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Novel chitosan-based pH-sensitive interpenetrating network microgels for the controlled release of cefadroxil ☆

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Abstract

This paper addresses development of novel pH-sensitive interpenetrating polymeric network (IPN) microgels (MGs) based on chitosan, acrylamide-*grafted*-poly(vinyl alcohol) and hydrolyzed acrylamide-*grafted*-poly(vinyl alcohol) that are crosslinked with glutaraldehyde and used in the controlled release (CR) of cefadroxil, an antibiotic drug. The MGs formed were characterized by Fourier transform infrared spectroscopy to confirm the grafting and crosslinking reaction, scanning electron microscopy to understand the surface morphology and differential scanning calorimetry to conform a uniform distribution of the drug in the polymer matrix. Swelling experiments on hydrogels provided important information on drug diffusion properties. In vitro release results performed in acidic and basic media affected the drug release characteristics. Release data have been analyzed using an empirical equation to understand about the transport of drug containing solution through the polymeric matrices. Extent of crosslinking was studied in terms of size of MGs as well as their release characteristics. Effect of drug loading on encapsulation efficiency was investigated to find a linear manner. IPN matrices of this study were able to extend the release rates from conventional dosage release times to more than 10 h.

Keywords: Chitosan; Poly(vinyl alcohol); Microgels; Cefadroxil; Controlled release

1. Introduction

Biodegradable or bioerodible carbohydrate polymers are useful as drug delivery devices due to their major advantages in eliminating surgical operations to remove the implanted delivery devices after when the release system is exhausted. Of particular interest are the biodegradable hydrogels that are crosslinked three-dimensional network polymers, which swell, but do not dissolve when in contact with water. In the swollen state, hydrogels become soft and rubbery resembling those of the living tissues (Beena, Chandi, & Sharma, 1995; Pourjavadi, Mahdavinia, & Zohuriaan-Mehr, 2003; Yazdani & Retuert, 2004). Hydrogels are known to exhibit good biocompatibility and are highly responsive to external media such as temperature, pH, electrical pulses, etc. (Chandy & Sharma, 1992; Chuang, Young, Yao, & Chiu, 1999; Kim, Park, Kim, Shin, & Kim, 2002). However, the main disadvantage of hydrogels is their poor mechanical properties as a result of extensive swelling. To alleviate this problem and to convert them as controlled release (CR) devices, attempts have been made to modify their structures by physical blending with other polymers (Amiji, 1995; Cascone, Sim, & Downes, 1995; Chandy & Sharma, 1992; Chuang et al., 1999; Koyano, Koshizaki, Umehara, Nagura, & Minoura, 2000), grafting (Kumbar, Soppimath, & Aminabhavi, 2003; Yang, Huang, & Yeh, 1999; Yang, Wang, Hsu, Chang, & Lo, 1998; Yang, Jong, Hsu, & Chang, 1998), developing interpenetrating polymer networks (IPNs) (Bischoff & Cray, 1999; Kim & Sperling, 1997; Kurkuri, Kulkarni, Kariduraganavar, &

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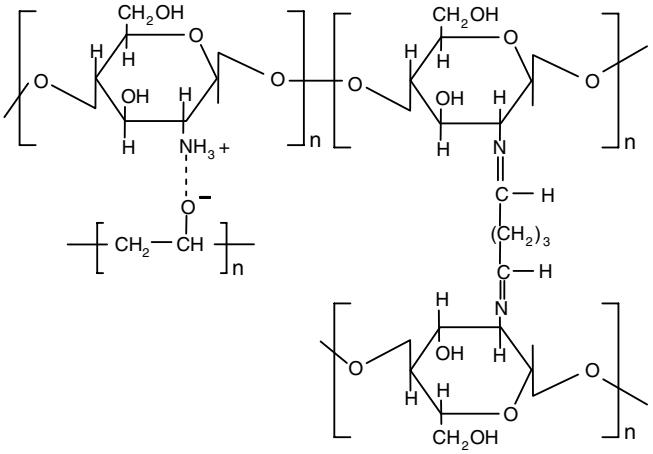
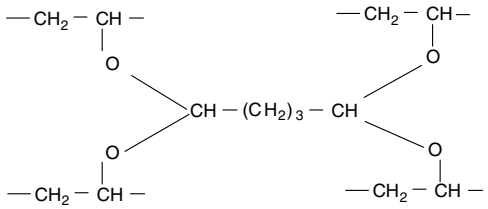
Aminabhavi, 2001; Peniche et al., 1999) or by crosslinking (Kumbar, Kulkarni, & Aminabhavi, 2002).

Among the many polymeric systems studied in the literature, chitosan, being a biodegradable carbohydrate polymer, has been quite widely used as a matrix material in pharmaceutics (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Muzzarelli, Jeuniaux, & Gooday, 1986). The hydrophilicity of chitosan, due to the presence of amine and hydroxy functional groups in its repeat unit, makes the polymer soluble in dilute acidic solutions and yields a rubbery hydrogel in water. Poly(vinyl alcohol) (PVA), on the other hand, has several desirable physical properties such as elasticity and high hydrophilicity (Schellekens & Bastiansen, 1991), making it appropriate to blend with other polymers such as chitosan. Chitosan and modified chitosan hydrogels have several advantages over conventional polymers because of their nontoxicity, biocompatibility and biodegradability. The propensity of chitosan to absorb water and swell into soft rubbery material makes it a good matrix material for incorporating hydrophilic drugs like cefadroxil. Logically, blending of chitosan with PVA or acrylamide-*grafted*-PVA would produce a biodegradable blend system that can be used in the CR of drugs. Blends of chitosan with PVA have been reported in the earlier literature (Kurkuri & Aminabhavi, 2004a; Miya, Iwamoto, & Mima, 1984; Nakatsuka & Andrady, 1992) to study their reversible volume change properties in response to external stimuli such as pH or temperature.

Over the past decade, we have been developing newer polymers or blends of polymers or grafted copolymers for applications in the CR of drugs of various kinds as well as pesticides (Aminabhavi, Kulkarni, Soppimath, Dave, & Mehta, 1999; Dave, Mehta, Aminabhavi, Kulkarni, & Soppimath, 1999; Kumbar et al., 2002, 2003; Kulkarni, Soppimath, Aminabhavi, Dave, & Mehta, 2000; Kurkuri et al., 2001). In recent years, researchers are concentrating on developing modified polymers for the CR of antibiotics (Kulkarni, Soppimath, Aminabahvi, & Rudzinski, 2001). As a further contribution in this area, we report here the development of novel pH-sensitive and biologically compatible IPN microgels of chitosan with PVA, acrylamide-*grafted*-PVA as well as hydrolyzed acrylamide-*grafted*-PVA. Thus, the IPNs of chitosan developed in this work have two different polymers, each in a network form that are crosslinked in the presence of each other to give a three-dimensional network hydrogel structure, producing extra free volume for easy encapsulation of water soluble drug like cefadroxil, an antibiotic drug, which has a biological half-life of 1.2–2 h for a dose containing 0.5 and 1.5 g. It is demonstrated one can enhance the short half-life of cefadroxil by using IPNs prepared in this study. Cefadroxil is produced by chemical coupling of the side chain (D)-*p*-hydroxy phenyl glycine to β-lactam nucleus (see Structure 1). It may be noted that cephalosporin antibiotics used in the treatment of bacterial infections are already available in the market for more than two decades; these contain β-lactam nucleus and a D-amino acid side chain.



Crosslinked chitosan



Crosslinked PVA

Structure 1. Structure of cefadroxil.

To the best of our knowledge, no previous reports are available on the development of IPNs of chitosan with acrylamide-*grafted*-PVA and hydrolyzed acrylamide-*grafted*-PVA matrices for the CR of cefadroxil in both acidic and basic media. The present investigation therefore, deals with the in vitro release studies on IPN microgel formulations loaded with different amounts of cefadroxil. Drug-loaded microspheres were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). Release characteristics of the formulations were studied for their encapsulation efficiency, polymer morphology, drug diffusion rates as well as extent of crosslinking.

2. Materials

2.1. Materials

Cefadroxil (USP grade) was received from Bio-ethicals, Hubli, India. The 75–85% deacetylated chitosan having viscosity of 20-200 cP was purchased from Aldrich Chemical Company, Milwaukee, WI, USA. Analytical Reagent grade samples of acrylamide (Am), poly(-vinyl alcohol) ($M_W = 125{,}000$), hydrochloric acid, glutaraldehyde (25% aqueous solution) (GA), acetic acid and sodium hydroxide were purchased from s.d. Fine Chemicals, Mumbai, India. Chemicals were used without further purification.

2.2. Synthesis of PVA-grafted-acrylamide (PVA-g-AAm)

Acrylamide-*grafted*-PVA, hereafter designated as PVA-*g*-AAm was prepared by mixing PVA with acrylamide at 60 °C using ceric ammonium nitrate (CAN) as an initiator (Kumbar et al., 2003). In brief, 2% aqueous solution of PVA was prepared by dissolving PVA in water overnight under constant stirring conditions. The solution was degassed by passing nitrogen gas for 30 min. To this solution, 0.108 mol of acrylamide was added and stirred thoroughly for 1 h at 60 °C. The initiator solution containing 5.47×10^{-4} mol of CAN was added to the above mixture and stirred at 60 °C for 5 h. The reaction mixture was then cooled and a pinch of hydroquinone was added to quench the reaction. The PVA-*g*-AAm copolymer formed was precipitated in acetone and washed with water–methanol mixture to remove the polyacrylamide formed. The solid obtained was dried in an electrically controlled oven at 40 °C and weighed.

2.3. Hydrolysis of poly(acrylamide-grafted-PVA)

A 2.5 wt% solution of PVA-*g*-AAm was prepared and maintained at 60 °C. To this mixture, 50 mL of equimolar concentration of sodium hydroxide solution was added, the mixture was stirred for 5 h at 60 °C and then cooled to room temperature. To this solution, 1 N HCl solution was added with constant stirring until the solution becomes slightly acidic. The polymer was precipitated in acetone, washed with aqueous solution of methanol, dried and kept in a desiccator for further use.

2.4. Drug loading

About 4 g of polymer and a required amount of cefadroxil were dissolved in 2% acetic acid solution. The solution was then mixed with light liquid paraffin dispersed in a 500 mL beaker and stirred at 500-rpm speed using a high-speed stirrer (Ultra Turrex T-50, IKA Labortechnik, Germany). The water-in-oil (w/o) emulsion formed was stabilized by adding 1% Tween 80 solution. After 10 min, aqueous phase of the emulsion was hardened to produce MGs, which were crosslinked with glutaraldehyde. The MGs thus produced were separated by filtration, washed repeatedly with hexane followed by water to remove paraffin oil, acid and excess of crosslinking agent. Totally, 12 formulations were prepared by varying the amount of drug and blend ratios of chitosan with PVA, PVA-*g*-AAm as well as hydrolyzed PVA-*g*-AAm matrices. Different loadings of drug with different polymeric blends along with their formulation codes are given in Table 1.

2.5. Swelling studies

Dynamic swelling of the chitosan-based IPNs prepared by using three different crosslink densities as well as three different drug loadings was studied in water by mass uptake measurements with time. Swelling experiments performed in 7.4 pH buffer solutions produced no significant changes and hence, we studied the swelling of microspheres in water (Kurkuri & Aminabhavi, 2004b). To perform swelling experiments, microspheres were soaked in water; several of them were removed from the swelling bottles at different time intervals and

Table 1
Formulation codes

Formulation	Code
Different loadings of drug in plain chitosan MGs crosslinked with 5 mL of GA	
25% cefadroxil loaded	CS-25
50% cefadroxil loaded	CS-50
75% cefadroxil loaded	CS-75
100% cefadroxil loaded	CS-100
Different loadings of drug in chitosan + PVA (50:50) blend MGs crosslinked with 5 mL of GA	
25% cefadroxil loaded	CS-PVA-25
50% cefadroxil loaded	CS-PVA-50
75% cefadroxil loaded	CS-PVA-75
100% cefadroxil loaded	CS-PVA-100
Different loadings of drug in chitosan + PVA-g-AAm (50:50) blend MGs crosslinked with 5 mL of GA	
25% cefadroxil loaded	CS-PVA-*g*-AAm-25
50% cefadroxil loaded	CS-PVA-*g*-AAm-50
75% cefadroxil loaded	CS-PVA-*g*-AAm-75
100% cefadroxil loaded	CS-PVA-*g*-AAm-100
Different loadings of drug in chitosan + hydrolyzed PVA-g-AAm (50:50) blend MGs crosslinked with 5 mL of GA	
25% cefadroxil loaded	CS-PVA-*g*-AAm (H)-25
50% cefadroxil loaded	CS-PVA-*g*-AAm (H)-50
75% cefadroxil loaded	CS-PVA-*g*-AAm (H)-75
100% cefadroxil loaded	CS-PVA-*g*-AAm (H)-100

blotted carefully (without pressing hard) to remove the surface-adhered water. The MGs were then weighed (w_1) on an electronic microbalance (Mettler, AT 120, Switzerland) accurate to ±0.00001 g. The MGs were then dried to a constant weight (w_2) in an oven maintained at 60 °C for 5 h. Swelling experiments were repeated thrice for each sample and average values were used in data analysis. The standard deviations (SD) in all cases were <5%. The weight % water uptake was calculated as:

$$\%\ \text{Water uptake} = \left(\frac{\text{Weight of swollen MGs } (w_1) - \text{Weight of dry MGs } (w_2)}{\text{Weight of dry MGs } (w_2)}\right) \times 100. \quad (1)$$

2.6. Dissolution experiments

Dissolution experiments were performed at 37 °C using the dissolution tester (Dissotest, LabIndia, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 mL phosphate buffer solution (pH 1.2 or 7.4) was used as the dissolution media to simulate gastrointestinal tract (GIT) conditions. A 5 mL aliquot was used each time for analyzing the cefadroxil content at the fixed interval of time. The dissolution media was replenished with a fresh stock solution. The amount of cefadroxil released was analyzed by UV spectrophotometer (Secomam, model Anthelie, France).

2.7. Drug content assay and entrapment efficiency

Microspheres were evaluated for cefadroxil content by incubating the known weight of MGs with 5 mL of water for complete swelling. The swollen MGs were crushed in an agate mortar with a pestle and the homogeneous solution formed was sonicated (Ikasonic U50, IKA Labortechnik, Germany) for 2 min at 60 MHz frequency. About 20 mL of methanol was added to precipitate the blend polymer, which was then removed with methanol using a high-speed centrifuge (Remi, R24, India) for 5 min at the rotation speed of 10,000 rpm. The amount of cefadroxil was analyzed using UV spectrophotometer at the λ_{max} value of 230 nm. The % drug loading and % encapsulation efficiency were calculated using the following equations, respectively:

$$\%\ \text{Drug loading} = \left(\frac{\text{Weight of drug in MGs}}{\text{Weight of MGs}}\right) \times 100, \quad (2)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical loading}}\right) \times 100. \quad (3)$$

These data are included in Table 2.

2.8. Fourier transform infrared (FTIR) measurements

FTIR spectra were recorded on a Nicolet, Model Impact 410 (USA). About 2 mg of the samples were ground thor-

Table 2
Results of % encapsulation efficiency, mean particle size of the microgels at stirring speed of 500 rpm and% equilibrium swelling data in different external pH media of the uncrosslinked and crosslinked matrices at 37 °C

Formulation code	% Drug loading	% Encapsulation efficiency ± SD	Mean particle size (μm) ± SD	1.2 pH (0.1 HCl)	7.4 pH (phosphate buffer)
CS-25	25	82.1 ± 0.8	42 ± 0.2	202	182
CS-50	50	84.7 ± 0.3	55 ± 0.6	205	183
CS-75	75	84.0 ± 1.2	69 ± 0.3	207	184
CS-100	100	86.0 ± 1.2	82 ± 0.9	207	185
CS-PVA-25	25	85.0 ± 0.6	94 ± 1.2	241	250
CS-PVA-50	50	87.3 ± 0.9	118 ± 0.6	242	252
CS-PVA-75	75	89.7 ± 1.4	132 ± 1.8	243	255
CSP-PVA-100	100	91.9 ± 0.8	144 ± 0.8	244	254
CS-PVA-*g*-AAm-25	25	88.7 ± 0.8	124 ± 0.6	248	290
CS-PVA-*g*-AAm-50	50	89.0 ± 1.5	137 ± 1.4	250	289
CS-PVA-*g*-AAm-75	75	91.2 ± 1.6	148 ± 1.1	252	291
CS-PVA-*g*-AAm-100	100	92.0 ± 0.9	160 ± 0.9	252	292
CS-PVA-*g*-AAm (H)-25	25	89.6 ± 0.6	141 ± 0.2	242	360
CS-PVA-*g*-AAm (H)-50	50	90.2 ± 0.4	157 ± 0.5	242	365
CS-PVA-*g*-AAm (H)-75	75	93.8 ± 1.2	169 ± 0.8	241	363
CS-PVA-*g*-AAm (H)-100	100	95.3 ± 0.8	182 ± 0.6	242	367
CS-PVA-*g*-AAm (H)-25-(2.5 GA)	25	93.2 ± 1.2	159 ± 0.8	243	393
CS-PVA-*g*-AAm (H)-25-(5.0 GA)	25	89.6 ± 0.6	141 ± 1.6	242	360
CS-PVA-*g*-AAm (H)-25-(7.5 GA)	25	85.0 ± 0.9	117 ± 0.5	239	337

SD, Standard deviation; GA, glutaraldehyde crosslinked.

oughly with KBr and pellets were prepared using a hydraulic press under a pressure of 600 kg/cm^2. Spectra were scanned between 4000 and 400 cm^{-1}.

2.9. X-ray diffraction (X-RD) studies

X-RD diffractograms of the placebo MGs, plain cefadroxil and cefadroxil-loaded MGs were recorded on a Rigaku Geigerflex diffractometer equipped with a Ni-filtered CuKα radiation ($\lambda = 1.5418$ Å). The dried beads of uniform size were mounted on a sample holder and X-RD scans were recorded in the angle range of 10°–50° at a speed of 5°/min to estimate crystallinity of the samples.

2.10. Differential scanning calorimetric (DSC) studies

DSC thermograms of pure chitosan MGs, plain drug and drug-loaded MGs were recorded by using a Rheometric Scientific, Model DSC-SP, UK. Initially, the moisture was removed by heating the samples and then, thermograms were recorded from 30 to 400 °C at the heating rate of 10 °C/min.

2.11. Scanning electron microscopic (SEM) studies

SEM images of the MGs were recorded using a JSM 6400 scanning electron microscope (Japan) at the required magnification. Working distance of 39 mm was maintained and the acceleration voltage used was 20 kV with the secondary electron image (SEI) as a detector.

3. Results and discussion

3.1. FTIR of chitosan and chitosan–PVA complex blend

FTIR studies were carried out to confirm grafting of acrylamide as well as crosslinking of MGs. FTIR spectra of the plain PVA, PVA-*g*-AAm and hydrolyzed PVA-*g*-AAm are shown in Fig. 1. A broad band appearing at ~3430 cm^{-1} corresponds to associated –OH stretching vibrations of the hydroxyl group (see curve A). In the spectra of the grafted copolymer, a new peak at ~3200 cm^{-1} was observed and the related peak at 1665 cm^{-1} corresponds to –NH bending vibrations of the primary amides (see curve B) of acrylamide. A relatively high intense peak at 2925 cm^{-1} corresponds to aliphatic –CH stretching vibrations in the grafted copolymer, which confirmed the grafting reaction of acrylamide on PVA. In the spectra of hydrolyzed PVA-*g*-AAm (see curve C), the shoulder peak has completely disappeared, but two new peaks have appeared around ~1670 and ~1567 cm^{-1}, which are due to antisymmetric vibrations of COO- groups. The peak observed at 1406 cm^{-1} is due to the symmetric vibration of the ionized complex, which confirms the conversion of amide groups into carboxylic groups.

FTIR spectra of blends of the chitosan with PVA, PVA-*g*-AAm and the hydrolyzed PVA-*g*-AAm are shown in

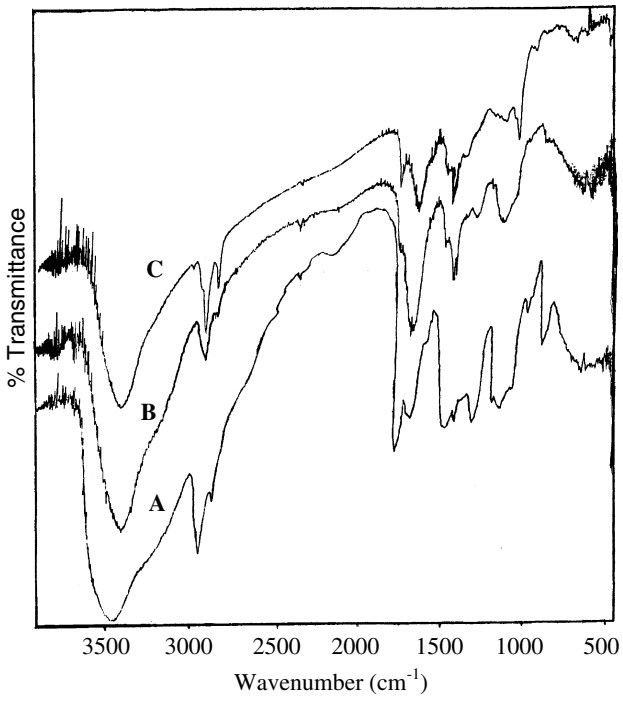


Fig. 1. FTIR spectra of (A) poly(vinyl alcohol), (B) poly(vinyl alcohol)-*g*-acrylamide and (C) hydrolyzed poly(vinyl alcohol)-*g*-acrylamide.

Fig. 2. The spectrum of plain chitosan powder (curve A) has shown two peaks around 894 cm^{-1} and 1153 cm^{-1}, corresponding to saccharide structure (Yoshioka, Hirano, Shioya, & Kako, 1990). Despite several peaks clustering in the secondary amide peak ranging from ~1516 to ~1565 cm^{-1}, the absorption peaks observed at ~1653 and ~1322 cm^{-1} are respectively, characteristics of chitin and chitosan moieties. These could be from the primary and tertiary amide peaks (Pearson, Marchessault, & Liang, 1960). The observed sharp peaks at ~1382 and ~1413 cm^{-1} are assigned to CH_3 symmetrical deformation mode (Peng, Yao, Chen, & Goosen, 1994; Sannan, Kurita, Ogura, & Iwakura, 1978). A broad band appearing around ~1073 cm^{-1} indicates the C–O stretching vibration of chitosan. Another broad band at ~3450 cm^{-1} is due to the amine N–H symmetric stretching vibration, which might be due to deacetylation of chitosan. Peaks observed at ~2845 and ~2919 cm^{-1} are typical of C–H stretching vibrations. A new peak appearing at ~1563 cm^{-1} due to imine bonds (–C=N) was formed as a result of crosslinking reaction between amino groups in chitosan and aldehydic groups in glutaraldehyde (Bellamy, 1980; Lee et al., 1999) (see curve B). A reaction leading to the formation of crosslinks is depicted in Structure 2. However, this is due to the overlapping of peaks corresponding to –NH stretching vibrations in –NH–C=O–CH_3 of the original chitosan with that of >C=N stretching of the newly formed Schiff base complex between –NH_2 group of chitosan and –CHO group of glutaraldehyde.

FTIR spectrum of blends of chitosan with PVA (see curve C) is different than that of chitosan because of the

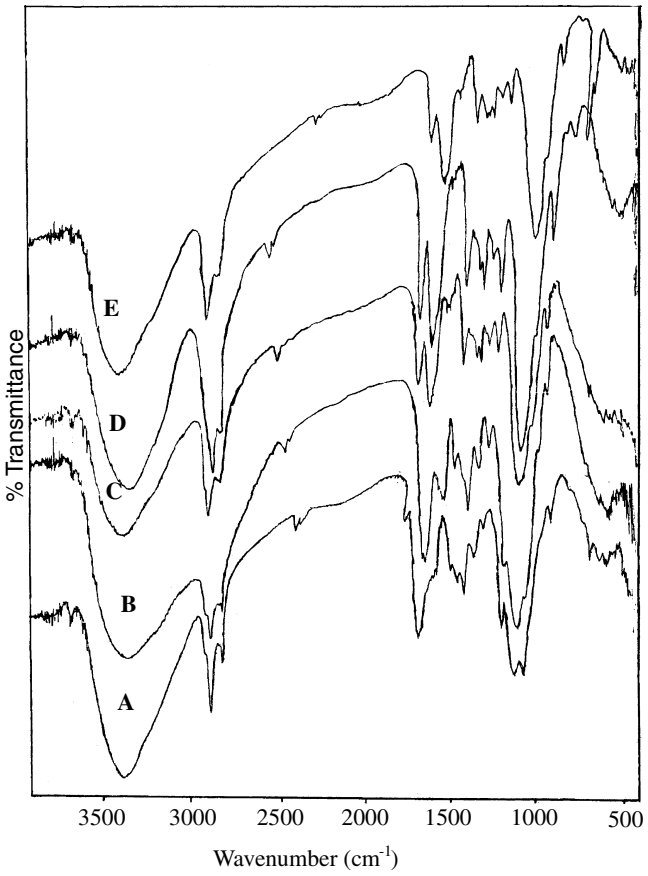
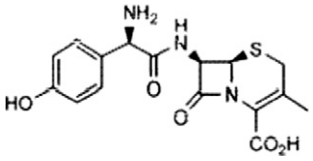


Fig. 2. FTIR spectra of pure (A) plain chitosan, (B) crosslinked chitosan, (C) chitosan blend with poly(vinyl alcohol), (D) chitosan blend with acrylamide-*g*-poly(vinyl alcohol) and (E) chitosan blend with hydrolyzed acrylamide-*g*-poly(vinyl alcohol).



Cefadroxil drug

Structure 2. Crosslinking structure of chitosan with aldehyde.

ionization of primary amino groups in the chitosan–PVA complex. The crosslinking reaction between hydroxyl groups of PVA and aldehydic groups in glutaraldehyde was further confirmed by the formation of acetal group and the ether linkage observed at ~1109 cm^{-1}. However, the spectrum of chitosan–PVA-*g*-AAm complex (see curve D) is smaller than that observed for chitosan–PVA blend, probably due to an increase in peak intensity at ~1733 cm^{-1}. There are two distinguishing peaks at ~1400 and ~1548–1560 cm^{-1}. Of these, the spectrum of chitosan–hydrolyzed PVA-*g*-AAm (see curve E) observed around ~1548–1560 cm^{-1} is due to symmetric deformation of $-NH_3^+$, resulting from the ionization of primary amino groups in the presence of carboxylic group of the hydrolyzed polymer (Bellamy, 1980). On the other hand, the peak at 1408 cm^{-1} indicates the presence of carboxylic acid of the polymers. In the presence of amine group, carboxylic group behaves as a dimer and these peaks are observed at ~1733 cm^{-1}. However, the presence of carboxylic dimer was due to the formation of chitosan complex with carboxylic acid.

3.2. X-RD studies

X-RD diffractograms of (A) placebo MGs, (B) cefadroxil-loaded MGs and (C) plain cefadroxil drug are displayed in Fig. 3. These traces reveal the crystallinity of the drug even after encapsulation in the crosslinked MGs. Cefadroxil has shown characteristic intense peaks between 2θ of 10° and 25° due to its crystalline nature. However, peaks for the plain drug were masked in the drug-loaded IPN matrix. X-RD diffractograms recorded for placebo MGs and cefadroxil-loaded MGs did not show any characteristic peak of the drug, indicating that the encapsulated drug is in the amorphous state.

3.3. DSC studies

DSC thermograms of the (A) plain cefadroxil, (B) chitosan–PVA IPN microgels containing cefadroxil and (C) placebo chitosan–PVA beads are displayed in Fig. 4. Cefadroxil exhibits a sharp endothermic peak at 207 °C, but cefadroxil-loaded beads did not show this feature and no characteristic peaks of loaded drug were observed. The cefadroxil peaks are almost identical to those of the empty microspheres, indicating that most of the drug is molecularly dispersed within the microgel matrix, a fact that was also seen in X-ray diffractograms (Fig. 4).

3.4. SEM studies

SEM images of the single MG taken at 1.50 K [×] and few MGs taken at 4.0 K [×] magnifications are shown in Fig. 5. MGs are spherical without forming agglomeration (Fig. 5A) and their surfaces are smooth (Fig. 5B). However, polymeric debris seen around some particles (Fig. 5A) could be due to the method of particle production (i.e., simultaneous particle production and formation of IPNs). MGs produced by blending of different polymers did not show any effect on surface properties.

3.5. Swelling studies

Swelling studies are important to understand drug release characteristics through MGs. It depends upon the nature and extent of interaction between solvent molecules and polymer chains in addition to porosity of MGs and nature of hydrophilic groups on the polymer. Swelling results of both uncrosslinked and crosslinked matrices are compared along with other pertinent data in Table 2. These will be discussed below.

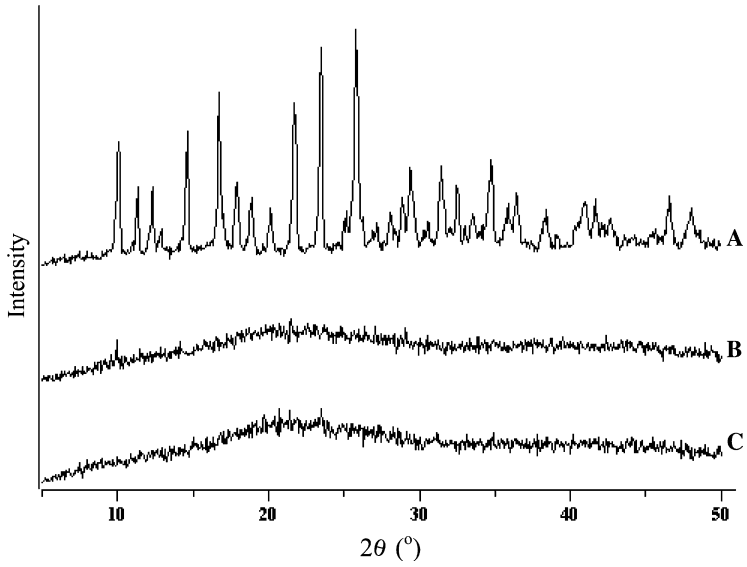


Fig. 3. X-RD diffractograms of (A) cefadroxil, (B) cefadroxil loaded CS-PVA blend MGs and (C) placebo CS-PVA blend MGs.

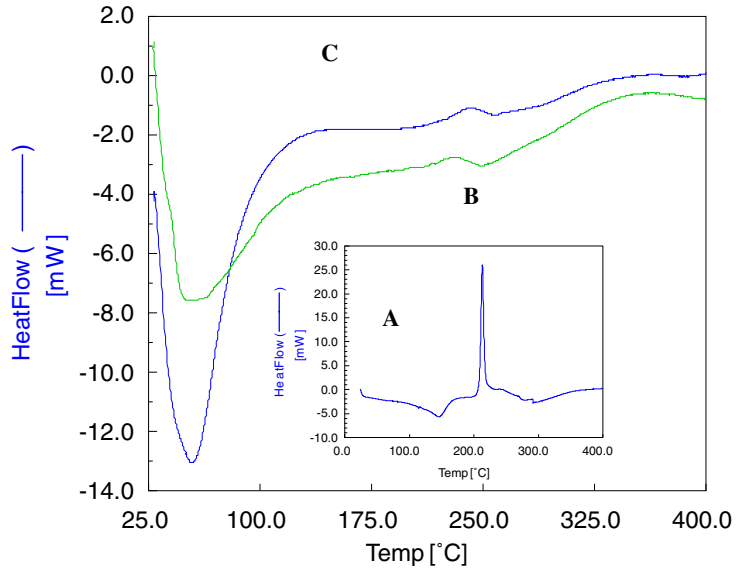


Fig. 4. DSC curves of (A) cefadroxil, (B) cefadroxil containing microspheres and (C) placebo microspheres.

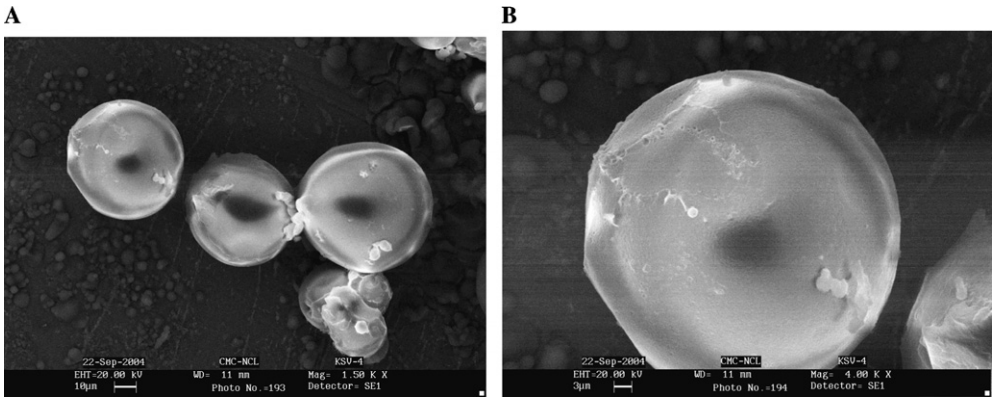


Fig. 5. SEM micrographs of the microspheres.

3.5.1. Plain chitosan microparticles

For plain chitosan MGs in pH 1.2 media, % equilibrium swelling of MGs is higher than in pH 7.4, phosphate buffer. These data for plain chitosan MGs in both pH 1.2 and pH 7.4 increased slightly with increasing amount of drug (CS-25 to CS-100). Swelling increased from 182% to 185% in pH 7.4 media, while in pH 1.2 media, it increased from 202% to 207%. In general, swelling of MGs at pH < 6 involves protonation of amino/imine group of MGs, due to relaxation of the polymer chains. Swelling occurs in two stages. In the first stage, amino/imine groups of the MG surface are protonated with the dissociation of hydrogen bonds between amino/imine groups. This will further facilitate the solvent transport from the MG surface, thereby creating a sharp moving boundary by separating the unsolvated polymer region from the swollen region. In the second stage, protons and counter ions will diffuse into MGs to protonate the inner amino/imine groups. This will allow the protonation to continue until chitosan coil will be collapsed due to solvation.

3.5.2. Chitosan/PVA or modified PVA complex blends

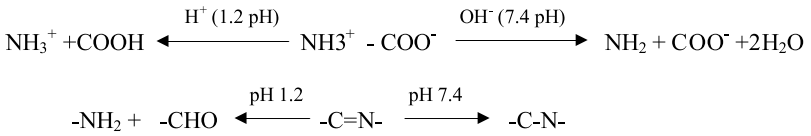
The % equilibrium swelling data for the blend MGs of CS and PVA are higher than the plain chitosan MGs in both the pH media, due to higher hydrophilic nature of the IPN matrix than the plain chitosan. By increasing drug loading in the blends, % equilibrium swelling also increased in both the pH media. For instance, from CS-PVA-25 to CS-PVA-100, % equilibrium swelling increased from 241 to 244 in pH 1.2 and 250 to 254 in pH 7.4 media. The % equilibrium swelling has increased from 249 to 252 in pH 1.2 and 290 to 292 in pH 7.4 media when loading is increased from 25% to 100%, i.e., CS-PVA-*g*-AAm-25 to CS-PVA-*g*-AAm-100 blend matrices. Such an increase in swelling of the blends is due to incorporation of hydrophilic PVA segments along with polyacrylamide chains into the IPN matrix. In all blend systems, % equilibrium was higher in pH 7.4 media than observed in pH 1.2 media due to the presence of functional (amide) groups on PVA backbone as a result of hydrophilic interactions between amide group and water molecules. In case of blend MGs of CS with hydrolyzed PVA-*g*-AAm, % equilibrium swelling reached the constant maximum value of 242, due to the complexation of amine group of CS and acidic group of the hydrolyzed acrylamide-*grafted*-PVA copolymer in pH 1.2 media. In pH 7.4 media, % equilibrium swelling increased from 360 to 367 with increasing drug loading, i.e., from CS-PVA-*g*-AAm (H)-25 to CS-PVA-*g*-AAm (H)-100. Hydrolyzed amide groups in these MGs are responsible to increase water uptake capacity (Soppimath, Kulkarni, & Aminabhavi, 2002). Such effects are attributed to the presence of acidic groups, because ion–dipole interactions between acid and water molecules are responsible because hydrophilic groups are incorporated into the IPN matrix as per the Scheme 1 shown below.

3.5.3. Effect of crosslinking agent on swelling

In hydrogels, the extent of crosslinking depends upon the amount of crosslinking agent used. In the present study, different amounts of GA were added as crosslinking agent to the hydrolyzed MGs of CS-PVA-*g*-AAm blend matrix containing 25 wt% of cefadroxil. These data are also presented in Table 2. Extent of crosslinking is dependent upon the pH of the media as well as equilibrium swelling. For instance, in pH 7.4 media, % equilibrium swelling decreased from 393 to 337 with increasing amount of GA from 2.5 to 7.5 mL [CS-PVA-*g*-AAm (H)-25-(2.5 GA) to CS-PVA-*g*-AAm (H)-25-(7.5 GA)]. But, in pH 1.2 media, the observed decrease in equilibrium swelling is smaller and it varied from 243 to 239. This is due to increased crosslink density and decreased pore volume of the IPNs (Patel, Patel, & Kansara, 1994; Vinogradove & Malkin, 1980) with increasing amount of GA in the matrix. Since PVA is a water-soluble polymer, it is readily miscible with CS and hence, blending of PVA with CS will increase matrix swelling due to higher water uptake. The blend itself remains essentially insoluble in non-acidic aqueous media due to hydrogen bonding between hydroxyl groups of PVA and amine group of CS. There is ample evidence from the published reports (Kim, Shin, Lee, & Kim, 2003) that the blend membranes of PVA/chitosan could change their ability to absorb water when the pH is altered. However, it is shown that the amount of crosslinking agent of the blend membrane affects degree of swelling.

3.5.4. Particle size

For development of any successful formulation, it is important to balance the relationship between particle size and encapsulation efficiency. In this work, we have measured the particle size of MGs by an optical microscope and results are included in Table 2. The size of MGs depends upon the amount of drug loaded, the extent of blending and crosslinking. All the MGs produced in this work were spherical in nature with diameters ranging from 42 to 182 μm. Particle size of the plain chitosan MGs is smaller than MGs produced from the blends of CS with PVA or even with the modified PVA. The size of MGs increased from 144 to 182 μm for 100% drug containing



Scheme 1. Protonation of chitosan complex in different pH media and different forms of Schiff bases formed in different pH media.

blend MGs (CS-PVA-100, CS-PVA-*g*-AAm-100 and CS-PVA-*g*-AAm (H)-100). This can be explained on the basis of hydrodynamic viscosity, i.e., during polymer modification, interfacial viscosity of the polymer droplets in emulsion phase have increased. Thus, when particle size is increased (as noticed by higher uptake capacity of the amide and acidic groups in modified PVA matrix), this will tend to break the dispersed phase into smaller size particles during emulsification. For all formulations, with increasing amount of drug in MGs, particle size also increased (see Table 2). In case of plain chitosan, when drug content was increased from 25% to 100%, size of the MGs increased from 42 to 82 μm (CS-25 to CS-100). For PVA-incorporated blend MGs, particle size increased from 94 to 114 μm (CS-PVA-25 to CS-PVA-100). Similar trends were observed for all other formulations shown in Table 2. This suggests that drug particles have occupied the free volume spaces of the IPN matrix, thereby hindering further inward shrinkage of the polymer matrix (Soppimath et al., 2002). However, 100% drug-loaded hydrolyzed PVA-*g*-AAm containing MGs (CS-PVA-*g*-AAm (H)-100) have shown a maximum size of 182 μm than all the remaining formulations studied.

Extent of crosslinking also has a considerable effect on particle size (see Table 2). For MGs produced from blends of chitosan with hydrolyzed PVA-*g*-AAm loaded with 25% drug, particle size decreased from 159 to 117 μm with increasing amount of GA from 2.5 to 7.5 mL CS-PVA-*g*-AAm (H)-25-(2.5 GA) to CS-PVA-*g*-AAm (H)-25-(7.5 GA). This could be due to the fact that at higher amount of GA in the matrix, a possible shrinkage of the MGs has occurred (Korsmeyer & Peppas, 1981; Soppimath et al., 2002). Since pK_a of the hydrolyzed polymer is 4.6 ± 0.1 (Soppimath et al., 2002) and when the pH is less than pK_a, the H^+ion strength will be high, which will effectively suppress the ionization of carboxylic acid groups. Since hydrogel is neutral and flexibility of the polymer chain is rather low, so carboxylic acid groups within the polymeric network will ionize and attract cations into the hydrogel region to replace H^+ ions, since the pH of the solution is above its pK_a value. This effectively increases the concentration of free ions inside the hydrogel. Thus, ionic swelling-based osmotic pressure will build up inside the matrix and so does swelling. When the hydrogel expands, it will minimize the repulsion between the ionized carboxylic groups.

3.5.5. Encapsulation efficiency

Results of encapsulation efficiency for different formulations are also included in Table 2 as a function of extent of drug loading. It is observed that % encapsulation efficiency is higher for MGs of the blends of hydrolyzed PVA-*g*-AAm with chitosan as compared to all other formulations. The % encapsulation efficiency increased systematically with increasing drug loading from 25% to 100% in the matrix for all the formulations. Comparatively, for unhydrolyzed microspheres of chitosan with PVA grafted acrylamide, % encapsulation efficiency was lower than observed for hydrolyzed microspheres. However, plain chitosan microspheres have lower values of % encapsulation efficiency and mean particle size compared to their blends. In an effort to study the effect of extent of crosslinkng on % encapsulation efficiency, we have chosen the microspheres of chitosan with hydrolyzed PVA-*g*-AAm in which we have added 2.5, 5.0 and 7.5 mL of GA. These results are also presented in Table 2. It is observed that with an increasing amount of GA in the matrix, % encapsulation efficiency decreased due to lesser free volume space available in the matrix. This could be because at higher crosslink density, microspheres would become more rigid.

In the case of blend MGs of CS with hydrolyzed PVA-*g*-AAm, the % drug loading was smaller in pH 1.2 media than in pH 7.4 media, due to the complexation of amine group of CS and acidic group of the hydrolyzed acrylamide-*grafted*-PVA copolymer. Polymeric chains of MGs will become more rigid due to complexation occurring in pH 1.2 media, resulting in lower encapsulation efficiency.

3.6. Drug diffusion through MGs

Results of equilibrium swelling diameter, D_∞ normalized to initial diameter, D_0, of the MGs with different amounts of GA for chitosan–PVA-*g*-AAm (H)-25 are presented in Table 3. Triplicate measurements gave standard errors within 3%. Dynamic swelling data were obtained by monitoring changes in diameter, D_t, of the spherical MGs with a lapse of time by using an optical microscope. Fig. 6 displays the normalized diameter, D_t/D_0 as a function of time for placebo MGs of the hydrolyzed PVA-*g*-AAm (i.e., without cefadroxil) crosslinked with different amounts of GA (i.e., formulations: CS-PVA-*g*-AAm (H)-2.5 GA, CS-PVA-*g*-AAm (H) and CS-PVA-*g*-AAm (H)-7.5 GA). As the amount of GA in the MGs increased from 2.5 to 7.5 mL, the equilibrium normalized diameter decreased from 3.08 to 2.17 in 7.4 pH media, while in pH 1.2 media, it decreased from 1.93 to 1.41 (see Table 3). These data are used to compute diffusion coefficients of drug molecules in different media.

Dynamic volume changes, i.e., ΔV_t of the MGs as a function of time were calculated from the initial volume, V_0. Using these data, diffusion coefficient, D_v, of the drug

Table 3
Transport results of MGs containing hydrolyzed PVA without drug and crosslinked with different amounts of GA in 7.4 and 1.2 pH media

pH	Amount of GA added (mL)	Equilibrium normalized diameter (D_∞/D_0)	$D_v \times 10^5$ (cm^2/s)
7.4	2.5	3.08	6.01
	5.0	2.46	4.18
	7.5	2.17	3.03
1.2	2.5	1.93	3.47
	5.0	1.55	2.89
	7.5	1.41	1.29

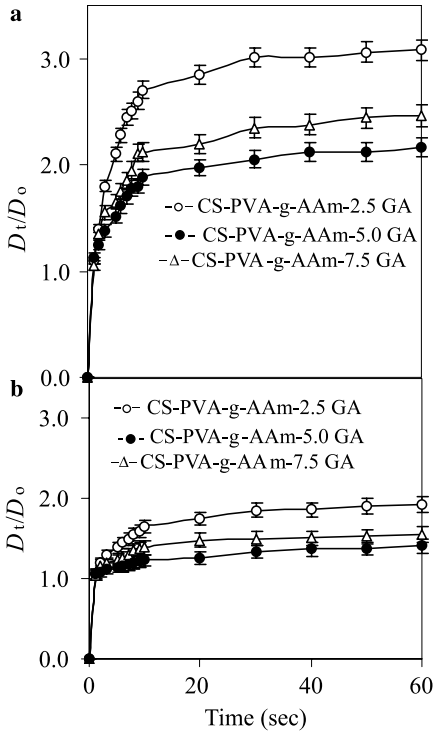


Fig. 6. Dimensional swelling of placebo MGs of CS-PVA-*g*-AAm (H) (a) in pH 7.4 media and (b) in pH 1.2 media.

in aqueous media was computed (Harogoppad & Aminabhavi, 1992) using:

$$\left(\frac{\Delta V_t}{V_0}\right) = \left[\frac{4\left(\frac{\Delta V_\infty}{V_0}\right)}{D_0}\right]\left(\frac{D_v}{\pi}\right)^{1/2} t^{1/2}, \quad (4)$$

$$D_v = \left[(1.773 \times \text{slope})\frac{V_0 D_0}{4\Delta V_\infty}\right] \quad (5)$$

Here, ΔV_∞ is the volume change at equilibrium and V_∞ is volume of the MG at equilibrium swelling. Eq. (5) was used to calculate D_v from the slope of the initial linear plots of $\Delta V_t/V_0$ vs $t^{1/2}$. These results are also included in Table 3. Diffusion coefficients thus calculated varied systematically with the amount of GA used, i.e., D_v decreased with increasing amount of GA in the MGs containing the hydrolyzed polymer. This is due to the fact that at higher crosslinking, free volume of the matrix will decrease, thereby hindering the small molecular diffusion through the MG matrix. The D_v values in 7.4 pH media are higher than those observed in pH 1.2 media. This indicates that in acidic media, more solvent molecules will transport through the hydrogel matrix than in the basic media. This property correlates well with the drug release characteristics of the MGs developed. The crosslinking of polymer network generally leads to a reduction in molecular transport, with the reduced value of diffusion coefficient due to a decrease in crystallinity as a result of introduction of more number of junction points. In the present system, exceptedly, the amount of liquid transported at any given time decreased with increasing crosslink density.

3.7. In vitro drug release

In an effort to study the feasibility of using the modified PVA blends with chitosan as a hydrophilic drug (cefadroxil) carrier, drug-loaded MGs were prepared by w/o emulsion method. Fig. 7 compares the release profiles of chitosan with all other blends. In both the pH media, release was much faster in plain chitosan MGs as compared to blend MGs. Nearly, 100% release of cefadroxil occurred at 10 h for CS-25 in pH 1.2 media (see Fig. 7a), whereas in pH 7.4 media, only 60% drug release was observed at 10 h (see Fig. 7b). Thus, blending of PVA, PVA-*g*-AAm and hydrolyzed PVA-*g*-AAm with chitosan appears to be important in controlling the release of cefadroxil over an extended period of time. Since PVA is water-soluble and hence, it is readily miscible with chitosan, which is more hydrophilic, but the blend will be insoluble in non-acidic aqueous media due to hydrogen bonding effect between hydroxyl groups of PVA and amine group of chitosan. From Fig. 7a and b, it is seen that the blend microgels have shown longer drug release rates than the plain chitosan microgels. But, there is a drastic difference in the release rates of the formulated blend MGs in pH 1.2 and pH 7.4 conditions. Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. For instance, only 40% drug was released at 10 h (see Fig. 7b) for CS-PVA-*g*-AAm (H)-25 when compared to 62% drug release in pH 7.4 media (see Fig. 7a) for the same time. In the case of hydrolyzed blend polymer,

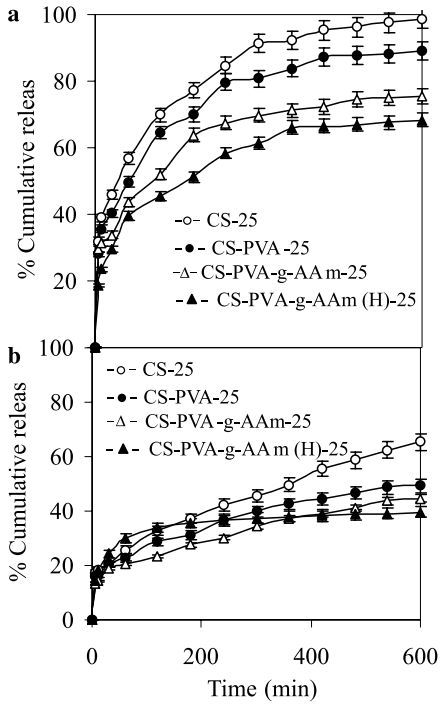


Fig. 7. % Cumulative release vs time for different formulations loaded with 25% of drug in (a) pH 7.4 media and (b) in pH 1.2 media. Symbols: (○) CS-25, (●) CS-PVA-25, (△) CS-PVA-*g*-AAm and (▲) CS-PVA-*g*-AAm (H).

it is likely that there is a complexation of amino group of chitosan, but in pH 1.2 media, there could be a deformation of the complex ($NH_3^+COO^-$) formed as shown in Scheme 1.

The MGs of chitosan and the hydrolyzed PVA-*g*-AAm containing 5 mL of GA were chosen to study the effect of drug loading and extent of crosslinking on the release rates, because these blend MGs have shown lower release rates of drug than other blend MGs containing 25% of drug. Fig. 8 displays the% cumulative release of different amount of drug loaded MGs in pH 7.4 media. As the amount of drug increased from 25% to 100%, the % cumulative release rate also increased from 72% to 85%. This is obvious because as the amount of drug increases in the matrix, swelling of MGs also increases, which facilitates the diffusion of drug through the polymer matrix.

Fig. 9 displays the release profiles of MGs crosslinked with different amounts of GA containing 25% of drug in pH 7.4 media prepared with blends of chitosan and hydrolyzed PVA-*g*-AAm. The % cumulative release of MGs crosslinked with 7.5 mL of GA is lower, while intermediate values are observed for 5 mL containing GA microgels. This is due to the decrease in swelling rate as the amount of GA increased in the MGs. In all cases, % cumulative release data were obtained in triplicate. Error bars on all these curves indicate the standard deviations within 3%, but curves are drawn through average data points.

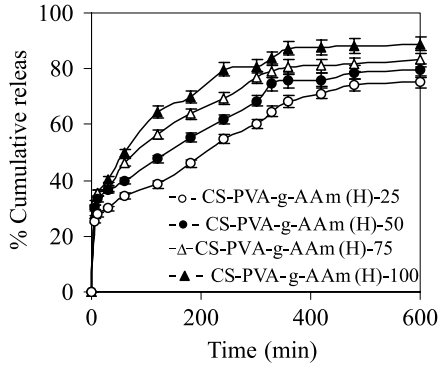


Fig. 8. % Cumulative release vs time for CS-PVA-*g*-AAm (H) loaded with different amounts of drug in pH 7.4 media. Symbols: (○) CS-PVA-*g*-AAm (H)-25, (●) CS-PVA-*g*-AAm (H)-50, (△) CS-PVA-*g*-AAm (H)-75 and (▲) CS-PVA-*g*-AAm (H)-100.

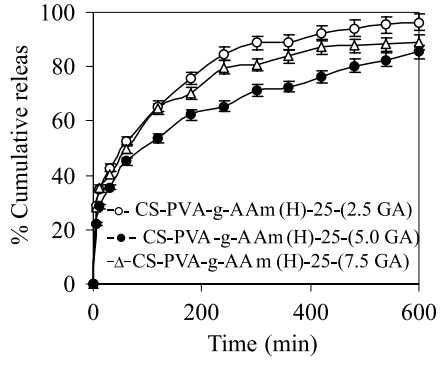


Fig. 9. % Cumulative release vs time for formulations of CS-PVA-*g*-AAm (H)-25 MGs crosslinked with different amount of GA in pH 7.4 media. Symbols: (○) CS-PVA-*g*-AAm (H)-25 (2.5 GA), (●) CS-PVA-*g*-AAm (H)-25 and (△) CS-PVA-*g*-AAm (H)-25 (7.5 GA).

4. Conclusions

The hydrophilic nature of polyacrylamide modified poly(vinyl alcohol) was utilized to develop blend microspheres with another hydrophilic carbohydrate polymer viz., chitosan. Thus, three types of pH sensitive interpenetrating network microgels were prepared to investigate the controlled release characteristics of cefadroxil drug. The blend microgels of chitosan with PVA have shown favorable CR characteristics (the release was more than 10 h) than plain chitosan MGs. Variations in swelling and drug release characteristics of the matrices in different pH media are attributed to morphological changes of the hydrogels. Compared to all formulations developed, blend microgels of chitosan with hydrolyzed poly(vinyl alcohol)-*grafted*-acrylamide matrices are quite promising for controlled release of cefadroxil.

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