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Preparation and characterization of hybrid pH-sensitive hydrogels of chitosan-co-acrylic acid for controlled release of verapamil

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Abstract In the present work crosslinked hydrogels based on chitosan (CS) and acrylic acid (AA) were prepared by free radical polymerization with various feed compositions using *N*,*N* methylenebisacrylamide (MBA) as crosslinking agent. Benzoyl peroxide was used as catalyst. Fourier transform infrared spectra (FTIR) confirmed the formation of the crosslinked hydrogels. This hydrogel is formed due to electrostatic interaction between cationic groups in CS and anionic groups in AA. Prepared hydrogels were used for dynamic and equilibrium swelling studies. For swelling behavior, effect of pH, polymeric and monomeric compositions and degree of crosslinking were investigated. Swelling studies were performed in USP phosphate buffer solutions of varying pH 1.2, 5.5, 6.5 and 7.5. Results showed that swelling increased by increasing AA contents in structure of hydrogels in solutions of higher pH values. This is due to the presence of more carboxylic groups available for ionization. On the other hand by increasing the chitosan content swelling increased in a solution of acidic pH, but this swelling was not significant and it is due to ionization of amine groups present in the structure of hydrogel. Swelling decreased with increase in crosslinking ratio owing to tighter hydrogel structure. Porosity and sol-gel fraction were also measured. With increase in CS and AA contents porosity and gel fraction increased, whereas by increasing MBA content porosity decreased and gel fraction increased. Furthermore, diffusion coefficient (D) and the network parameters i.e., the average molecular weight between crosslinks (M_c), polymer volume fraction in swollen state (V_{2s}), number of repeating units between crosslinks (M_r) and crosslinking density (q) were calculated using Flory-Rehner theory. Selected samples were loaded with a model drug verapamil. Release of verapamil depends on the ratios of CS/AA, degree of crosslinking and pH of the medium. The release mechanisms were studied by fitting experimental data to model equations and calculating the corresponding parameters. The result showed that the kinetics of drug release from the hydrogels in both pH 1.2 and 7.5 buffer solutions was mainly non-Fickian diffusion.

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1 Introduction

Hydrogels are three-dimensional crosslinked polymeric structures that are able to swell in an aqueous environment. Due to its specific properties such as hydrophilicity, swellability in water, biocompatibility and nontoxicity, hydrogels have been used in a wide range of biological, medical and pharmaceutical applications [1]. These gels have the ability to respond to changes in their external environmental conditions like pH [2, 3], temperature [4] and ionic strength [5]. Among these systems pH-responsive hydrogels have been exclusively studied in biomedical field for the controlled drug delivery because this factor can be easily controlled [6].

It is known that the oral route is the most convenient and comfortable way of administrating drugs. Owing to different pH in segments in gastrointestinal tract, various types of ionic hydrogels are used for site specific drug delivery. The pH of the medium affects the chain relaxation that leads to swelling and modifies the drug release behavior. Swelling and water contents are the important properties of the ionic hydrogels and these depend upon the fraction of ionizable groups, degree of crosslinking and composition of the medium in which the hydrogels are placed. Swelling of these hydrogels increases as the pH of the medium increases. pKa of the buffer components and pKa of the gel play a very important role in the ionization of ionic gels [7].

AA is a principle commercial super-absorbent and a typical pH-responsive polyelectrolyte. AA based materials offer vast potential for biomedical applications because gels formed from AA can be formulated at varying concentrations. They can be easily fabricated in a wide array of sizes and shapes. Other materials can be incorporated into the AA prior to gel formation and AA is biocompatible while eliciting little antigenic reaction in vivo [8]. In living cells, AA polymers exhibited high tolerance [9]. In addition, carboxylic groups of AA can form hydrogen bonds with mucin, a glycoprotein secreted locally that coats the mucosal surfaces [10]. This bioadhesive property makes AA a good candidate for many different delivery routes including oral, ocular, and nasal. More importantly, carboxylic groups in AA can interact with various groups thus creating attachments sites for wide range of therapeutics [11]. Many investigations have shown that these interactions exert strong influence on the swelling behavior of hydrogels and there is a great potential for their application in pharmaceutical preparations, particularly in site-specific drug delivery systems [8]. MBA has been used as crosslinking agent in many polymeric networks and showed good biocompatibility without any deterious effects on cell viability and functionality [12].

The utilization of natural polysaccharides in drug delivery continues to be a subject of intense investigation because of their biodegradability and biocompatibility. Many polymeric networks based on polysaccharides e.g. dextran, guar gum and konjac glucomannan with acrylic acid have been prepared [13, 14]. CS is a cationic polymer, it is a linear copolymer polysaccharide consisting of a (1-4)-linked 2-amino-2-deoxy-D-glucose and 2-acetoamido-2-deoxy-D-glucose units. Its properties like nontoxicity, biocompatibility, biodegradability, mucoadhesiveness and pH-sensitive behavior make it a useful vehicle for drug delivery [15]. pH-sensitive behavior of chitosan is due to the large quantities of amino groups on its chain. CS dissolves easily at low pH while it is insoluble at higher pH values. The mechanism of pH sensitive swelling involves the protonation of amine groups of CS under low pH conditions. This protonation leads to chain repulsion, diffusion of proton and counter ions together with water inside the gel and dissociation of secondary interactions [16]. This property has helped its use in the drugs delivery to the stomach [17]. Drugs which are delivered to the intestine undergoes enzymatic degradation in stomach, resulting in a poor bioavailability, this property pauses a limitation. Because, as the matrix gets dissolved in the stomach, the drugs undergo degradation, resulting in low bioavailability and less therapeutic effects.

Present research focused on delivering that drug to small intestine which is degraded in stomach by developing a novel pH-sensitive chitosan-co-acrylic acid (CS-co-AA) using *N*,*N* methylenebisacrylamide (MBA) as a crosslinking agent by free radical polymerization. In this respect, various samples with varying polymeric, monomeric compositions and degree of crosslinking are prepared to investigate their effect on the dynamic and equilibrium swelling for site specific drug delivery. Release of the model drug verapamil from the CS-co-AA hydrogels in USP phosphate buffer of varying pH is studied. Hydrogels are characterized by SEM and FTIR to investigate their surface morphology and structure respectively. Network parameters, sol-gel fraction and porosity of hydrogels are also determined.

2 Materials and methods

2.1 Materials

For the preparation of graft copolymer, acrylic acid (AA), chitosan (CS) (Fluka, Buchs, Switzerland), *N*,*N* methylenebisacrylamide (MBA) as crosslinking agent (Merck, F.R Germany) and benzoyl peroxide (Merck, F.R Germany) as initiator were used. All solvents used were of analytical grade.

2.2 Synthesis of hybrid network of chitosan/acrylic acid (CS/AA)

In present work a series of crosslinked hydrogels were synthesized after the modification of procedure reported earlier [18, 19]. A weighed amount of CS was added into rapidly stirred 1% acetic acid solution (30 ml) at room temperature. Varying amounts of MBA and benzyl peroxide were dissolved in AA. After adding required quantities both solutions were mixed and ethanol was added to make the final weight up to 100 g. The resultant solution was poured into polyethylene tube having 16 mm internal diameter and 150 mm length. The air above the solution was removed with nitrogen as it was bubbled through the

reaction mixture for 15–20 min, which act as free radical scavenger. The capped tubes were placed in the water bath and the temperature was increased gradually to avoid bubble formation. The temperature program for CS/AA was 45°C for 1 h, 50°C for 2 h, 55°C for 3 h, 60°C for 4 h, 65°C for 12 h. The hydrogels obtained were cut into discs of 8 mm length and immersed into 50:50 v/v ethanol-water mixture at room temperature, these gel discs were thoroughly washed until the pH of mixture was same as the mixture before washing. The hydrogels obtained were dried at 40°C to constant weight and stored in the desiccators for further use. The presumptive structure of prepared gel is shown in Fig. 1. A list of different formulations of chitosan/AA is given in Table 1.

Table 1 Different formulations of Chitosan/AA hydrogel

Name of the samples	CS/100 g solution	AA/100 g solution	AA/CS (wt%)	MBA/100 g solution
S1	0.15	37.50	99.60/0.39	0.30
S2	0.30	37.50	99.20/0.79	0.30
S3	0.60	37.50	98.40/1.57	0.30
S4	0.60	18.75	96.89/3.10	0.30
S5	0.60	25.00	97.65/2.34	0.30
S6	0.60	31.25	98.11/1.88	0.30
S7	0.60	31.25	98.11/1.88	0.05
S8	0.60	31.25	98.11/1.88	0.10
S9	0.60	31.25	98.11/1.88	0.15

2.3 Swelling characterization

2.3.1 Dynamic and equilibrium swelling

The dynamic and equilibrium swelling ratio were studied in 100 ml medium of pH 1.2, 5.5 6.5 and 7.5. Dried hydrogels were left to swell in a solution of desired pH (1.2–7.5), at a temperature of 37°C. For dynamic swelling, swollen gels removed from the medium, blotted with filter paper, weighed, at regular intervals for 8 h and placed in the same flask. The swelling ratio of each hydrogel was calculated from the following relation [20].

$$q = W_h/W_d \quad (1)$$

where W_h is the weight of swollen gel at time t, and W_d is the initial weight of dry gel.

Swelling ratio was equilibrium when hydrogel reached a constant weight. For equilibrium swelling the swollen gels were weighed regularly to a constant weight which takes 2–3 weeks.

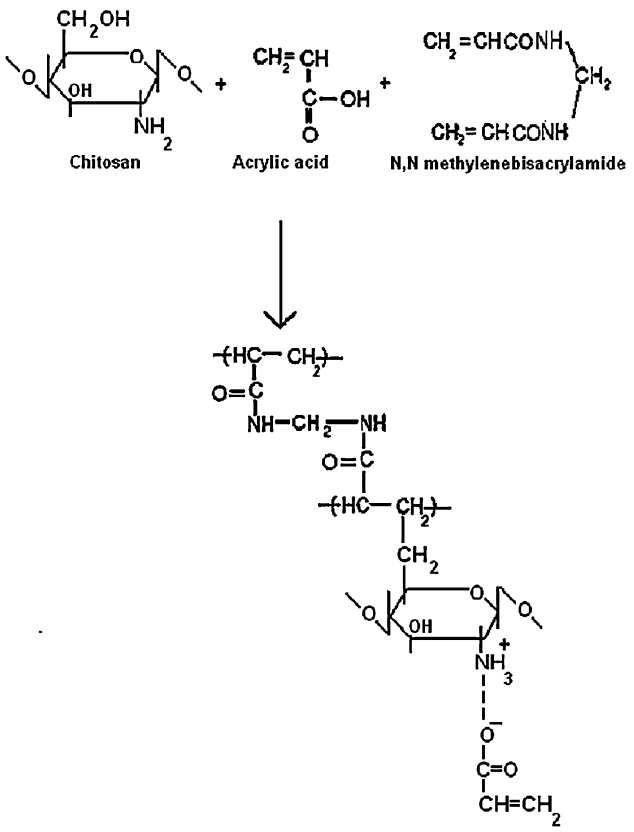

Fig. 1 Semi interpenetrating polymeric networks of CS/AA hydrogels

2.3.2 Diffusion coefficient

Release of drug from the hydrogels generally occurs by a diffusion mechanism. Water diffusion coefficients of hydrogels were calculated by the following equation:

$$D = \pi\left(\frac{h.\theta}{4.Q_{eq}}\right)^2 \quad (2)$$

where D is diffusion coefficient of the hydrogels, Q_{eq} is the swelling of the gel at equilibrium, θ is the slop of the linear part of the swelling curves and h is the initial sample thickness before swelling [21].

2.4 Characterization of network structure of CS/AA hydrogels

2.4.1 Solvent interaction parameters (χ)

Solvent interaction parameters (χ) are calculated by Flory-Huggins theory. Equation used to calculate χ values is given below:

$$\chi = \frac{\ln(1 - v_{2,s}) + v_{2,s}}{v_{2,s}^2} \quad (3)$$

$V_{2,s}$ (ml/mol) is volume fraction of the swollen hydrogel in the equilibrium state and x is the Flory-Huggins polymer solvent interaction parameters [21].

2.4.2 Molecular weight between crosslinks (M_c)

Flory-Rehner theory is used to calculate the values of M_c between interpenetrating polymeric networking CS/AA hydrogels. According to this theory M_c values increased with the increase of swelling ratio of hydrogels. Molecular weight between crosslinks is calculated by the following equation [22];

$$Mc = -\frac{d_p v_s \left(v_{2,s}^{1/3} - v_{2,s}/2\right)}{\ln(1 - v_{2,s}) + v_{2,s} + x v_{2,s}^2} \quad (4)$$

2.4.3 Volume fraction of polymer

Volume fraction of the polymer (V_{2s}) in the swollen gel is a measure of the amount of fluid that a hydrogel can incorporate into its structure. It is calculated by the following equation [23];

$$V_{2,s} = \left[1 + \frac{d_p}{d_s}\left(\frac{M_a}{M_b} - 1\right)\right]^{-1} \quad (5)$$

where d_p and d_s are densities (gm/ml) of the hydrogel and solvent respectively. M_a and M_b are the masses (gm) of the swollen and dry hydrogels respectively. $V_{2,s}$ (ml/mol) is volume fraction of the swollen hydrogel in the equilibrium state and χ is the Flory-Huggins polymer solvent interaction parameters.

2.4.4 Crosslinked density (q)

Crosslinked hydrogels are characterized by crosslinked density [24]. The equation used for crosslinked density is given below;

$$q = \frac{M_C}{M_r} \quad (6)$$

where M_r is molar mass of the repeat unit and is calculated as;

$$M_r = \frac{m_{CS}M_{CS} + m_{AA}M_{AA} + m_{MBA}M_{MBA}}{m_{CS} + m_{AA} + m_{MBA}} \quad (7)$$

where m_{CS}, m_{AA} and m_{MBA} are the masses of the polymer CS, AA and MBA respectively. While M_{CS}, M_{AA} and M_{MBA} are the molar masses of CS, AA and MBA respectively.

2.5 Sol-gel fraction

Hydrogel samples were cut into pieces with a diameter of 3–4 mm, dried in a vacuum oven at 45°C to a constant weight (W_o), and subjected to Soxhlet extraction for 4 h with deionized water as solvent. Uncrosslinked polymer was removed with this extraction from the gel structure. Extracted gels were dried again in a vacuum oven at room temperature to constant weight (W_1). The gel fraction was calculated according to the following equations [25];

$$\text{Sol fraction}(\%) = \left[\frac{W_o - W_i}{W_o}\right] \times 100 \quad (8)$$

$$\text{Gel fraction}(\%) = 100 - \text{Sol fraction} \quad (9)$$

2.6 Porosity measurement

For porosity measurement, the solvent replacement method was used. Dried hydrogels were immersed in absolute ethanol over night and weighed after blotting excess ethanol on the surface. The porosity was calculated from the following equation:

$$\text{Porosity} = \left[\frac{M_2 - M_1}{pV}\right] \times 100 \quad (10)$$

where M_1 and M_2 are the mass of the hydrogel before and after immersion in ethanol, respectively: p is density of absolute ethanol and V is the volume of the hydrogel [26].

2.7 Loading of verapamil into crosslinked CS/AA hydrogel

Samples which showed maximum swelling were used for drug loading and release studies. The drug loading into the disks of hydrogel was achieved by soaking them for 1 week in solution of the drug. A 1% w/v verapamil solution in ethanol–water mixture (50:50% v/v) was used for drug loading. After achieving the equilibrium value, swelled hydrogels were removed from the drug solution, blotted with filter paper, first dried at room temperature and then they were placed in an oven at 40–45°C for 1 week for removing the absorbed solvent.

For determining the percent drug-loading, weighed drug loaded sample was extracted repeatedly using phosphate buffer solution of pH 7.5 up to exhaustion and the concentration of the drugs in pooled extract was determined spectrophotometrically at λ_{max} 271 nm. The quantity of drug loaded into the hydrogels was also determined by swelling method.

2.8 Release of verapamil from crosslinked CS/AA hydrogel

Drug release was measured with a flow-through dissolution apparatus (Pharmatest; type PT-DT 7, Germany) associated with UV-Vis. spectrophotometer (IRMECO,

UV-Vis. U2020). The weighed polymer disk was immersed in 500 ml dissolution medium and dissolution medium was stirred at a rate of 100 rpm for maintaining a uniform drug concentration in the medium. The dissolution media was maintained at 37°C. With each sampling, the solution was changed with fresh medium, maintaining the total volume constant. The determination of verapamil released was carried out at λ_{max} 271 nm with readings taken up to 12 h. Verapamil released was conducted in solutions of USP phosphate buffer 0.2 M (pH 1.2 and pH 7.5).

2.9 Analysis of drug release kinetics

To get an insight into the solute release mechanism zero-order [27], first order [28], Higuchi [29] and Korsmeyer-Peppas [30] models are used. Equations used for these models are as follows:

$$\text{zero-order kinetics} : F_t = K_0 t \quad (11)$$

where F represents the fraction of drug release in time t and K_0 is the apparent rate constant of zero-order release constant.

$$\text{first-order kinetics} : \ln(1 - F) = -K_1 t \quad (12)$$

where F represents the fraction of drug release in time t and K_1 is the first-order release constant.

$$\text{Higuchi model} : F = K_2 t^{1/2} \quad (13)$$

where F represents the fraction of drug release in time t and K_2 is the Higuchi constant.

$$\text{Korsmeyer-Peppas model} : Mt/M_\infty = K_3 t^n \quad (14)$$

where K_3 is a constant incorporating the structural and geometric characteristics of the gels and n is the release exponent. When $n = 0.45$ order of release is Fickian, $n = 0.89$ corresponds to case II transport, while $0.45 < n > 1.0$, the diffusional mechanism, is non-Fickian. No kinetic data or n values were calculated when swelling and drug release was not significant.

2.10 Scanning electron microscopy

The morphology of the hydrogel sample was investigated using, scanning electron microscope S3400-N (Hitachi).

2.11 FTIR spectroscopic studies

Hydrogel sample was crushed with pestle in an agate mortar. The crushed material was mixed with potassium bromide (Merck IR spectroscopy grade) in 1:100 proportions and dried at 40°C. The mixture was compressed to 12 mm semitransparent disk by applying a pressure of 65 kN (Pressure gauge, Shimadzu) for 2 min. The FTIR spectrum over the wavelength range 4,000–400 cm^{-1} was recorded using FTIR spectrometer (FT-IR 8400 S, Shimadzu).

3 Results and discussion

3.1 Effect of pH on swelling and on drug release

CS and AA containing hydrogels were used for swelling and drug release studies in solutions of different pH. This hydrogel is a polyampholytic type hydrogels containing both amine and carboxylic groups. The dynamic and equilibrium swelling ratio is high in solutions of pH 6.5 and 7.5. At low pH, it showed little swelling. The swelling at low pH is due to the protonation of amino groups of CS. As the amino groups are protonated, they are ionized. This ionization causes swelling due to electrostatic repulsion [31]. On increasing the pH of the medium above the pKa values of the AA (4.26), its hydrophilicity increases after ionization. As the pH of the medium increases above the pKa values of the acidic component of the polymer, it starts swelling due to the ionization of the carboxyl groups. The ionization in turn stretches the coiled molecules to an extant which depends on the percent ionization of the carboxylic groups [32]. The swelling ratio of hydrogels in acidic medium is always less than that in alkaline medium.

For drug loading, samples (S4–S9) which showed substantial swelling were selected. Verapamil was used as model drug due to its water solubility. Table 2 shows the amount of verapamil loaded g/g of dry gel in samples with varying amount of AA and MBA. Samples with varying amount of CS are not selected for drug loading because these samples did not show any significant swelling.

To investigate the effect of pH on the drug release, drug loaded samples were immersed in buffer solutions of pH i.e. 1.2 and 7.5 at 37°C. In all samples drug release increased, as the pH of the medium was increased. The results of drug release can be correlated with the results of swelling which showed that water uptake by the polymer increased with increasing pH of the medium.

3.2 Effect of polymeric and monomeric composition on swelling and on drug release

Effect of polymeric and monomeric composition on swelling was studied in solutions of varying pH. Figure 2 shows the effect of polymer concentration and Fig. 3 shows the effect of monomer concentration on the dynamic swelling keeping the degree of crosslinking constant. Table 3 shows the effect of polymer concentration,

Table 2 Amount of verapamil loaded in different samples of CS/AA hydrogels

Samples name	Composition CS/AA	Degree of crosslinking MBA % W/W	Amount of verapamil loaded (g/g of dry gel)	
			By swelling	By extraction
S4	0.60/18.75	0.30	0.028	0.025
S5	0.60/25	0.30	0.029	0.027
S6	0.60/31.25	0.30	0.030	0.028
S7	0.60/31.25	0.05	0.048	0.045
S8	0.60/31.25	0.10	0.046	0.044
S9	0.60/31.25	0.15	0.044	0.041

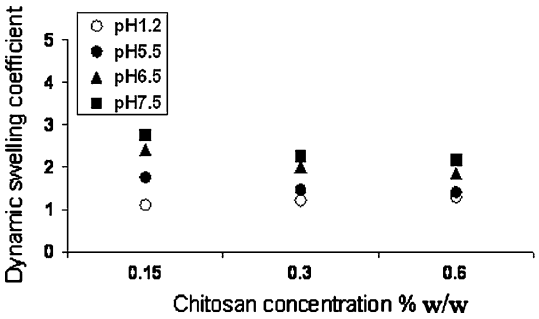

Fig. 2 Dynamic swelling after 8 h of CS/AA copolymer with different chitosan concentrations i.e. (0.15%, 0.3%, 0.6%) using 0.30% MBA as crosslinking agent in solutions of various pH at 37°C. The pH values are: pH 1.2 (*open circle*), pH 5.5 (*filled circle*), pH 6.5 (*filled triangle*) and pH 7.5 (*filled square*)

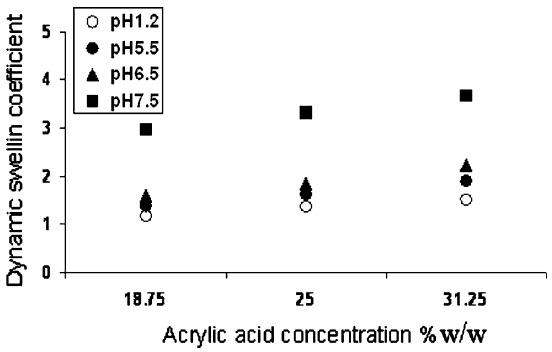

Fig. 3 Dynamic swelling after 8 h of CS/AA copolymer with different acrylic acid concentrations i.e. (18.75%, 25%, 31.25%) keeping concentration of MBA constant at 0.30%, in solutions of various pH at 37°C. The pH values are: 1.2 (*open circle*), pH 5.5 (*filled circle*), pH 6.5 (*filled triangle*) and pH 7.5 (*filled square*)

monomer concentration and crosslinking agent on equilibrium swelling in solutions of different pH.

It was observed that by increasing the CS content keeping AA concentration constant as shown in Table 3, the swelling ratio (both dynamic and equilibrium) increases in solution of acidic pH (1.2). This could be attributed to the presence of amine groups and these groups ionize in low pH. This ionization increased swelling because of electrostatic repulsions. But at high pH (7.5), increase in CS concentration resulted in decrease in swelling ratio. This could be attributed by the fact as number of amine groups increased more carboxylic groups are linked to it as a result less number of carboxylic groups are available for ionization, ultimately decrease in swelling ratio with increase in CS concentration.

AA is anionic monomer, containing carboxylic group. It was observed that by increasing the AA content keeping CS content constant as shown in Table 3, the swelling increases in a solution of high pH as well as in solution of low pH but it was not substantial at low pH. Swelling at higher pH values is due to the presence of more carboxylic groups available for ionization.

To investigate the effect of monomer on the drug release three samples of CS/AA 0.6/18.75, 0.6/25 and 0.6/31.25 using 0.30% w/w MBA as crosslinking agent were selected. Release profile of verapamil from selected samples for 12 h in phosphate buffer solution of pH 1.2 and 7.5 at 37°C are presented in Fig. 4. Drug release increased as the contents of AA were increased at low as well as at high pH. Huang et al. [8] prepared guar gum/poly(acrylic acid) hydrogels and observed the similar swelling and drug release behavior. They reported that the swelling and ketoprofen release increased with increase in PAA content in the gel structure. This phenomenon can be explained on the basis of more swelling due to enhanced number of ionizable groups as concentration of AA increases at pH 7.5, which leads to increased polymer chain relaxation hence increased swelling and drug release.

3.3 Effect of degree of crosslinking on swelling and on drug release

Figure 5 and Table 3 show the effect of concentration of crosslinking agent on the dynamic and equilibrium swelling respectively while keeping CS/AA composition constant i.e. 0.6/31.25. It was observed that increasing the crosslinking percentage as shown in Table 3, the swelling decreases both at low as well as at high pH. This effect was

Table 3 Equilibrium swelling coefficient of CS/AA gels using MBA as crosslinking agent

Sample code	Composition CS/AA	Degree of crosslinking MBA % W/W	pH of the solution			
			1.2	5.5	6.5	7.5
S1	0.15/37.50	0.30	1.34	1.99	2.84	[a]
S2	0.30/37.50	0.30	1.42	1.50	2.98	[a]
S3	0.60/37.50	0.30	1.51	1.46	2.11	[a]
S4	0.60/18.75	0.30	1.55	1.85	2.26	[a]
S5	0.60/25	0.30	1.70	2.22	2.46	[a]
S6	0.60/31.25	0.30	1.82	2.35	2.85	[a]
S7	0.60/31.25	0.05	1.84	2.48	[a]	[a]
S8	0.60/31.25	0.10	1.58	2.42	[a]	[a]
S9	0.60/31.25	0.15	1.46	2.37	[a]	[a]

[a] The samples broke

significant in dynamic swelling. For equilibrium swelling some samples broke at higher pH values. Mechanistically the effect of increased crosslinking can be explained by two reasons: firstly, it is due to decrease mesh size of the hydrogels; secondly, high crosslinked polymers are less acidic as compared to non-crosslinked polymers having same composition because as mesh size is reduced the carboxylic groups are concealed and higher degree of crosslinking hinders the ionization process hence lower relaxation ability of polymeric chains [33].

Three samples with same CS/AA composition (0.6/31.25) but different concentration of crosslinking agent (MBA) (i.e. 0.05%, 0.10%, 0.15%) were used to investigate the effect of concentration of crosslinking agent on the drug release behavior. It was observed that increase in MBA concentration resulted in the decrease in drug release as indicated in Fig. 6. This could be explained by the fact that increase in concentration of crosslinking agent results in the increase in the entanglement due to hydrogen bonding between chitosan and acrylic acid which provided elastic restrained forces to retard the expending of network hence decreased chain relaxation and decreased drug release.

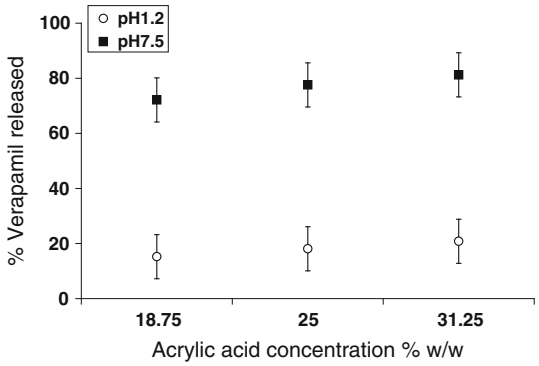

Fig. 4 Effect of monomeric concentration on the verapamil release after 12 h from chitosan-co-acrylic acid (CS/AA) hydrogels using 0.30% MBA as crosslinking agent in solution of pH 1.2 (*open circle*) pH 7.5 (*filled square*). [Loading: S6 = 2.8% w/w; S5 = 2.7% w/w; S4 = 2.5% w/w]

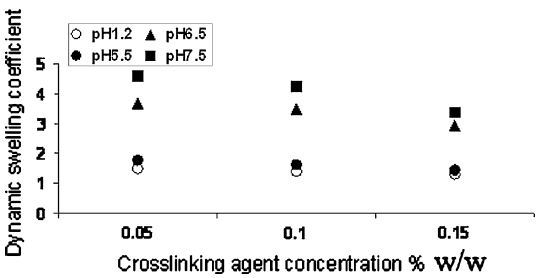

Fig. 5 Dynamic swelling after 8 h of CS/AA copolymer with various crosslinking agent concentrations i.e. (0.05%, 0.10%, 0.15%) keeping concentration of chitosan and acrylic acid constant, in solutions of various pH at 37°C. The pH values are: pH 1.2 (*open circle*), pH 5.5 (*filled circle*), pH 6.5 (*filled triangle*) and pH 7.5 (*filled square*)

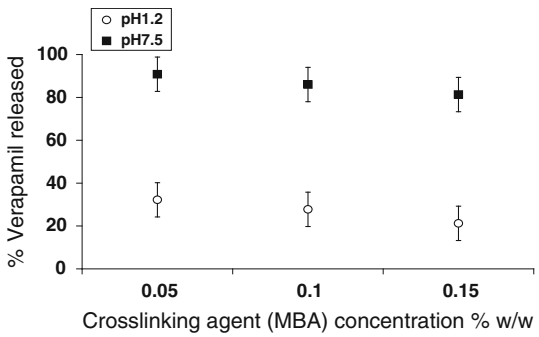

Fig. 6 Effect of crosslinking agent concentration on the verapamil release after 12 h from chitosan-co-acrylic acid (CS/AA) hydrogels, keeping chitosan and acrylic acid concentration constant, in a solution of pH 1.2 (*open circle*) pH 7.5 (*filled square*). [Loading: S9 = 4.1% w/w; S8 = 4.4% w/w; S7 = 4.5% w/w]

3.4 Diffusion coefficient of polymers (D)

Diffusion of water from the gels determines the release of the drugs from them, therefore diffusion coefficient phenomena has been investigated for only selected samples which showed maximum swelling at high pH. Fick's law of diffusion was applied. It was observed that diffusion coefficient decreased by increasing the concentration of AA because swelling of hydrogel increased as the AA concentration increased. Table 4 shows that diffusion coefficient changes with varying concentration of the AA.

3.4.1 Molecular weight between crosslinks (M_c) and solvent interaction parameters (χ)

The values of Mc and χ are shown in the Table 4. It was observed that values of χ decreased by increasing contents of AA in the gels. This represents the hydrophilic nature of the chains and strong interaction with solvent. From these χ values, M_c and $V_{2,s}$ values were calculated. The results show that there is an increase in M_c values with increasing the AA content in the gels. This effect indicates that AA imparts hydrophilicity by ionization of COOH groups, which is added into the chains that resulted in an increase in M_c values and subsequently show higher swelling. The values of n are related to the number of average molecular weight between crosslinks. According to this approach as AA contents in the gel increases, chain length also increases which results in the increase of M_c values [22].

3.5 Gel fraction

Figures 7, 8, 9 show gel fraction of different formulations of CS/AA. It was observed that by increasing the concentration of CS (S1–S3), AA (S4–S6) and MBA (S7–S9), the sol fraction tend to decrease while gel fraction increased which results in more grafting. Dergunova et al. [34] prepared hydrogel of chitosan and polyvinyl pyrrolidone and showed that by increasing CS content the gel fraction increases. Similar trend observed with increased AA. As more the amount of crosslinking agent in the initial solution more will be the crosslinked hydrogels which increased gel fraction.

Table 4 Flory-Huggins network parameters of chitosan/acrylic acid hydrogels

Sample code	V_{2s}	χ	M_c	M_r	q	D 10^{-6} (cm^2/s)
S4	0.11	0.57	1897.63	77.26	24.56	7.7
S5	0.09	0.55	2054.47	81.78	25.12	6.1
S6	0.06	0.54	2139.14	82.36	25.97	5.3

V_{2s} Volume fraction of the polymer at swelling equilibrium in USP phosphate buffer solution, χ solvent interaction parameter, M_c number of average molecular weight between crosslinks, Mr molar mass of repeat unit, q crosslinking density, D duffusion coefficient

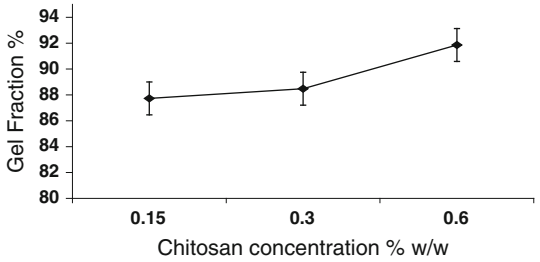

Fig. 7 The effect of chitosan concentration on the Gel fraction of CS/AA Hydrogel

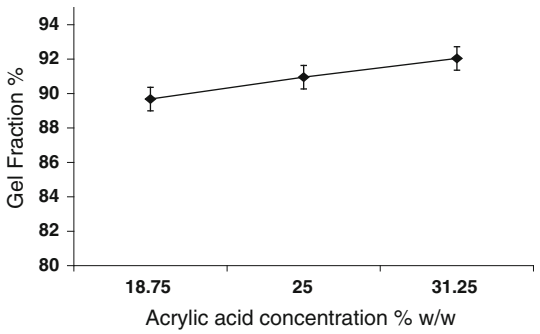

Fig. 8 The effect of acrylic acid concentration on the Gel fraction of CS/AA Hydrogel

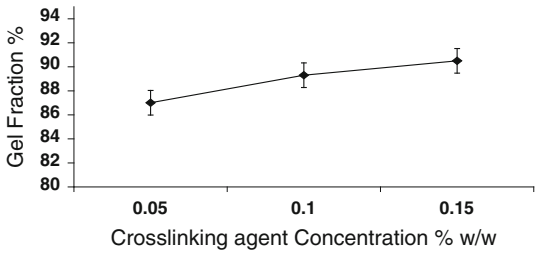

Fig. 9 The effect of crosslinking agent (MBA) concentration on the Gel fraction of CS/AA Hydrogel

3.6 Porosity

Table 5 shows the porosity of different formulations of CS/AA. It was observed that by increasing the CS or AA contents in the samples (S1–S6) porosity increases. This could be attributed by the fact that by increasing CS or AA contents viscosity of solution enhanced, which efficiently prevented the bubbles escaping from the solution thus forming interconnected channels thus increased porosity. On the other hand by increasing the MBA content (S7–S9)

Table 5 Porosity of different formulations of CS/AA hydrogels

Sample code	Composition CS/AA (100 g solution)	Porosity (%)
S1	0.15/37.50	18.09
S2	0.30/37.50	19.22
S3	0.60/37.50	20.10
S4	0.60/18.75	14.66
S5	0.60/25.00	21.54
S6	0.60/31.25	27.22
S7	0.60/31.25	53.05
S8	0.60/31.25	39.20
S9	0.60/31.25	25.30

porosity decreases. This could be explained by the fact that increasing in crosslinking density molecular entanglement between CS and AA enhanced which result in decreased mesh size of the hydrogel, less pore formation and ultimately decreased porosity [35].

3.7 Drug release mechanism

The model that best fits the release data was evaluated by the correlation coefficient (r), a criterion for selecting the most appropriate model was based on the ideal fit indicated by the values of correlation coefficient (r) nearer to 1.

The correlation coefficient (r) obtained for CS/AA at varying content of AA (18.75%, 25% and 31.25%) and degree of crosslinking (0.05%, 0.10% and 0.15%) are given in Tables 6 and 7, respectively. The value of r obtained for zero-order release rate constants were found higher compared with those of first-order rates indicating that drug release from the samples of varying monomeric compositions and degree of crosslinking were according to zero-order release.

The r value of Higuchi model at different mononmeric composition and at different degree of crosslinking indicated that the drug release mechanism was diffusion controlled.

Effects of monomer composition and degree of crosslinking on order of release (n) and r values are given in Tables 8 and 9 respectively. Non-Fickian behavior was observed in all samples. This indicates that the water transport mechanism becomes non-Fickian as gel ionization becomes prominent.

3.8 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed to see the morphology of hydrogels. Figure 10a showing sponge like fibrilar surface. This structure could be due to electrostatic interaction between cationic groups of chitosan

Table 6 Effect of monomer composition on release kinetics of CS/AA in the solution of different pH at fixed crosslinking percentage i.e. 0.30% w/w

Sample code	AA contents (%)	pH	Zero order kinetics		First order kinetics		Higuchi equation	
			$K_0(h^{-1})$	r	$K_1(h^{-1})$	r	$K_2(h^{-1})$	r
S4	18.75	1.2	1.0965	0.9444	0.1547	0.7998	4.2191	0.9949
		7.5	5.2438	0.8740	0.4034	0.6353	21.149	0.9650
S5	25	1.2	1.2297	0.8802	0.1449	0.7069	4.9422	0.9686
		7.5	5.5460	0.8784	0.1942	0.6330	22.276	0.9657
S6	31.25	1.2	1.3326	0.8713	0.4460	0.6800	5.3474	0.9573
		7.5	5.4341	0.8533	0.1857	0.6034	22.069	0.9519

Table 7 Effect of degree of crosslinking on release kinetics of CS/AA (0.6/31.25) in the solution of different pH

Sample code	MBA contents (%)	pH	Zero order kinetics		First order kinetics		Higuchi equation	
			$K_0(h^{-1})$	r	$K_1(h^{-1})$	r	$K_2(h^{-1})$	r
S7	0.05	1.2	2.1803	0.9620	0.1602	0.6749	8.4813	0.9857
		7.5	5.6775	0.8559	0.1751	0.5906	23.056	0.9590
S8	0.10	1.2	1.6879	0.8907	0.1457	0.6565	6.6768	0.9647
		7.5	5.4410	0.8587	0.1720	0.5694	22.062	0.9533
S9	0.15	1.2	1.6040	0.9279	0.1715	0.7700	6.2550	0.9592
		7.5	5.4930	0.8611	0.1776	0.5834	22.350	0.9905

Table 8 Effect of monomer composition on drug release mechanism of CS/AA in the solution of different pH at fixed crosslinking percentage i.e. 0.30% w/w

Sample code	AA contents (%)	pH	Release exponent (n)	*r*	Order of release
S4	18.75	1.2	0.4909	0.7774	Non-Fickian
		7.5	0.6024	0.6099	Non-Fickian
S5	25	1.2	0.4638	0.6918	Non-Fickian
		7.5	0.6095	0.6085	Non-Fickian
S6	31.25	1.2	0.4631	0.6657	Non-Fickian
		7.5	0.5751	0.5723	Non-Fickian

Table 9 Effect of degree of crosslinking on drug release mechanism of CS/AA (0.6/31.25) in the solution of different pH

Sample code	MBA contents (%)	pH	Release exponent (n)	*r*	Order of release
S7	0.05	1.2	0.4761	0.6216	Non-Fickian
		7.5	0.5075	0.5043	Non-Fickian
S8	0.10	1.2	0.4561	0.6016	Non-Fickian
		7.5	0.5030	0.5011	Non-Fickian
S9	0.15	1.2	0.4819	0.7704	Non-Fickian
		7.5	0.5210	0.5244	Non-Fickian

and anionic groups of acrylic acid. Figure 10b showing drug particles in hydrogel at high magnification (×5,000).

3.9 FT-IR spectroscopy

Figure 11 represents the FTIR spectra CS, CS/AA polymer complex, CS/AA polymer complex with drug and PAA. CS has characteristic peaks at 1643 and 1558 cm^{-1} assigned to amide I and II. PAA has carbonyl absorption band at 1715 cm^{-1} [8]. The bands at 1563 (asymmetrical COO^{-}) and 1460 cm^{-1} (symmetrical COO^{-}) present in the spectrum of hydrogel, together with the band at 1707 cm^{-1} (attributed to the formation of NH_3^{+}) are indicative of complexation reaction between amino groups of CS and the carboxylic groups of AA. Other characteristics peaks at 2853 and 2923 cm^{-1} represent CH_2 groups. The band at 3438 cm^{-1} represents OH stretch. From the FTIR it is clear that there is no significant shift in major peaks, which indicates that there is no chemical interaction between the polymer and the drug used.

4 Conclusion

pH-sensitive hydrogels composed of CS and AA in the presence of MBA as crosslinking agent have been synthesized by free radical polymerization. This prepared hydrogel is biocompatible nontoxic and suitable for controlled drug release. Swelling ratio of crosslinked hydrogels was more in alkaline media than in acidic media. Samples with high porosity showed high swelling at low as well as at high pH values. Gel fraction increased as the CS, AA and degree of crosslinking increased. The equilibrium swelling of CS-co-AA graft copolymer hydrogels decreased with increasing crosslinking ratio. The SEM revealed the sponge like porous structure. Verapamil was loaded as model drug. In all samples drug release increased, as pH of medium increased. Hydrogels with high content of acrylic acid showed more drug release than those gels with low content of AA. Whereas decreasing trend in drug release was observed with increasing degree of crosslinking. The analysis of release showed that verapamil might be released from CS/AA hydrogels by non-Fickian diffusion mechanism. The results of the release experiment of verapamil from gel indicated that CS/AA could be a potential pH-sensitive carrier for a colon-specific drug delivery system.

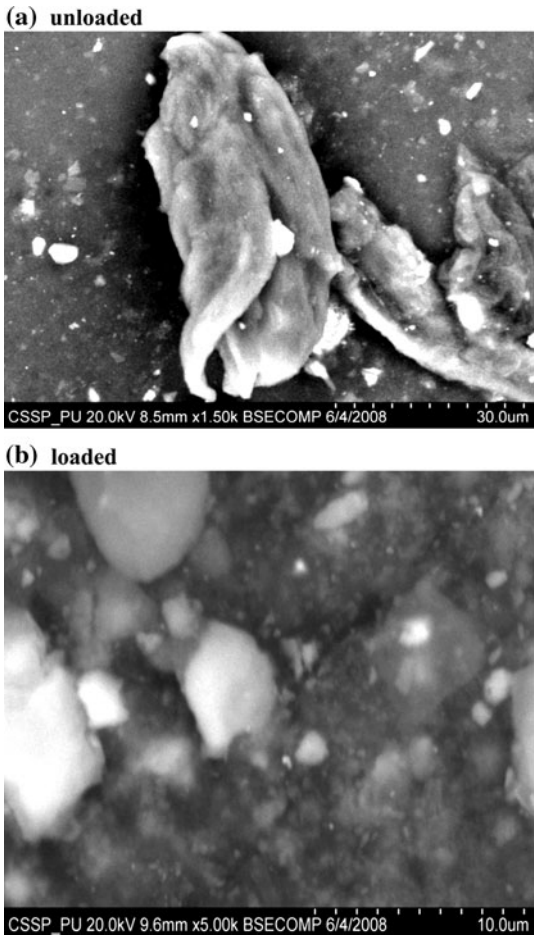

Fig. 10 Scanning electron micrographs of sample S7. **a** Magnification ×1,050, **b** Magnification ×5,000

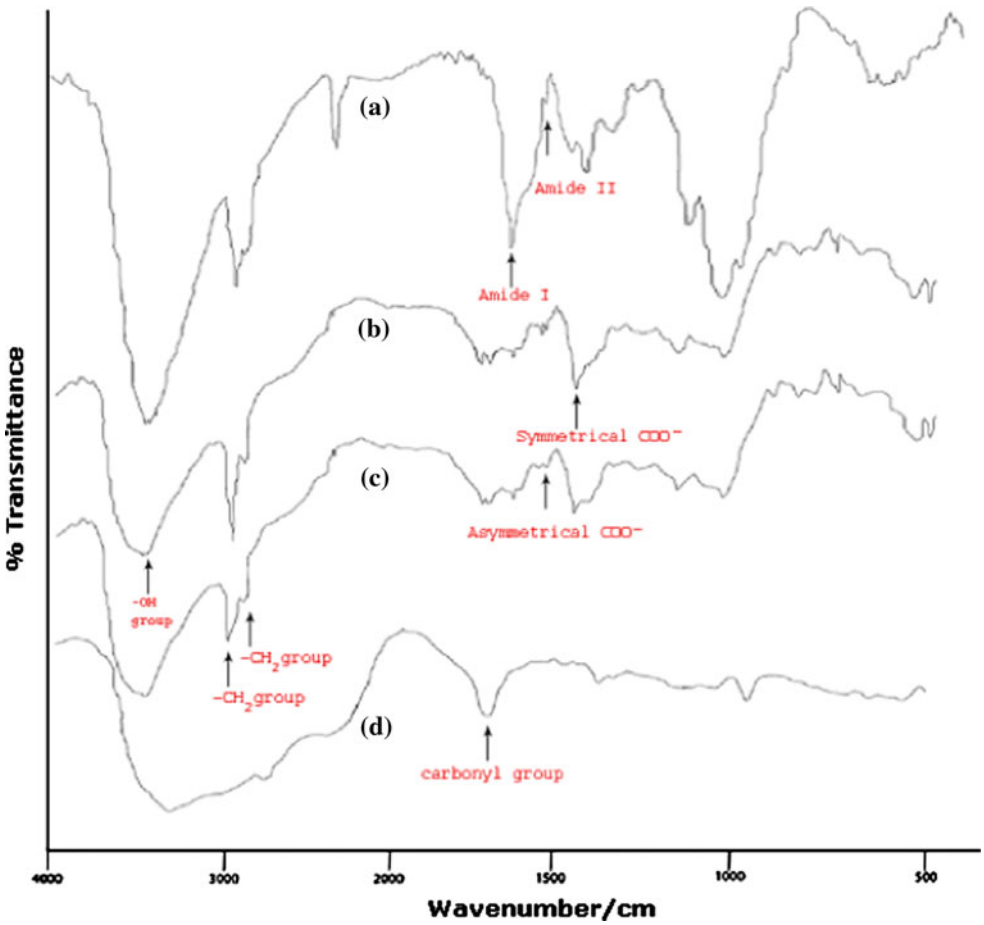

Fig. 11 FTIR spectra *a* chitosan, *b* CS/AA hydrogel without drug, *c* CS/AA hydrogel with drug, *d* acrylic acid

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