Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 69 (2007) 678–687
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Novel interpenetrating polymer network microspheres of chitosan and methylcellulose for controlled release of theophylline ☆

Ajit P. Rokhade, Namdev B. Shelke, Sangamesh A. Patil, Tejraj M. Aminabhavi *

Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University, Dharwad 580 003, India

Received 8 October 2006; received in revised form 3 February 2007; accepted 8 February 2007
Available online 17 February 2007

Abstract

Interpenetrating polymer network (IPN) microspheres of chitosan (CS) and methylcellulose (MC) were prepared by emulsion-crosslinking in the presence of glutaraldehyde (GA) as a crosslinker. Theophylline (THP), an antiasthmatic drug was encapsulated into IPN microspheres under varying ratios of MC and CS, % drug loading and amount of GA added. IPNs have shown better mechanical properties than pure CS. Cross-link density of the matrices was significantly affected by the amount of GA and MC. Microspheres were characterized by Fourier transform infrared (FTIR) spectroscopy to assess the formation of IPN structure and to confirm the absence of chemical interactions between drug, polymer and crosslinking agent. Particle size was measured by laser light scattering technique. Microspheres with the average particle sizes ranging from 119 to 318 μm were produced. Differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of drug after encapsulation into IPN microspheres. Theophylline encapsulation of up to 82% was achieved as measured by UV spectrometer. Equilibrium swelling was performed in distilled water. *In vitro* release studies were performed in both 0.1 N HCl and pH 7.4 buffer solutions. These data indicated a dependence of drug release on the extent of crosslinking and amount of MC added during the preparation of microspheres. The release was extended up to 12 h and release rates were fitted to an empirical equation to compute the diffusional parameters, which indicated a slight deviation from the Fickian trend for the release of theophylline.

Keywords: Carbohydrate polymers; Chitosan; Methylcellulose; Interpenetrating networks; Microspheres; Theophylline

1. Introduction

The importance of biocompatible and biodegradable carbohydrate polymers is continuously increasing in pharmaceutical applications (Shariati & Peters, 2003) because of their propensity to form crosslinked three-dimensional network hydrogels that tend to swell in water or biological fluids. Such systems have been the potential candidates to deliver bioactive molecules, particularly in controlled release applications (Itokazu et al., 1997; Kawaguchi, 2000; Yao, Peng, Feng, & He, 1994). Hydrogels based on carbohydrate polymers swell in water and retain a significant fraction of water within their network structures. They are insoluble in water at physiological temperature, pH and ionic strengths (Ratner, 1981). Soft tissue biocompatibility and an open porous structure allows for on easy transport of the incorporated drugs in a controlled manner. Self-regulated release from such hydrogels would enhance the drug release with a sustained action. However, biocompatibility is the major determinant for their successful functioning, since noncompatible materials can elicit inflammatory responses *in vivo* and thus limit their usage in living systems.

An interpenetrating polymer network (IPN) is a combination of two polymers exhibiting varied characteristics. Whenever an IPN hydrogel is formed from two polymers

☆ This paper is Center of Excellence in Polymer Science communication # 170.

* Corresponding author. Tel.: +91 836 277 8279; fax: +91 836 277 1275.
E-mail addresses: patil1956@yahoo.com (S.A. Patil), aminabhavi@yahoo.com (T.M. Aminabhavi).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.02.008

at a given temperature, the physical phase separation between the component polymers would be almost impossible because of the infinite zero-viscosity of the gel. IPN is also attractive in producing synergistic properties from the component polymers. For example, when a hydrophilic gelling polymer is interpenetrated with a relatively hydrophobic gelling polymer, the resultant IPN hydrogel is expected to have an improved capability of immobilizing a drug. This would open up new avenues to use IPN in designing the novel drug release systems (Rokhade et al., 2006; Rokhade, Patil, & Aminabhavi, 2007).

Even though several polymers have been used in pharmaceutical industry, chitosan (CS) has been one of the most widely used polymers due to its reduced toxicity and better patient compliance (Reverchon, Porta, De Rosa, Subra, & Letourneur, 2000). Drug release from such matrices can be controlled and enhanced by the addition of another water-soluble or water swellable carbohydrate polymer such as methylcellulose (MC) (Handbook of Pharmaceutical Excipients, 2003). Methylcellulose, a polyhydroxy water-soluble carbohydrate polymer can be chemically crosslinked with dialdehyde in the presence of a strong acid to generate a hydrogel (Horkay & Zrinyi, 1982; Park, Park, & Ruckenstein, 2001; Tomihata & Ikada, 1997). On the other hand, chitosan is a unique cationic polymer with excellent gel and film-forming properties. This polymer has been investigated extensively for many years in the pharmaceutical area (Berger, Reist, Mayer, Felt, & Gurny, 2004; Dodane & Vilivalam, 1998; Felt, Buri, & Gurny, 1998; Illum, 1998; Paul & Sharma, 2000).

In continuation of our earlier research on the development of controlled release (CR) devices utilizing carbohydrate polymers, we herein present the work on hydrogel microspheres of chitosan and methylcellulose. The effect of composition of these polymers on the CR of theophylline (THP) was investigated. THP is an effective drug used in the treatment of asthma and pulmonary disease (Yu, Schwartz, & Sugita, 1996) and has been widely used as a model drug in various CR studies (Antal, Zelk'o, Roczey, Plachy, & R'acz, 1997; Coviello, Grassi, Lapasin, Marino, & Alhaique, 2003; Katime, Novoa, & Zuluaga, 2001; Shozo, Wataru, & David, 2000). The release data have been measured in 0.1 N HCl and pH 7.4 buffer solutions. Diffusion anomalies of the drug through the developed matrices have been investigated.

2. Experimental

2.1. Materials

Theophylline was purchased from Loba chemicals, Mumbai, India. High molecular weight chitosan (degree of deacetylation 85% and viscosity 800–2000 cPs) was procured from Aldrich Chemical Company, Milwaukee, WI, USA. Methylcellulose (viscosity, 350–550 cPs) was obtained from s.d. fine Chemicals, Mumbai, India. Analytical reagent grade glutaraldehyde solution 25% (v/v), *n*-hexane and light liquid paraffin were all purchased from s.d. fine Chemicals, Mumbai, India. Span®-80 was purchased from Loba Chemicals, Mumbai, India. All the chemicals were used without further purification.

2.2. Preparation of IPN microspheres

IPN hydrogel microspheres of CS and MC were prepared by emulsion-crosslinking method (Rokhade et al., 2006). Briefly, CS was dissolved in 2% aqueous acetic acid solution by continuously stirring until a homogeneous solution was obtained. After this, MC was dispersed in CS solution and stirred overnight to obtain a homogeneous solution. Then, THP was dissolved in the above polymer solution. This solution was added slowly to a light liquid paraffin (100 g, w/w) containing 1% (w/w) Span®-80 under constant stirring at 400 rpm speed for 10 min. To this w/o emulsion, GA containing 1 N HCl was added slowly and stirring was continued for 3 h. The hardened microspheres were separated by filtration and washed with *n*-hexane. Finally, the microspheres were washed with 0.1 M glycine solution to mask the unreacted GA (Hejazi & Amiji, 2003) and distilled water to remove the unreacted GA. The microspheres were vacuum dried at 40 °C for 24 h and stored in a desiccator until further use. Totally, twelve formulations were prepared and the assigned formulation codes are given in Table 1. A schematic representation of the synthesis of IPN is given in Fig. 1.

2.3. Drug content

Estimation of drug content was done according to the method adopted earlier (Rokhade et al., 2006). Microspheres of known weight (~10 mg) were crushed using an agate mortar and extracted with 50 mL of water and then sonicated using a probe sonicator (UP 400 s, dr. hielscher, GmβH, Germany) for 15 min to break the microspheres. The whole solution was centrifuged (Jouan, MR23i, France) to remove the polymeric debris. The polymeric debris was washed twice to extract the drug completely. The clear supernatant solution was analyzed by UV spectrophotometer (Secomam, model Anthelie, France) at the λ_{max} value of 270 nm. Encapsulation efficiency was calculated as:

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical drug loading}}\right) \times 100 \quad (1)$$

These data for various formulations are given in Table 2.

2.4. Particle size measurements

Particle size and size distributions were measured using a laser light scattering technique (Mastersizer-2000, Malvern, UK). Particle size was measured using the dry sample adopter and volume mean diameter (V_d) was recorded. These data are also included in Table 2.

Table 1
Various formulation parameters used in the preparation of microspheres

Formulation code	Methyl cellulose (% w/w)	Chitosan (% w/w)	Drug loading (%)	GA (mL)	% Entrapment efficiency	Volume mean particle size (μm)	*n*
*F*1	10	90	25	5	68.54	209	0.522
*F*2	15	85	25	5	61.17	238	0.549
*F*3	20	80	25	5	58.29	269	0.563
*F*4	10	90	50	5	72.44	282	0.508
*F*5	15	85	50	5	66.10	301	0.459
*F*6	20	80	50	5	62.33	318	0.363
*F*7	10	90	25	10	76.60	119	0.494
*F*8	15	85	25	10	72.23	156	0.504
*F*9	20	80	25	10	70.16	175	0.502
*F*10	10	90	50	10	82.06	219	0.532
*F*11	15	85	50	10	77.72	233	0.414
*F*12	20	80	50	10	72.19	251	0.398
CF	0	100	25	10	68.15	274	0.314

CF, Control Formulation.

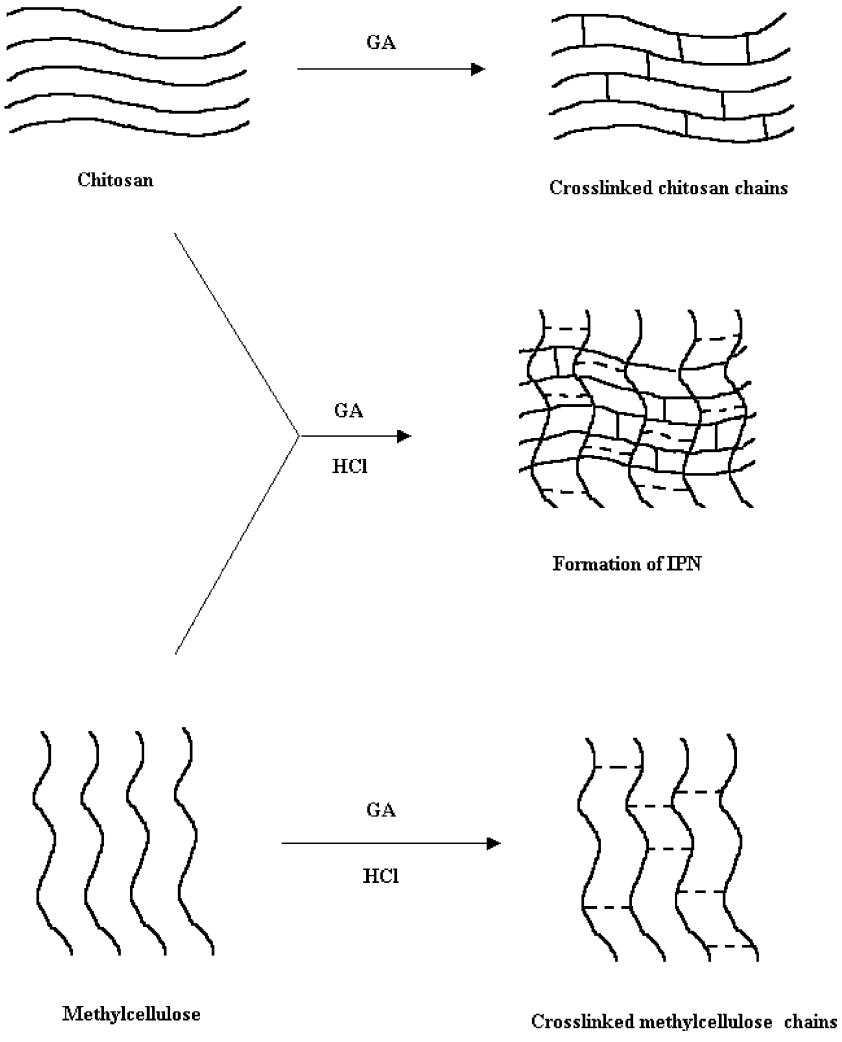

Fig. 1. Schematic representation of synthesis of IPN.

2.5. Fourier transform infrared (FTIR) spectral studies

FTIR spectral data were taken on a Nicolet (Model Thermo 5700, USA) instrument to confirm the formation of IPN structure and also to find the chemical stability of the drug in the microspheres. FTIR spectra of the neat CS, neat MC, placebo microspheres, drug-loaded microspheres and pristine theophylline were obtained. Samples

Table 2
Results of % entrapment efficiency, volume mean particle size, % water-uptake and n values, r values and diffusion coefficient

Formulation code	% Entrapment efficiency	Volume mean particle size (μm)	% Water uptake	n	r	$D \times 10^5$ (cm^2/s)
*F*1	68.54	209	217	0.522	0.994	2.64
*F*2	61.17	238	281	0.549	0.994	3.24
*F*3	58.29	269	304	0.563	1.0	5.57
*F*4	72.44	282	231	0.508	1.0	2.83
*F*5	66.10	301	254	0.459	1.0	3.74
*F*6	62.33	318	289	0.363	0.999	6.21
*F*7	76.60	119	148	0.494	0.993	1.04
*F*8	72.23	156	178	0.504	0.995	2.67
*F*9	70.16	175	192	0.502	0.995	3.33
*F*10	82.06	219	140	0.532	0.995	1.95
*F*11	77.72	233	182	0.414	1.0	2.92
*F*12	72.19	251	197	0.398	0.994	4.16

were crushed with KBr to get pellets at 600 kg/cm^2 pressure. Spectral scanning was done in the range between 4000 and 500 cm^{-1}.

2.6. Differential scanning calorimetric (DSC) study

DSC (Rheometric Scientific, UK) was performed on placebo microspheres, drug-loaded microspheres and pristine theophylline. Samples were heated from 25 to 400 °C at the heating rate of 10 °C min^{-1} in nitrogen atmosphere (flow rate, 20 mL/min).

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

Crystallinity of theophylline after the encapsulation was evaluated by X-ray diffraction (X-RD) measurements recorded for pristine theophylline, placebo microspheres and drug-loaded microspheres using X-ray diffractometer (x-Pert, Philips, UK). Scanning was done up to 2θ of 50°.

2.8. Scanning electron microscopic (SEM) studies

SEM images were taken on CS-MC IPN hyrogel microspheres prepared by crosslinking with 5 mL of glutaraldehyde and loaded with 25% of THP. Microspheres were sputtered with gold to make them conducting and placed on a copper stub. Scanning was done using JEOL model JSM-840A, Japan instrument available at Indian Institute of Science, Bangalore, India. The thickness of the gold layer accomplished by gold sputtering was about 15 nm.

2.9. Swelling studies

Equilibrium water uptake of the crosslinked microspheres loaded with the drug was determined by measuring the extent of swelling of the matrix in water. To ensure complete equilibration, samples were allowed to swell for 24 h. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to an accuracy of 0.01 mg using an electronic microbalance (Mettler, AT 120, Switzerland). The hydrogel microspheres were then dried in an oven at 60 °C for 5 h until there was no change in the dried mass of the samples. The % equilibrium water uptake was calculated as:

$$(\text{Weight of swollen microspheres} - \text{Weight of dry microspheres})/(\text{Weight of dry microspheres}) \times 100 \quad (2)$$

2.10. Tensile strength measurements

Young's modulus of the crosslinked CS and MC membranes was used to estimate the average molecular weight between network crosslinks (M_c) and the effective crosslink density (V_e). To measure Young's modulus, membranes were prepared from aqueous solutions of CS, MC and other formulations (without the drug); the formulation compositions are given in Table 3. The solution casting method was used to prepare the membranes on a clean glass plate. Membranes formed were peeled off from the glass plate and crosslinked by immersing in methanol:water

Table 3
Results of tensile properties of various formulations

Formulation code	CS (% w/w)	MC (% w/w)	GA (mL)	ρ (g/cm^3)	E (MPa)	M_c (kg/mol)	$V_e \times 10^{-5}$ (mol/cm^3)
Crosslinked CS	0	100	5	0.67	3.4 ± 0.1	14.94 ± 1.45	4.45 ± 0.32
Crosslinked MC	100	0	5	1.30	7.1 ± 0.21	10.67 ± 0.44	12.2 ± 0.43
*F*1	90	10	5	0.733	3.65 ± 0.13	15.22 ± 1.12	4.8 ± 0.91
*F*2	85	15	5	0.765	8.06 ± 0.31	8.67 ± 1.06	7.2 ± 0.58
*F*3	80	20	5	0.80	10.0 ± 0.50	6.06 ± 0.89	13.2 ± 0.88
*F*7	90	10	10	0.733	7.98 ± 0.26	6.96 ± 0.72	10.5 ± 0.62
*F*8	85	15	10	0.765	11.97 ± 0.29	4.84 ± 0.34	15.8 ± 0.74
*F*9	80	20	10	0.80	21.25 ± 1.87	2.85 ± 0.17	28.0 ± 1.07

(80:20, v/v) mixture containing the same quantities of GA and 5 N HCl as used in the preparation of microspheres. The crosslinking period was the same as that used in microsphere preparation, i.e., 3 h. Films were dried at ambient temperature and used for the measurements. Test specimens were prepared by cutting the membranes to 10 mm wide and 100 mm long strips using a precise cutter. Tensile testing was done on a Hounsfield universal testing machine (Model H25KS, Surrey, UK). Two ends of the specimen were placed between the upper and lower jaws of the instrument, leaving a length of 50 mm of the film in between the two jaws. Extension speed of the instrument was 10 mm/min.

2.11. In vitro release studies

Drug release from the IPN microspheres with different % drug loading, polymer composition and different extent of crosslinking were investigated in 0.1 N HCl for the initial 2 h, followed by phosphate buffer pH 7.4 until the completion of dissolution. These experiments were performed using a fully automated dissolution tester coupled with a UV system (Logan Instruments Corp., Model D 800, Piscataway, NJ, USA) equipped with six baskets at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500 mL of dissolution medium maintained at 37 °C. The instrument automatically measures the concentration of the drug released at particular time intervals by a UV spectrophotometer coupled with flow-through cells attached to the instrument; it then puts the solution back into the dissolution bowl. Theophylline concentration was determined by UV spectrophotometer at λ_{max} of 270 nm. These studies were performed in triplicate for each sample, but the average values were considered in data analysis.

3. Results and discussion

3.1. Preparation and characterization of IPN microspheres

In the present research, theophylline-loaded IPN microspheres based on two well-known carbohydrate polymers viz., chitosan and methylcellulose were prepared by crosslinking with GA. By this method, % encapsulation efficiency was found to be in the range between 58.29 and 82.06. The % encapsulation efficiency showed a dependence on MC content, extent of crosslinking and % drug loading. By increasing the amount of MC, a slight decrease in % encapsulation efficiency was observed, which is due to the formation of a loose network that allows for leaching out of more of drug particles during microsphere preparation. The % encapsulation efficiency also shows a dependence on % drug loading. The formulations loaded with higher amount of drug exhibited higher encapsulation efficiencies (see Table 1). This is due to the accumulation of more amount of drug particles at higher % drug loading. The effect of crosslinking on % encapsulation efficiency showed a significant effect. As the concentration of crosslinking agent increased, an increase in % encapsulation efficiency was observed. This could be due to higher extent of crosslinking, resulting in the formation of a more rigid network. This caused retention of more drug particles during the microsphere preparation.

Particle size (see Table 2) revealed an increase with increasing amount of MC. It was found that particle size of *F*3 (20%, w/w, MC) was higher than that of *F*2 (15%, w/w, MC) and particle size of *F*2 was greater than *F*1. Similar findings were observed for other formulations. This could be due to the higher amount of MC present, leading to viscosity increase in polymer solution, thereby producing bigger droplets during emulsification that were later hardened in the presence of GA. Particle size also showed a dependence on drug loading. Formulations containing 50% drug loading exhibited higher particle sizes as compared to formulations containing 25% drug loading. This could be due to the accumulation of more drug particles during microsphere preparation. Another interesting observation is that particle size decreased with an increase in crosslinking extent. It is observed that particle size of *F*1 (5 mL GA added) is higher than that of *F*7 (10 mL GA added). Similar findings were observed for *F*2, *F*3, *F*4, *F*5 and *F*6 formulations as compared to *F*7, *F*8, *F*9, *F*10, *F*11 and *F*12. This could be due to the formation of more rigid network structures at higher crosslinking. Microspheres of this study were spherical with smooth surfaces and also have shown surface adhered drug particles as revealed by SEM micrographs as shown in Fig. 2.

3.2. Fourier transform infrared (FTIR) spectral studies

FTIR spectra of plain CS, plain MC, placebo microspheres, drug-loaded microspheres and plain THP were studied to investigate the effect of crosslinking and chemical stability of the drug after encapsulation into the matrix. Fig. 3 depicts the FTIR spectra of (a) plain chitosan, (b) plain methylcellulose and (c) placebo microspheres. In case of CS, a broad band at 3422 cm^{-1} is attributed to N—H stretching vibrations. Bands at 2922 and 2810 cm^{-1} represent the aliphatic C—H stretching vibrations. Three bands observed at 1649, 1594 and 1379 cm^{-1} indicate amide-I, amide-II and amide-III, respectively. The MC showed a broad band at 3457 cm^{-1} due to O—H stretching vibrations. Two bands at 2930 and 2834 cm^{-1} show the presence of C—H aliphatic stretching vibrations. In case of placebo microspheres, all the bands of both CS and MC were observed in addition to a new band observed at 1647 cm^{-1}, which confirmed the C—N stretching vibration of the imine group of Schiff base. The band at 1014 cm^{-1} is due to the presence of an acetal group, which is formed due to the reaction of GA with hydroxyl groups of MC. Thus, FTIR confirms the crosslinking reaction of GA with CS and MC.

FTIR spectral data were also used to confirm the chemical stability of THP in IPN microspheres. For instance, FTIR spectra of (a) placebo microspheres, (b) drug-loaded

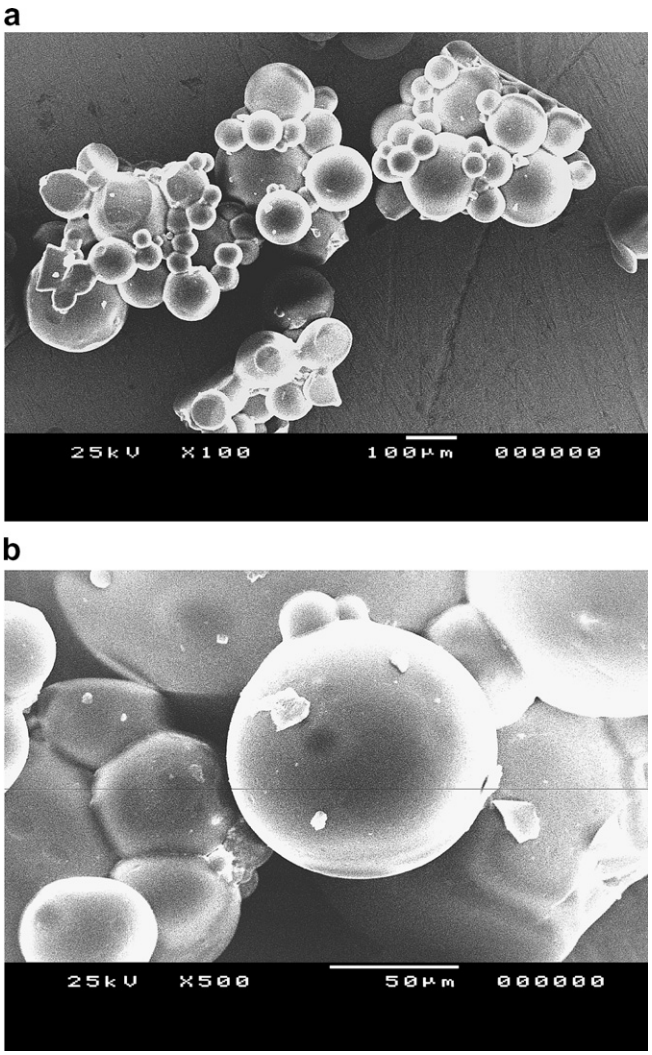

Fig. 2. SEM micrographs of (a) group of microspheres and (b) a single microsphere.

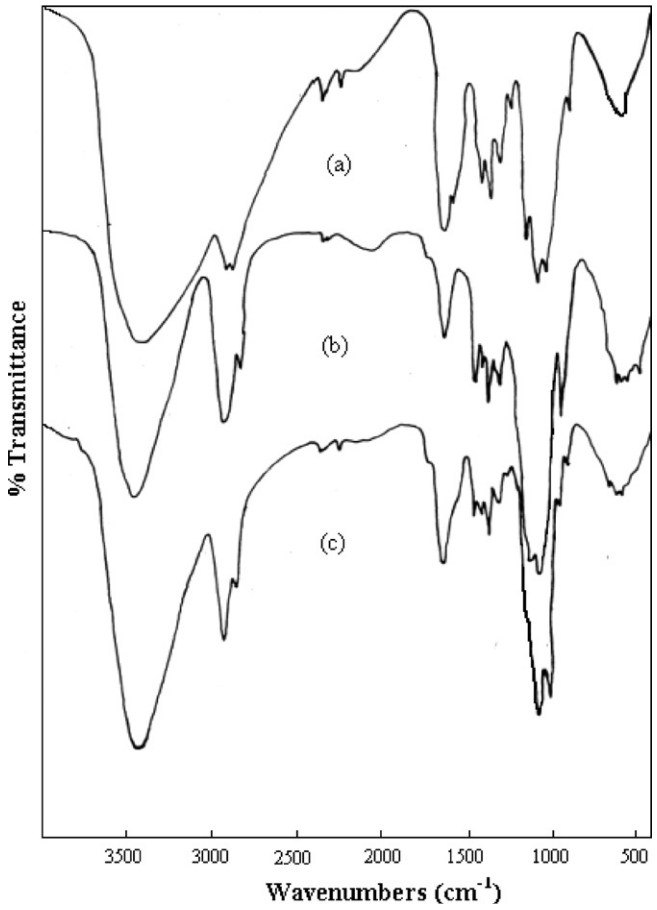

Fig. 3. FTIR spectra of (a) plain chitosan, (b) plain methylcellulose and (c) placebo microspheres.

microspheres and (c) pure THP are presented in Fig. 4. In case of THP, a broad band at 3432 cm^{-1} is due to N—H stretching vibrations. Bands at 3061, 2986, 2918 and 2827 cm^{-1} are attributed to both aromatic and aliphatic C—H stretching vibrations. A band at 1715 cm^{-1} represents the imide group stretching of the heterocyclic ring. A sharp band at 1670 cm^{-1} is due to tertiary amide group stretching vibrations. N—H bending vibration is represented by a band at 1566 cm^{-1}. A band at 1243 cm^{-1} shows C—N stretching vibrations. In case of drug-loaded microspheres, all the bands that were observed in THP have also appeared, indicating the chemical stability of THP after encapsulation into the polymer matrix.

3.3. Differential scanning calorimetric (DSC) study

DSC thermograms of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pure THP are displayed in Fig. 5. In case of placebo microspheres, a small peak and two broad peaks were observed at 35, 57 and 175 °C, respectively due to endothermic transition of the polymer matrix. Thermogram of THP showed a sharp peak at 277 °C, indicating the melting of the drug. In case of drug-loaded microspheres, three peaks were observed at 41, 74 and 181 °C due to endothermic transitions. However, there was no peak corresponding to THP, indicating the amorphous dispersion of THP into IPN matrix.

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

X-ray diffractograms of (a) placebo microspheres, (b) drug loaded microspheres and (c) pure THP are presented in Fig. 6. The diffraction pattern of THP has the characteristic intense peak at 2θ of 12.4°, identical to stable anhydrous theophylline crystal. This peak has disappeared in the THP-loaded microspheres, but only peaks observed in placebo polymer matrix were seen. X-RD peak depends on the crystal size; but in the present study, for all the drug-loaded matrices, the characteristic peak of THP could overlap with the noise of the coated polymer itself. Further, the loaded drug is amorphous, which is very difficult to measure at the detection limit of the crystal size in the present case. This indicates that the drug is dispersed molecularly in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

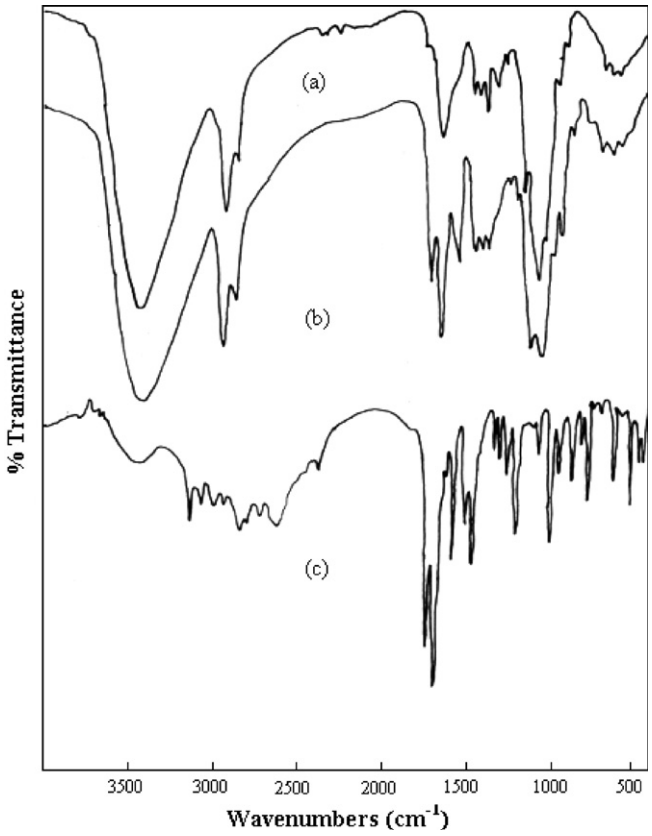

Fig. 4. FTIR spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.

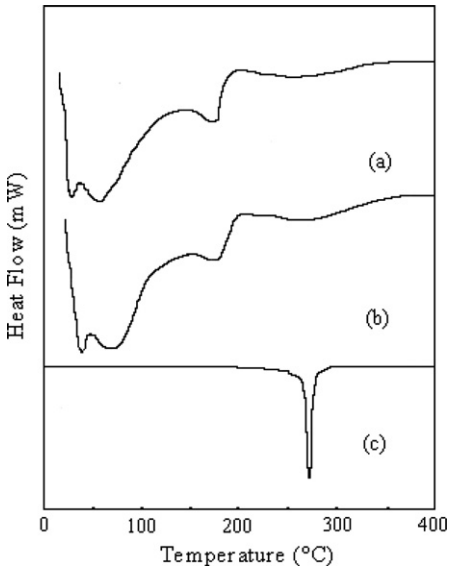

Fig. 5. DSC spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.

3.5. Swelling studies

The % equilibrium water uptake of the crosslinked microspheres presented in Table 2 indicate that, as the amount of GA in the matrices increased from 5 to 10 mL, the equilibrium water uptake decreased significantly from 304% to 140%. Such a reduction in water uptake capacity is due to the formation of a rigid network structure at higher concentration of crosslinking. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake as well as the release rates. Notice that formulations containing higher amounts of MC showed higher swelling rates than formulations containing lesser amount of MC. Thus, formulation *F*3 (20%, w/w, MC) exhibited a higher swelling than formulation *F*2 (15%, w/w, MC); similarly, formulation *F*2 exhibited a greater swelling than formulation *F*1, due to hydrophilic nature of MC, thereby leading to higher water uptake capacity.

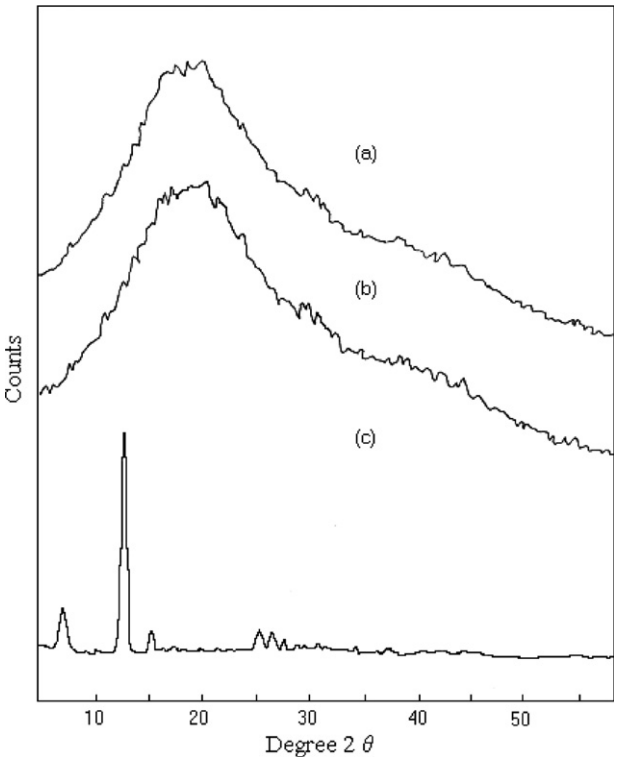

Fig. 6. X-RD spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.

3.6. Tensile strength measurements

Young's modulus of the crosslinked CS and MC membranes was used to estimate the average molecular weight between crosslinks (M_c) as well as the effective crosslink density (V_e). The M_c was determined using Eq. (3) derived from the rubber elasticity theory (Flory, 1953; Ward & Hadley, 1993):

$$M_c = \frac{3\rho RT}{E} \tag{3}$$

where ρ is specific density (g/cm^3), R is gas constant, T is absolute temperature and E is Young's modulus. The effective crosslink density was calculated using the following equation (Flory, 1953; Uzun, Hassnisaber, & Sen, 2003):

$$V_e = \frac{\rho}{M_c} \quad (4)$$

The results of Young's modulus, M_c and V_e estimated for CS and MC formulations are shown in Table 3. Here, we notice that the M_c values decreased with an increase in GA content of the formulation, since the network becomes denser. Also, the M_c values decreased with an increase in MC content of the formulation, indicating a dense structure. Similarly, GA and MC contents of the formulations significantly affected the crosslink density of IPNs. Thus, from the results of tensile strengths, the formation of IPN between CS and MC was confirmed.

3.7. In vitro release study

Drug release behavior of the formulations based on CS and MC polymers were evaluated by performing the *in vitro* release experiments in simulated gastric and intestinal pH conditions. Results of % cumulative release vs time for drug-loaded microspheres for formulations *F*1, *F*7, *F*2, *F*8, *F*3 and *F*9 are compared in Fig. 7 to investigate the extent of crosslinking on the *in vitro* release profiles. The *F*1 showed a higher release rate than *F*7 and similarly, *F*2 and *F*3 showed higher release rates than *F*8 and *F*9. This is attributed to an increase in the extent of crosslinking, leading to the formation of a denser network structure. Effects of MC content in formulations *F*7, *F*8, *F*9 and control formulation (CF) on the release rates are presented in Fig. 8. The % cumulative release is higher in case of *F*8 than *F*7 and similarly, *F*9 shows higher release rates than *F*8 (i.e., the trend is: $F9 > F8 > F7$). All the formulations showed higher release rates than CF, because, with an increasing MC content of the matrix, swelling of the matrix also increased due to more hydrophilic nature of MC. The effect of drug loading on *in vitro* release profiles for formulations *F*1, *F*4, *F*2, *F*5, *F*3 and *F*6 are displayed in Fig. 9, wherein it was observed that formulation *F*4 exhibited higher release rate than *F*1. Similarly, *F*5 and *F*6 formulations showed higher release rates than *F*2 and *F*3 indicating

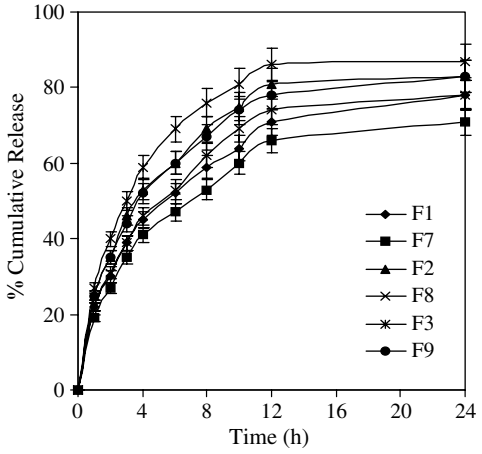

Fig. 7. Effect of crosslinking on *in vitro* release profile of formulations.

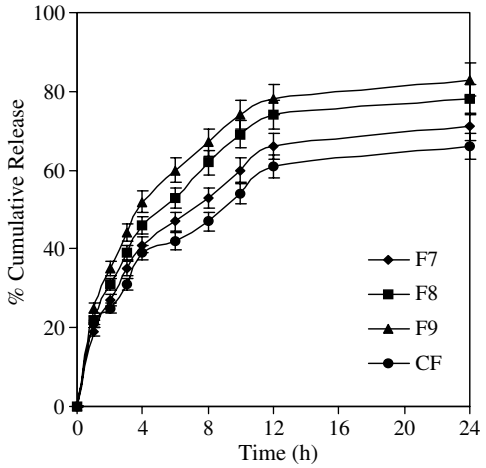

Fig. 8. Effect of polymer ratio on *in vitro* release profile of formulations.

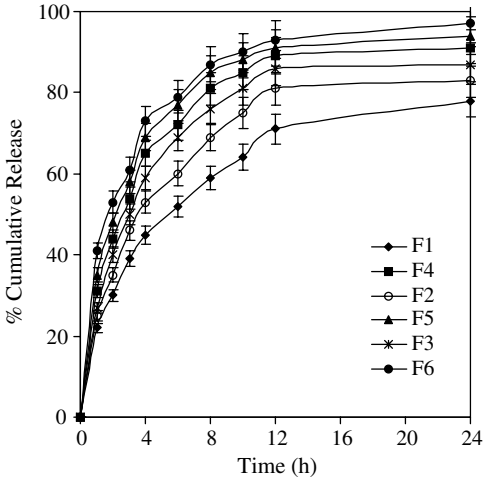

Fig. 9. Effect of % drug loading on *in vitro* release profile of formulations.

that the release rates vary depending upon the amount of drug loaded in the matrices. The release rate is higher in case of formulations containing higher amount of drug and similarly, drug release was lower for formulations having a lower amount of drug. Drug in the microspheres might also act as inert filler by occupying the available free volume of the swollen hydrogel. This might have created a tortuous path for water molecules to permeate through, but the degree of tortuosity depends upon the volume fraction of the filler. During the first two hours of release, the dissolution was performed in 0.1 N HCl, wherein we observed a burst release and the release of drug was extended up to 24 h.

The *in vitro* drug release rates were correlated to diffusion coefficients presented in Table 2. Diffusion coefficients are higher for formulations containing a higher amount of MC as compared to formulations containing a lower amount of MC. A similar trend was observed in drug release profiles i.e., formulations having a higher amount of MC showed a faster release rate as compared to

formulation with a lower amount of MC. Also, the crosslinking agent exhibited a perfect correlation between diffusion coefficient and the drug release behavior. It was found that formulations crosslinked with 5 mL of GA showed higher diffusion coefficients as compared to formulations crosslinked with 10 mL of GA. Similarly, the drug release was fast in those formulations that were crosslinked with 5 mL of GA as compared to formulations crosslinked with 10 mL of GA. In all the formulations, we observed the burst release, which varied depending upon the amount of MC, extent of drug loading and amount of GA present in the matrix. However, the release of drug was continued up to 24 h, but none of the formulations showed 100% release from the matrix.

The diffusion coefficient values were calculated using an empirical equation of the type:

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \tag{5}$$

where θ is slope of the linear portion of the plot of M_t/M_∞ vs. $t^{1/2}$, r is initial radius of the microspheres and M_∞ is the maximum value of drug release. It is noticed that diffusion coefficient values showed an increase with increasing content of MC from 10% to 20%. The diffusion coefficient values were higher in case of microspheres crosslinked with 5 mL of GA than those crosslinked with 10 mL of GA. This could be due to the hydrophilic nature of MC as well as its loose network structure at lower crosslinking concentration, leading to higher matrix swelling.

Drug release and molecular transport parameters were correlated using an empirical equation (Ritger & Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \tag{6}$$

Here, k is rate constant and n is an exponent parameter that represents the type of transport. The n values calculated by Eq. (6) included in Table 2 range from 0.363 to 0.563, indicating a slight deviation from the Fickian transport (Korsmeyer & Peppas, 1981; Rokhade et al., 2006; Soppimath, Kulkarni, & Aminabhavi, 2000). It is evident from Table 2 that the correlation coefficient (r) values approached unity, suggesting a best fit to the Fickian model.

4. Conclusions

This work demonstrates the successful use of two carbohydrate polymers viz., chitosan and methylcellulose to produce in the form of IPN microspheres for the effective encapsulation of THP by emulsification method. The IPNs of this demonstrated better mechanical properties than pure CS, indicating the suitability of IPNs for microsphere preparation. The crosslink density was significantly affected by the content of GA and MC in the formulations. FTIR confirmed the formation of IPN as well as chemical stability of THP in the microspheres. Microspheres with spherical shapes having smooth surfaces were produced. Microspheres with a narrow size distribution of sizes in the range of 119–318 μm were obtained. Swelling kinetics was dependent on the extent of crosslinking and the amount of MC used. The release of THP was found to depend on the extent of matrix crosslinking, amount of drug loading and MC content of the matrix. The release mechanism showed a slight deviation from the Fickian behavior. The microspheres of this study could be used as controlled release devices for the release of THP.

Acknowledgement

Authors thank University Grants Commission (UGC), New Delhi, India (F1-41/2001/CPP-II) for financial support to establish Center of Excellence in Polymer Science.

References

Antal, I., Zelk'o, R., Roczey, N., Plachy, J., & R'acz, I. (1997). Dissolution and diffuse reflectance characteristics of coated theophylline particles. *International Journal of Pharmaceutics, 155*, 83–89.

Berger, J., Reist, M., Mayer, J. M., Felt, O., & Gurny, R. (2004). Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics, 57*, 35–52.

Coviello, T., Grassi, M., Lapasin, R., Marino, A., & Alhaique, F. (2003). Scleroglucan/borax: characterization of a novel hydrogel system suitable for drug delivery. *Biomaterials, 24*, 2789–2798.

Dodane, V., & Vilivalam, V. D. (1998). Pharmaceutical applications of chitosan. *Pharmaceutical Science & Technology Today, 1*, 246–253.

Felt, O., Buri, P., & Gurny, R. (1998). Chitosan: a unique polysaccharide for drug delivery. *Drug Development and Industrial Pharmacy, 24*, 979–993.

Flory, P. J. (1953). *Principles of Polymer Chemistry*. New York: Cornell University Press, pp. 266, 432.

Handbook of Pharmaceutical Excipients, (2003) (4th ed.). Washington: American Pharmaceutical Association.

Hejazi, R., & Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *Journal of Controlled Release, 89*, 151–165.

Horkay, F., & Zrinyi, M. (1982). Studies on the mechanical and swelling behavior of polymer networks based on the scaling concept. 4. Extension of the scaling approach to gels swollen to equilibrium in a diluent of arbitrary activity. *Macromolecules, 15*, 1306–1310.

Illum, L. (1998). Chitosan and its use as a pharmaceutical excipient. *Pharmaceutical Research, 15*, 1326–1331.

Itokazu, M., Yamemoto, K., Yang, W. Y., Aoki, T., Kato, N., & Watanabe, K. (1997). The sustained release of antibiotics from freeze-dried fibrin-antibiotic compound and efficacies in rat model of osteomyelitis. *Infection, 25*, 359–363.

Katime, I., Novoa, R., & Zuluaga, F. (2001). Swelling kinetics and release studies of theopylline and aminophylline from acrylic acid/*n*-alkyl methacrylate hydrogels. *European Polymer Journal, 37*, 1465–1471.

Kawaguchi, H. (2000). Functional polymer microspheres. *Progress in Polymer Science, 25*, 1171–1210.

Korsmeyer, R. C., & Peppas, N. A. (1981). Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water-soluble drugs. *Journal of Membrane Science, 9*, 211–227.

Park, J. S., Park, J. W., & Ruckenstein, E. (2001). Thermal and dynamic mechanical analysis of PVA/MC blend hydrogels. *Polymer, 42*, 4271–4280.

Paul, W., & Sharma, C. P. (2000). Chitosan, a drug carrier for the 21st century: a review. *STP Pharma Science, 10*, 5–22.

Ratner, B. D. (1981). Biomedical applications of hydrogels: review and critical appraisal. In D. F. Williams (Ed.), *Biocompatibility of Clinical Implant Materials II*. Boca Raton, Florida: CRC Press.

Reverchon, E., Porta, G. D., De Rosa, I., Subra, P., & Letourneur, D. (2000). Supercritical antisolvent micronization of some biopolymers. *Journal of Supercritical Fluids, 18*, 239–245.

Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release. II Fickian and anomalous release from swellable devices. *Journal of Controlled Release, 5*, 37–42.

Rokhade, A. P., Agnihotri, S. A., Patil, S. A., Mallikarjuna, N. N., Kulkarni, P. V., & Aminabhavi, T. M. (2006). Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine. *Carbohydrate Polymers, 65*, 243–252.

Rokhade, A. P., Patil, S. A., & Aminabhavi, T. M. (2007). Synthesis and characterization of semi-interpenetrating microspheres of acrylamide grafted dextran and chitosan for controlled release of acyclovir. *Carbohydrate Polymers, 67*, 605–613.

Shariati, A., & Peters, C. J. (2003). Recent developments in particle design using supercritical fluids. *Current Opinion in Solid State Material Science, 7*, 371–383.

Shozo, M., Wataru, K., & David, A. (2000). Oral sustained delivery of theophylline using in situ gelation of sodium alginate. *Journal of Controlled Release, 67*, 275–280.

Soppimath, K. S., Kulkarni, A. R., & Aminabhavi, T. M. (2000). Controlled release of antihypertensive drug from the interpenetrating network poly(vinyl alcohol)-guar gum hydrogel microspheres. *Journal of Biomaterial Science, Polymer Edition, 11*, 27–43.

Tomihata, K., & Ikada, Y. (1997). Crosslinking of hyaluronic acid with glutaraldehyde. *Journal of Polymer Science Part A, 35*, 3553–3559.

Uzun, C., Hassnisaber, M., & Sen, M. (2003). Enhancement and control of cross-linking of dimethylaminoethyl methacrylate irradiated at low dose rate in the presence of ethylene glycol dimethacrylate. *Nuclear Instrumentation Methods Physics Research Section B: Beam Interaction of Materials and Atoms, 208*, 242–246.

Ward, I. M., & Hadley, D. W. (1993). *An Introduction to the Mechanical Properties of Solid Polymers*. Chichester: Wiley.

Yao, K. D., Peng, T., Feng, H. B., & He, Y. Y. (1994). Swelling kinetics and release characteristic of crosslinked chitosan: polyether polymer network (semi-IPN) hydrogels. *Journal of Polymer Science A: Polymer Chemistry, 32*, 1213–1223.

Yu, Z., Schwartz, J. B., & Sugita, E. T. (1996). Theophylline controlled release formulations: in vivo–in vitro correlations. *Biopharmaceutics Drug Dispos, 17*, 259–272.