour

Effect of polymer composition on Swelling

The effect of incorporation of Na-Alg chains in the GL gel network on swelling was investigated by evaluating swelling in H_2O and the results are displayed in Figure 4. The gel GL-Alg-1 containing Na-Alg swells to higher extent than the GL-1. The incorporation of Na-Alg increases the carboxylate content thereby enhancing swelling.

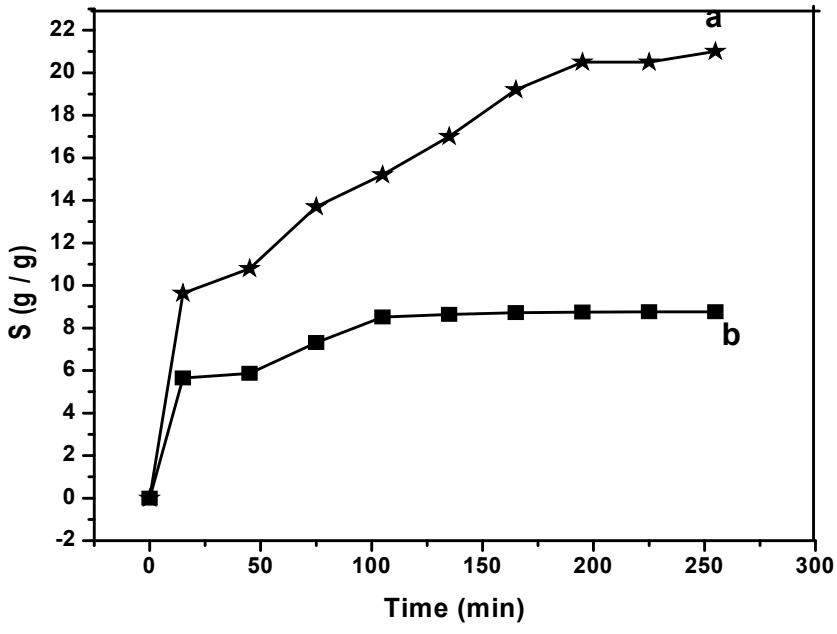


Figure 4. Swelling isotherms of (a) GL-Alg -1 and (b) GL-1 in H_20

Effect of concentration of crosslinker on swelling

The influence of extent of crosslinking on the gel characteristics was studied by measuring the swelling ratios of various samples made with different amounts of crosslinking agent. The swelling pattern observed under pH-6.8 is shown in Figure 5.

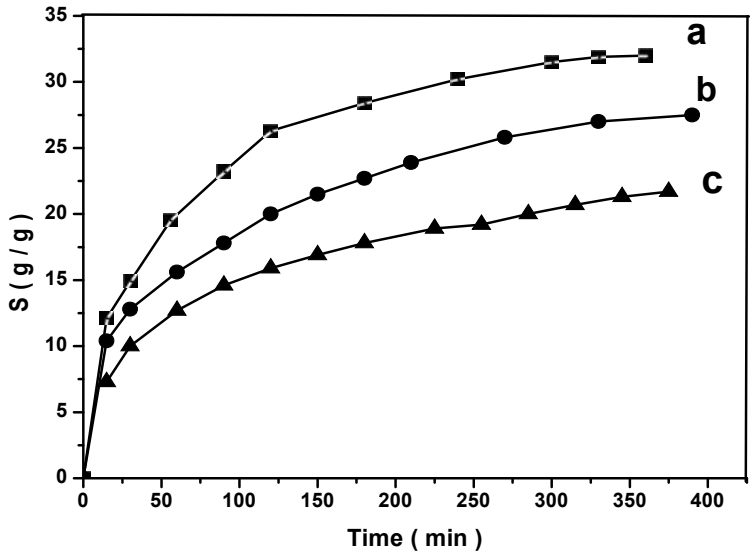


Figure 5. The swelling isotherms for a) GL-Alg-1 b) GL-Alg-2 & c) GL-Alg-3 at pH-6.8

The swelling ability of the gels follow the order GL-Alg-1 >GL-Alg-2 > GL-Alg-3 in agreement with the increasing amount of the crosslinker in the gel preparation mixture. The initial swelling rates of the gels are also affected by the crosslinker concentration. This is indicative of the fact that the swelling ability of the gels can be altered by 50% by changing the concentration of the crosslinker during the gel preparation process. The maximum water content in gels is found to vary in the same order, GL-Alg-1(98%), GL-Alg-2(96.4 %) and GL-Alg-3(94%). As these gels contain very high amount of water, they may be found suitable for use as biomaterials in medicine and pharmacy.

Effect of pH on Swelling

The effect of pH on the swelling behaviour has been studied for GL-Alg-1 and the results are displayed in Figure 6. GL-Alg-1 exhibits drastically low swelling in acidic medium whereas the swelling ability under neutral and basic conditions is incomparably high. As the carboxyl groups of the GL and Na-Alg which are in protonated form in acidic condition, ionize on increasing the pH, chain expansion between the crosslinks occur, increasing swelling. The swelling of gel network appears to be influenced by factors such as ionic strength of the swelling medium, in addition to the protonation effect.

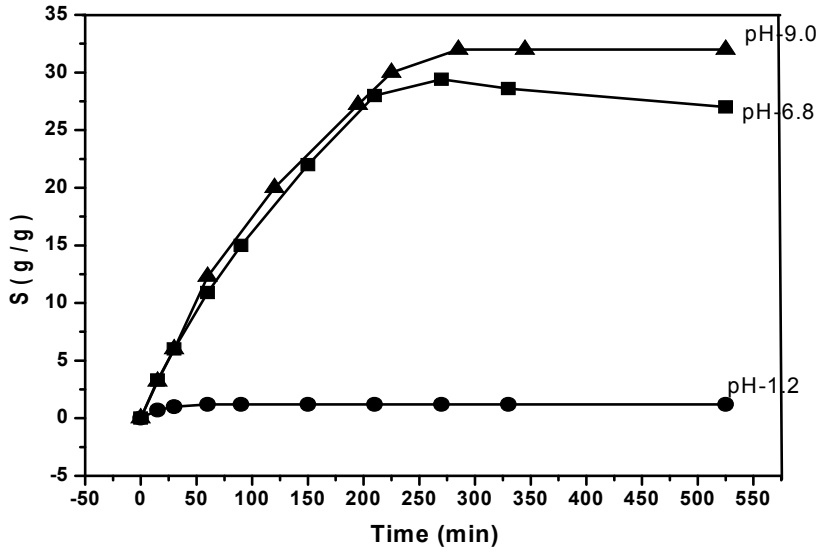


Figure 6. Swelling isotherms for GL-Alg-1 in different buffer media

Effect of salt concentration on water absorbency

In order to study the effect of ionic strength, swelling was carried out in sodium chloride solutions of different concentration, and results are shown in Figure 7. It is observed that with increase in ionic strength of the medium, the gel network shrinks and swelling is drastically reduced.

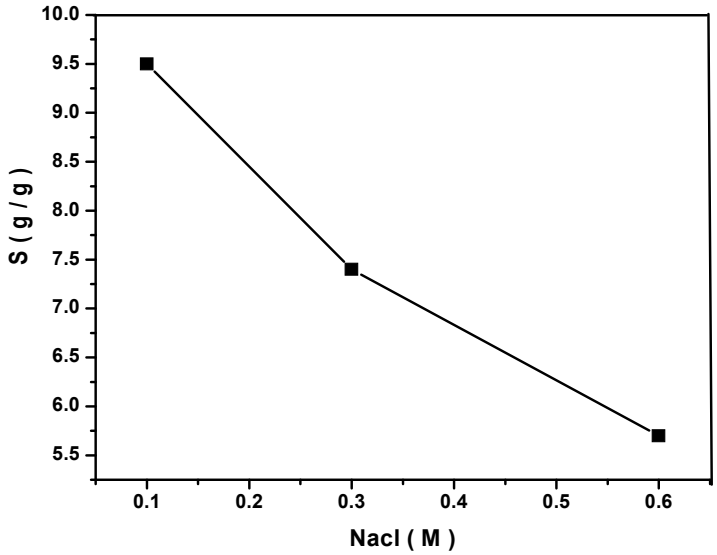


Figure 7. Swelling of GL-Alg-1 in NaCl.

The swelling data obtained from the chloride salts of various metal ions of identical concentrations were given in Figure 8. The swelling capacity decreased with increasing charge on the cation. The multivalent cations form tight network due to formation of ionic bonds involving carboxylate groups[29]. It is observed that the hydrogels turn very hard when placed in Ca^{2+} and Fe^{3+} solutions.

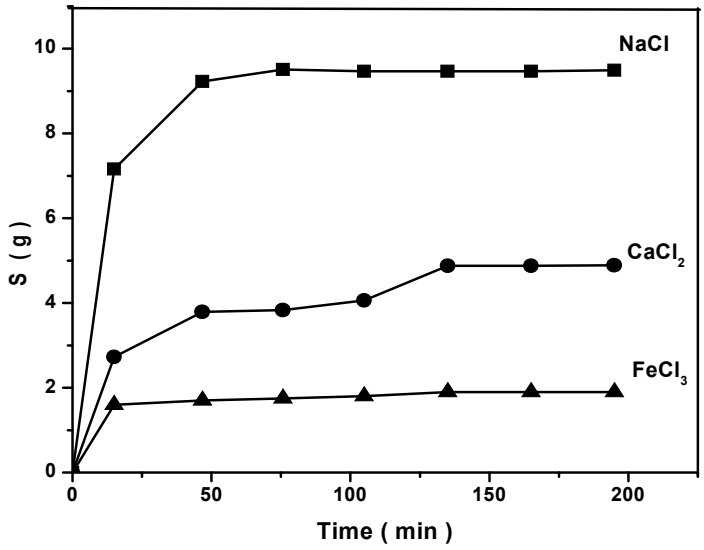


Figure 8. Swelling isotherms of GL-Alg-1 in different salt solutions

To achieve a comparative measure of sensitivity of the hydrogels to the nature of aqueous fluid, a dimensionless 'salt sensitivity factor', f, defined as follows, was evaluated.

$$f = 1 - \frac{(absorption\ in\ given\ fluid)}{(absorption\ in\ deionized\ water)} \qquad (3)$$

The absorption was measured in terms of equilibrium swelling ratio in the metal salt solutions and water. The 'f' values obtained for the presently studied gel has been compared with the similar gels reported in literature (Table 2). The values of 'f' show that the GL-Na-Alg hydrogel exhibits salt sensitivity, almost of the same order as Na-Alg/CMC gel and it increases with increasing charge on the metal ion.

Swelling and Diffusion Analysis

Swelling /shrinking and diffusion characteristics of hydrogels are presently being exploited for various applications including control of microfluidic flow, development of muscles-like actuators, filtration/separation and drug delivery. The mechanism of swelling process of hydrogels is determined experimentally by simple kinetic analysis using the following equation for a second order kinetic process[22,23].

$$\frac{ds}{dt} = R_s (S_{eq} - S)^2 \qquad (4)$$

Where S_{eq} & R_s denote the degree of swelling at equilibrium and swelling rate constant, respectively. The integration of Eq. 4 over the limits $S = S_0$ at $t = t_0$ and $S = S$ at $t = t$, gives the following equation:

$$\frac{t}{S} = A + Bt \qquad (5)$$

Where $A = 1 / (R_s S^2_{eq})$ is the reciprocal of the initial swelling rate

$B = 1 / S_{eq}$ is the inverse of the equilibrium swelling and

R_s is the swelling rate constant.

This relation represents swelling as a second order kinetic process. To check the applicability of this equation to the presently studied system, plots of t / S versus t were made for the GL-Alg samples and are shown in Figure 9.

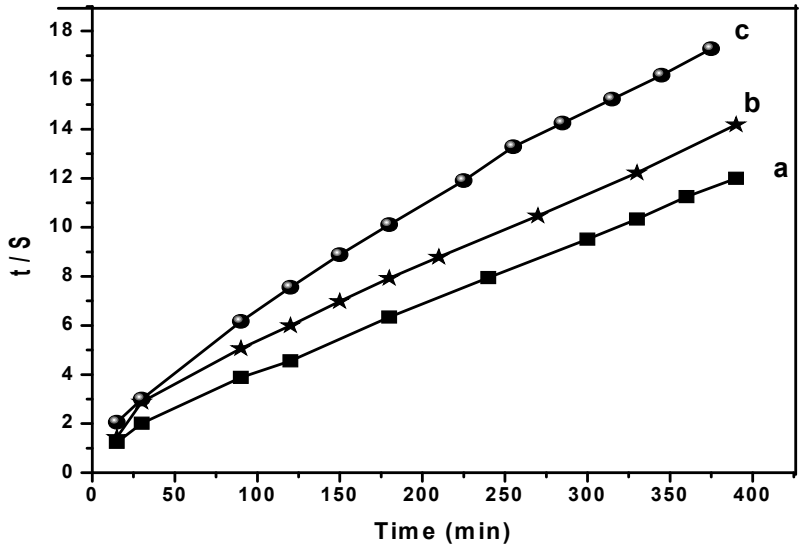


Fig. 9 t / s Vs t plots for (a) GL-Alg-1 (b) GL-Alg-2 & (c) GL-Alg-3

The linearity of the relationship between t/S and t indicate swelling to be a second order kinetic process. The swelling parameters, namely, initial swelling rate (Ri), maximum equilibrium swelling ratio (S_{max}) and swelling rate constant (R_s), were evaluated following methods reported earlier [24]. The results are tabulated in Table 3.

Table 3. Swelling characteristics of gels at pH 6.8 and 30 ^{0}C.

Gel code	GL-Alg-1	GL-Alg-2	GL-Alg-3
Equilibrium Swelling ratio (S_{eq})(g /g)	35.3	27.5	21.5
Equilibrium Water content (EWC %)	98	96.4	95
The initial swelling rate(Ri)[(g water / g gel)/min]	1.02	0.74	0.6
Swelling rate constant (Ks)x10^{-4} [(g gel/gwater)/min]	8.78	7.78	11.09
Theoretical S_{eq} (g / g)	34.5	29.6	23.0
Swelling Exponent (n)	0.29	0.29	0.37
Swelling Constant (K)	1.71	1.55	0.99

The slow rate of swelling of the gels is reflected in the lower values of initial swelling rate (Ri) and Swelling rate constant (Ks) obtained for these gels. The maximum equilibrium swelling ratios calculated theoretically are in good agreement with equilibrium swelling ratios obtained experimentally. The initial swelling rate and the overall swelling rate constants decrease with increasing crosslinking of the gels.

Hydrogels are considered as important materials in agricultural, biomedical, pharmaceutical, and environmental applications. The applicability of these hydrogels may be governed by the mechanism of water diffusion. The absorption process involves the diffusion of water molecules into the free spaces, increasing the segmental mobility leading to expansion of chain segment between crosslinks resulting in swelling. The dynamics of water sorption process was studied by analysing the swelling data obtained for initial 60% of swelling, using the following equation.

$$\text{Swelling ratio}(S) = K\,t^{n} \quad (6)$$

Where S is the swelling ratio defined in eqn.1, ' k' is a swelling constant related to the structure of the network and 'n' is the swelling exponent, which indicates the water transport mechanism. 'k' and 'n' were evaluated from the plots of 'ln S' versus 'ln t' shown in Figure 10.

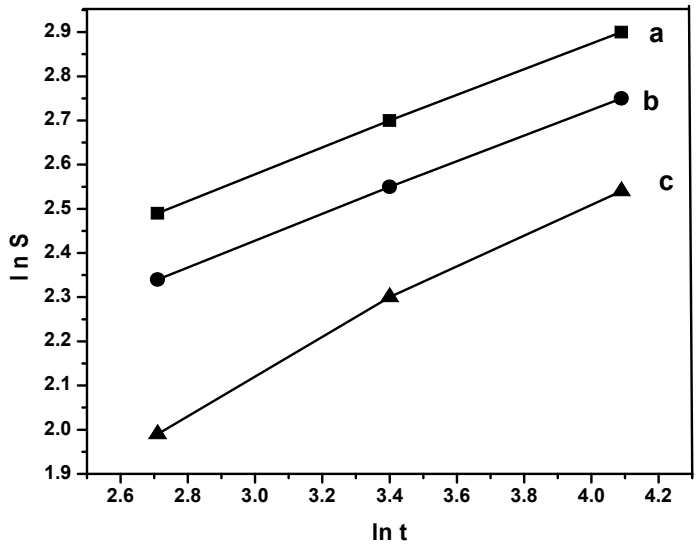


Fig. 10 ln S Vs ln t plots for (a) GL-Alg-1 (b) GL-Alg-2 & (c) GL-Alg-3

The n values were in the range 0.29–0.37. The exponent values are below 0.50 indicating that the diffusion of water is fickian in nature. The diffusion phenomenon of water into the hydrogel is important in various applications.

CONCLUSIONS

The swelling and diffusion characteristics of GL- Na-Alg hydrogels having different amounts of EGDMA were studied. Swelling results indicate that the Na-Alg component has appreciable contribution to the swelling capacity of the gels. The swelling can be considerably controlled by altering the amount of crosslinker in the gel preparation mixture. Further, the gels exhibit pH and salt sensitivity. Higher swelling was observed under neutral and basic conditions. Higher ionic strength and higher charge on metal ions of swelling medium decreased the swelling capacity. The kinetic analysis of swelling data indicates second order swelling. The swelling exponent 'n' is observed to be < 0.5 indicating fickian diffusion to be operative in the presently studied gels. As the presently studied gels contain high water content (> 95%), and pH and salt responsive swelling behavior, they may find various biomedical applications.

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