Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 65 (2006) 243–252
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine ☆

Ajit P. Rokhade [a], Sunil A. Agnihotri [a], Sangamesh A. Patil [a], Nadagouda N. Mallikarjuna [b,1], Padmakar V. Kulkarni [b], Tejraj M. Aminabhavi [a,*]

[a] *Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University, Dharwad 580 003, India*
[b] *University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75390-9058, USA*

Received 3 November 2005; received in revised form 8 January 2006; accepted 10 January 2006
Available online 18 April 2006

Abstract

Semi-interpenetrating polymer network (IPN) microspheres of natural polymers, viz., gelatin and sodium carboxymethyl cellulose (NaCMC) were prepared by using glutaraldehyde (GA) as a crosslinker. Ketorolac tromethamine (KT), an anti-inflammatory and analgesic agent, was successfully encapsulated into IPN microspheres. Various formulations were prepared by varying the ratio of gelatin and NaCMC, % drug loading, and amount of GA. Microspheres were characterized by Fourier transform infrared spectroscopy (FTIR) to understand the formation of IPN structure and to confirm the absence of chemical interactions between drug, polymer, and crosslinking agent. Scanning electron microscopy (SEM) was used to study the surface morphology of the microspheres. SEM showed that particles have slightly rough surfaces. Particle size as measured by using laser light scattering technique gave an average size ranging from 247 to 535 μm. Differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of the drug after encapsulation into IPN microspheres. Drug encapsulation of up to 67% was achieved as measured by the UV method. Both equilibrium and dynamic swelling experiments were performed in water. Diffusion coefficients (D) of water transport through the microspheres were determined using an empirical equation. Values of D decrease with increasing crosslinking as well as increasing content of NaCMC in the matrix. In vitro release studies indicated a dependence of release rate on both the extent of crosslinking and the amount of NaCMC used to produce microspheres, but slow release was extended up to 10 h. Cumulative release data were fitted to an empirical equation to compute diffusional exponent (n), which indicated the non-Fickian trend for drug release.

Keywords: Hydrogels; Crosslinking; Interpenetrating network; Drug delivery; Microspheres; Ketorolac tromethamine

1. Introduction

Carbohydrate polymers are extensively used in recent years in biomedical and pharmaceutical applications due to their biocompatibility and biodegradability (Tabata & Ikada, 1998). Biopolymeric hydrogels can be prepared as three-dimensional hydrophilic networks that are capable of imbibing large quantities of water or biological fluids and release drugs at the controlled rates. Such networks can be composed of homopolymers or copolymers and are insoluble in water because of the presence of chemical or physical crosslinks such as entanglements or crystallites (Peppas, Burns, Leobandung, & Ichikawa, 2000; Sperling, 1981). Polymeric hydrogels have been studied in a variety of areas such as in chemical engineering, medicine, pharmaceuticals, food, and agriculture (Dave, Mehta, Aminabhavi, Kulkarni, & Soppimath, 1999; Dong &

☆ This paper is CEPS communication # 78.
* 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).
[1] Present address: US Environmental Protection Agency, Cincinnati, OH, USA.

0144-8617/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2006.01.013

Hoffman, 1991; Peppas & Korsmeyer, 1987; Seigel & Firestone, 1990). However, the use of natural carbohydrate polymers like polysaccharides and proteins for biomedical applications has attracted the attention of many investigators (Davis & Huglin, 1990; Desai & Hubbell, 1992; Khare & Peppas, 1993). Such naturally abundant carbohydrate polymers even though exhibit some limitations in their reactivity and processibility have been still used after being modified by crosslinking, blending, etc. Many studies have been made in the literature to overcome these shortcomings by chemical and physical alterations of such natural carbohydrate polymers. Among these, development of hydrogels and interpenetrating polymer network (IPN) structures has received greater attention (Burugapalli, Bhatia, Koul, & Choudhary, 2001; Changez, Koul, Burugapalli, & Dinda, 2004).

Gelatin is derived from collagen, a natural protein, which is a fibrous material that occurs in skin, bones, and connective tissues of animals (Ferdinando, 2000). It is insoluble in water and is solubilized by hydrolysis. The raw materials used for its manufacture are obtained from the bovine bones or porcine skins. The reaction can be carried out at an acid pH level, yielding type A gelatin (which is primarily produced from skins) and at the basic pH level giving type B gelatin (primarily produced from the bovine bones). Gelatin is a heterogeneous product that is a mixture of molecular species, α-, β-, and γ-peptides. Their proportions and molecular weights are dependent upon the nature of the chemical process. Gelatin is biocompatible, biodegradable, edible, and soluble at the body temperature, which undergoes gelation at temperatures just above ambient (Tabata & Ikada, 1998), which makes it an ideal material for pharmaceutical applications.

Sodium carboxymethyl cellulose (NaCMC) is a carboxymethyl ether of cellulose, the ubiquitous polysaccharide, composing the fibrous tissue of plants. Hydroxyl groups on 2-glucopyranose residue of cellulose are replaced by carboxymethyl groups; the number of replacements is known as the degree of substitution (DS). Both DS and polymer chain length will determine solubility, viscosity, and gel strength of NaCMC. Various researchers have studied the gelatin-based IPN systems. Kosmala, Henthorn, and Peppas (2000) have studied the IPN of gelatin and dextran as biodegradable materials. They have blended gelatin with dextran and crosslinked to form enzymatically degradable IPNs as matrices for biodegradable implants. Changez, Koul, and Dinda (2005) have studied the in vivo efficacy of antibiotics-loaded IPN hydrogels based on poly(acrylic acid) and gelatin for the treatment of experimental osteomyelitis. Liang, Chang, Liang, Lee, and Sung (2004) have studied gelatin hydrogels crosslinked with genipin and water-soluble carbodiimide and investigated the crosslinking mechanism, extent of crosslinking and cytotoxicity.

Ketorolac tromethamine (KT) is a member of the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drug (NSAID). It is a racemic mixture of [−] *S* and [+] *R* forms, but its biological activity is associated with the *S*-form. The half-life of *S*-enantiomer is approximately 2.5 h (SD ± 0.4) compared with 5 h (SD ± 1.7) for the *R*-enantiomer. The half-life of racemate has been reported (Bhaskaran & Suresh, 2004) to be around 4–6 h. Among the various drug delivery systems, particulate drug delivery systems have gained much attention in the controlled release (CR) of pharmaceuticals (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Roney et al., 2005; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Specific advantages of multiparticulate systems, such as microspheres, beads, etc., over the conventional dosage forms like tablets and capsules have been discussed in our earlier paper (Agnihotri & Aminabhavi, 2004). Presently, our laboratory is actively involved in the development and evaluation of IPN-based polymeric systems for the CR of various types of drugs. Earlier, Soppimath, Kulkarni, and Aminabhavi (2000) have prepared the IPN of poly(vinyl alcohol)–guar gum hydrogel microspheres for the CR of nifedipine. Kurkuri and Aminabhavi (2004) have developed pH-sensitive sequential IPN microspheres of poly(vinyl alcohol) and poly(acrylic acid) for the delivery of diclofenac sodium to the intestine. Recently, Agnihotri and Aminabhavi (2005) have developed the IPN microspheres of gellan gum and poly(vinyl alcohol) for the CR of carvedilol. As a part of our ongoing program of research on the modification of natural carbohydrate polymers, the present work describes the preparation and characterization of semi-IPN microspheres for the CR of KT. The microspheres prepared have been characterized by different techniques to understand their drug release characteristics and morphological as well as chemical interactions.

2. Experimental

2.1. Materials

Ketorolac tromethamine was kindly received as a gift sample by Ranbaxy Laboratories, Ltd., New Delhi, India. Gelatin, sodium carboxymethyl cellulose, high viscosity grade (500–800 cPs), 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. Petroleum ether, b.p. 60–80 °C, was received from Ranbaxy Fine Chemicals Ltd., New Delhi, India. All chemicals were used without further purification.

2.2. Preparation of semi-IPN microspheres

Semi-IPN microspheres of gelatin and NaCMC were prepared by emulsion-crosslinking method (Tabata & Ikada, 1989). Briefly, gelatin was dissolved in distilled water by continuously stirring at 37 °C until a homogeneous

solution was obtained. After cooling to ambient temperature, NaCMC was dispersed in the above gelatin solution and stirred overnight to obtain a homogeneous solution. Then, KT was dissolved in the above polymer solution. This solution was added slowly to a mixture of petroleum ether and light liquid paraffin (40:60, w/w) containing 1% (w/w) Span®-80 under constant stirring at 400 rpm speed for 10 min. To this w/o emulsion, GA was added slowly and further stirred for 2 h. The hardened microspheres were separated by filtration, washed with *n*-hexane, vacuum dried at 40 °C for 24 h, and stored in a desiccator until further use. Totally, eight formulations were prepared and the assigned formulation codes are given in Table 1. A schematic representation of the synthesis of semi-IPN is given in Fig. 1.

2.3. Drug content

Estimation of drug content was done according to the method adopted earlier (Agnihotri & Aminabhavi, 2004). Microspheres of known weight were soaked in 50 mL of water for 30 min and sonicated using a probe sonicator (UP 400s, 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, Paris, France) at the maximum wavelength, λ_{max} value of 324 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 laser light scattering technique (Mastersizer-2000, Malvern, UK). Particle size was measured by using dry sample adopter and volume mean diameter (V_d) was recorded. These data are also included in Table 2.

Table 1
Formulation codes and different ingredients used to prepare microspheres

Formulation code	NaCMC (% w/w)	Gelatin (% w/w)	Drug loading (%)	GA (mL)
F1	10	90	20	5
F2	20	80	20	5
F3	10	90	40	5
F4	20	80	40	5
F5	10	90	20	10
F6	20	80	20	10
F7	10	90	40	10
F8	20	80	40	10

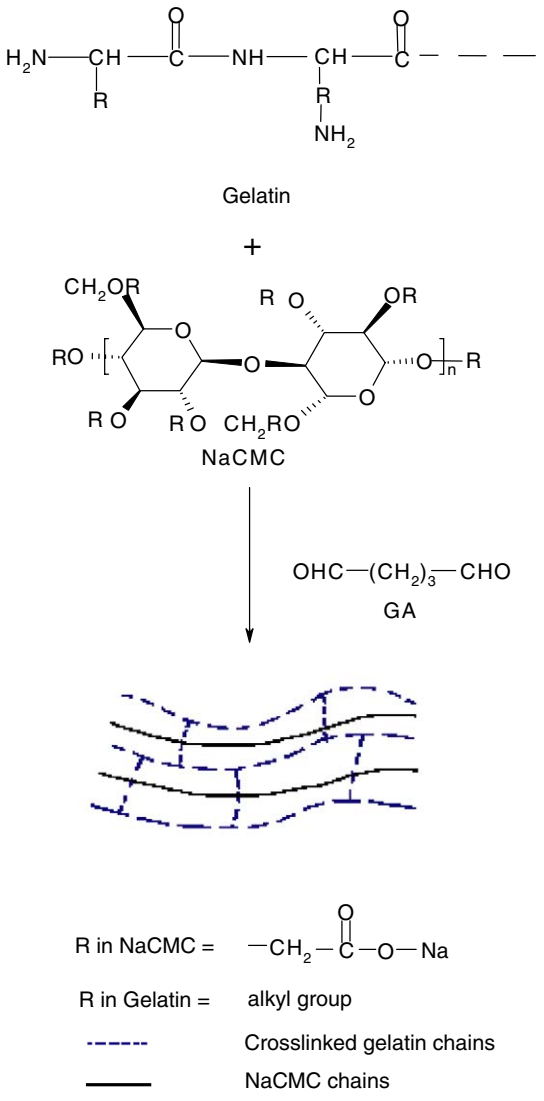

Fig. 1. Schematic representation of the synthesis of semi-IPN polymers.

2.5. Fourier transform infrared spectral studies

Fourier transform infrared (FTIR) spectral data were taken on a Nicolet (Model Impact 410, Milwaukee, WI, 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 pristine gelatin, pristine NaCMC, placebo microspheres, drug-loaded microspheres, and pristine drug were obtained. The samples were crushed with KBr to get pellets at 600 kg/cm^2. Spectral scanning was done in the range between 4000 and 500 cm^{-1}.

2.6. Differential scanning calorimetric study

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

Table 2
Results of % entrapment efficiency, volume mean particle size, % water uptake, and n values of Eq. (6) along with correlation coefficients, r, for various formulations

Formulation code	% Encapsulation efficiency Eq. (1)	Volume mean particle size (μm)	Water uptake (%, w/w)	n	r
F1	66.6	399	457.53	0.185	0.966
F2	60.9	535	458.60	0.229	0.984
F3	65.9	287	340.45	0.298	0.993
F4	62.7	334	380.51	0.357	0.932
F5	62.8	320	278.17	0.427	0.991
F6	60.2	426	319.97	0.459	0.947
F7	65.1	247	176.16	0.467	0.997
F8	61.6	292	245.05	0.481	0.963

2.7. X-ray diffraction studies

Crystallinity of KT after encapsulation was evaluated by X-ray diffraction (X-RD) measurements recorded for pristine KT, placebo microspheres, and drug-loaded microspheres using the X-ray diffractometer (x-Pert, Philips, Cambridge, UK). Scanning was done up to 2θ of 43°.

2.8. Scanning electron microscopic (SEM) studies

SEM micrograph of the semi-IPN microspheres prepared by crosslinking with 10 mL GA and loaded with 40% KT was taken. Microspheres were sputtered with gold to make them conducting and placed on a copper stub. Scanning was done using JEOL Model JSM-840A, Japan.

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 and the swollen microspheres were weighed to an accuracy of ±0.01 mg using an electronic microbalance (Mettler, AT 120, Griefensee, 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 then calculated as:

$$\left(\frac{\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}}{\text{Mass of dry microspheres}}\right) \times 100. \quad (2)$$

Drug release from the crosslinked hydrogels depends upon the extent of water penetration into the crosslinked hydrogel matrix. In order to understand the molecular transport of water into crosslinked microspheres, dynamic swelling studies were performed by the microscopic technique (Robert, Bun, & Peppas, 1985; Soppimath & Aminabhavi, 2002). Here, the change in diameter of the microspheres in the presence of distilled water was monitored as a function of time. Experiments were carried out in triplicate, but average values were considered for data treatment and calculations.

2.10. In vitro release studies

Drug release from the semi-IPN microspheres with different % drug loading and different extent of crosslinking was investigated in 0.1 N HCl for the initial 2 h, followed by phosphate buffer, pH 7.4, until the completion of dissolution. In vitro release experiments were performed using the USP apparatus-I dissolution tester (Dissotest, LabIndia, Mumbai, India) at a stirring speed of 100 rpm. Weighed quantities of the samples equivalent to 10 mg of the drug were placed in the basket, which was then immersed in 500 mL dissolution medium maintained at 37 °C. A 5 mL of sample aliquot was withdrawn at different time intervals and filtered through a 0.45 mm filter (Sartorius, Goettingen, Germany). The dissolution media were then replaced with 5 mL of fresh dissolution media. The KT concentration was determined spectrophotometrically at λ_{max} of 324 nm. These studies were performed in triplicate for each sample, but average values were considered in data analysis and graphical presentations.

In order to study the release of NaCMC from the IPN matrix during drug release studies, four formulations were prepared by varying the concentration of NaCMC, gelatin, and GA. The formulation code, polymer composition, and the amount of GA used are given in Table 3. The release of NaCMC from IPN microspheres was studied gravimetrically with respect to time. About 500 mg (initial weight) of the microspheres was accurately weighed and placed in a beaker containing about 100 mL of distilled water and stirred at 100 rpm speed on a electronically controlled magnetic stirrer (Jenway 1103, Essex, England). At a particular time interval (after 1 h), microspheres were filtered and dried in an oven at 40 °C for overnight and weighed to an accuracy of ±0.01 mg (final weight). The difference in initial and final weights of the microspheres gave the

Table 3
Formulation codes and different ingredients used for the preparation of microspheres for the release study of NaCMC

Formulation code	NaCMC (% w/w)	Gelatin (% w/w)	GA (mL)
F1	10	90	5
F2	20	80	5
F3	10	90	10
F4	20	80	10

amount of NaCMC released in the medium. The same procedure was repeated at 2, 3, 4, 6, 8, and 10 h for different formulations.

3. Results and discussion

3.1. Preparation and characterization of microspheres

In the present research, the KT-loaded semi-IPN microspheres based on gelatin and NaCMC were prepared and crosslinked with GA. By this method, % encapsulation efficiency was between 60 and 67, which did not show any dependence on % drug loading and extent of crosslinking. However, by increasing the amount of NaCMC, a slight decrease in % encapsulation efficiency was observed, due to the formation of loose network that allows the leaching out of more of drug particles during the production stage of the microspheres. Particle size (see Table 2) revealed an increase with increasing amount of NaCMC. It was found that particle size of F2 (20%, w/w, NaCMC) is higher than that of F1 (10%, w/w, NaCMC) and similar findings were observed for F4, F6, and F8 formulations as compared to F3, F5, and F7. This could be due to the fact that at higher amounts of NaCMC, the viscosity of polymer solution increased, thus producing bigger droplets during emulsification that were later hardened in the presence of GA. Another interesting observation is that particle size decreased with an increase in the amount of crosslinking agent. It is observed that particle size of F1 (with 5 mL GA) is higher than that of F5 (with 10 mL GA). Similar findings were observed for F2, F3, and F4 formulations as those of F6, F7, and F8 formulations, due to the formation of increased rigid network structure at higher extent of crosslinking. Agnihotri and Aminabhavi (2004) have also reported similar findings. Microspheres of this study were spherical with slightly rough surfaces as revealed by SEM images shown in Fig. 2.

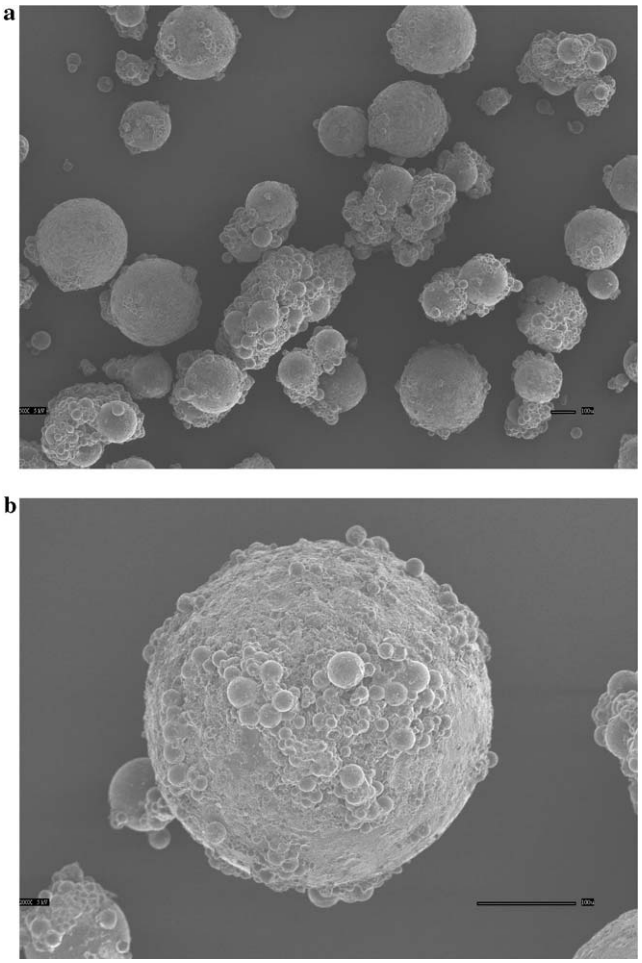

Fig. 2. SEM photographs of (a) group of microspheres and (b) single microsphere.

FTIR spectral data were used to confirm the crosslinking of gelatin chains by GA. FTIR spectra of the pristine gelatin (curve a), pristine NaCMC (curve b), and placebo microspheres (curve c) are compared in Fig. 3. In the case of pristine gelatin, a characteristic band due to N–H stretching is observed at 3413 cm^{-1}. The N–H bending vibration is indicated by a band observed at 1535 cm^{-1}. Aliphatic C–H stretching is observed at 2919 cm^{-1}, but aliphatic C–H bending vibrations are observed at 1450 and 1405 cm^{-1}. The band appearing at 1646 cm^{-1} indicates amide I band, while bands at 1338 and 1239 cm^{-1} indicate the C–N bond stretching vibrations. NaCMC shows bands at 3401 and 3314 cm^{-1} due to O–H stretching vibrations. Bands at 2937 and 2907 cm^{-1} show aliphatic C–H stretching vibrations, but those appearing at 1604 and 1425 cm^{-1} are, respectively, due to the asymmetric and symmetric stretchings of the carboxylate group. Bands found at 1108 and 1030 cm^{-1} represent C–O–C stretching vibrations. In the case of placebo microspheres, all the peaks appeared both in gelatin and NaCMC were observed. In addition, a new peak was observed at 1647 cm^{-1}, indicating the C=N stretching vibration of the imine group of Schiff base. This band confirms the formation of crosslink between gelatin chains by GA. It may be noted that in the case of placebo microspheres, the band observed at 1647 cm^{-1} was overlapped with that of amide I band of gelatin, which is evident from an increased intensity of the band.

FTIR spectra were also used to confirm the chemical stability of KT after the preparation of microspheres. The spectra of placebo microspheres (curve a), drug-loaded microspheres (curve b), and pristine KT (curve c) are presented in Fig. 4. For KT, a band at 3357 cm^{-1} shows N–H and O–H stretching vibrations. A band at 3061 and 731 cm^{-1} indicates aromatic C–H stretching and aromatic C–H bending vibrations, respectively. Aliphatic C–H stretching is indicated by bands at 2956 and 2919 cm^{-1} but, aliphatic C–H bending vibration is observed at 1382 cm^{-1}. Carboxylic acid C=O stretching vibration is represented by a band observed at 1610 cm^{-1}. Bands at 1586 and 1561 cm^{-1} are due to carbonyl C=O stretching vibrations, but a band at 896 cm^{-1} indicates the monosubstituted phenyl ring.

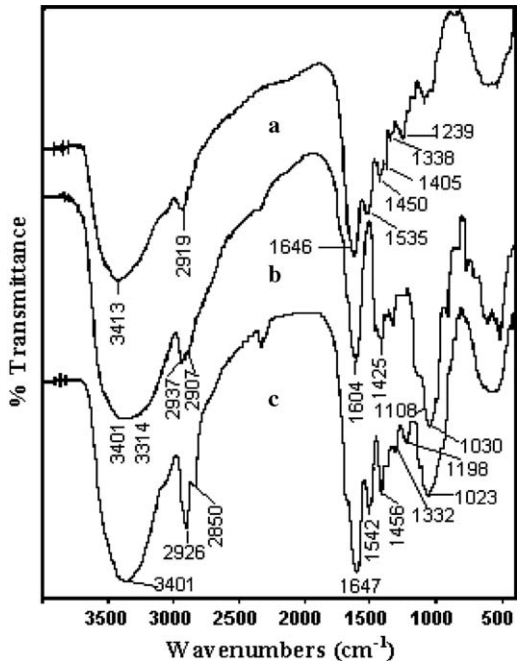

Fig. 3. FTIR spectrum of (a) pristine gelatin, (b) pristine NA-CMC, and (c) placebo microspheres.

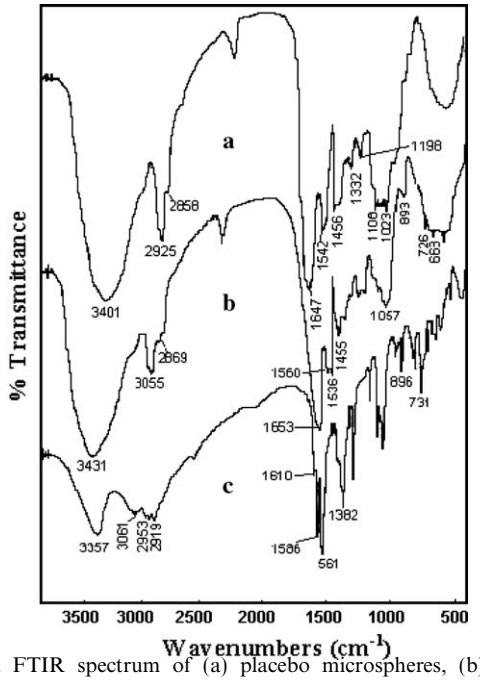

Fig. 4. FTIR spectrum of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pristine KT.

In case of drug-loaded microspheres, all the peaks present in placebo microspheres appeared. Along with this, the peaks observed for KT at 3055, 1560, 893, 727, and 668 cm^{-1} have also appeared. In addition, drug-loaded microspheres showed a broad peak at 1653 cm^{-1}, which is due to the merging of peaks of KT observed at 1610, 1586, and 1561 cm^{-1} with that of the peak of placebo microspheres at 1647 cm^{-1}. This confirms the presence of drug in the drug-loaded microspheres and also, the chemical stability of KT even after encapsulation.

3.2. Differential scanning calorimetric study

DSC thermograms of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pristine KT are presented in Fig. 5. In case of placebo microspheres, a broad peak was observed at 85 °C with two small peaks around 155 and 250 °C due to endothermic transition. Thermogram of KT showed a sharp peak at 172 °C, indicating the melting of the drug. In case of drug-loaded microspheres, all three peaks observed in placebo microspheres are noticed, but there was no peak corresponding to KT. This indicates the amorphous dispersion of KT into semi-IPN matrix.

3.3. X-ray diffraction studies

X-ray diffractograms of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pristine KT are presented in Fig. 6. These studies are useful to investigate the crystallinity of drug in the crosslinked microspheres. KT has shown characteristic intense peaks between 2θ of 18° and 20° due to its crystalline nature. However, these peaks have disappeared in the KT-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 formulations, characteristic peaks of KT have overlapped 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 drug is dispersed at the molecular level in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

3.4. Water uptake studies

Equilibrium uptake of the crosslinked microspheres exerts an influence on their release rates (Ritger & Peppas,

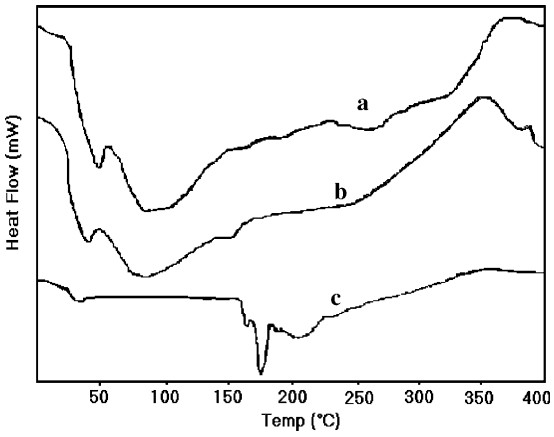

Fig. 5. DSC thermograms of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pristine KT.

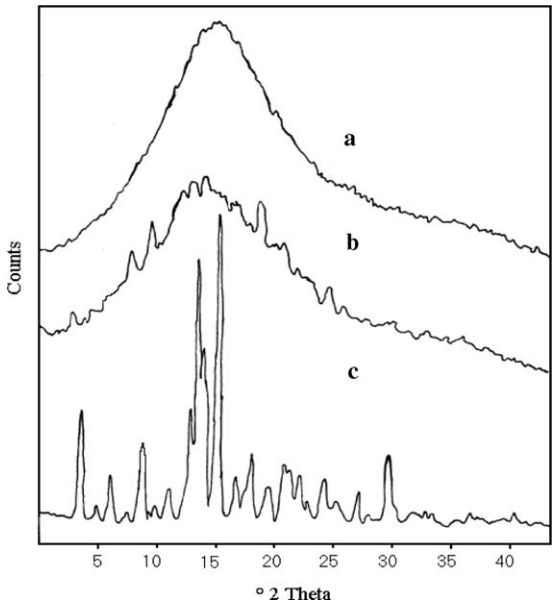

Fig. 6. X-RD patterns of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pristine KT.

1987). The % equilibrium water uptake data of the crosslinked microspheres presented in Table 2 indicate that, as the amount of GA in the matrices increases from 5 to 10 mL, the equilibrium water uptake decreases significantly from 459% to 176%. The reduction in water uptake capacity is due to the formation of a rigid network structure at the higher concentration of crosslinking. It is to be noted that formulations containing higher amounts of NaCMC showed higher swelling rates than formulations containing small amounts of NaCMC. Formulation F2 (20%, w/w, NaCMC) exhibits a higher swelling than F1 (10%, w/w, NaCMC). Results of swelling for formulations F4, F6, and F8 are almost identical to those observed for F3, F5, and F7 formulations, probably due to hydrophilic nature of NaCMC, thereby leading to higher water uptake.

Dynamic swelling studies were performed by monitoring the changes in microsphere diameter, D_t, as a function of time with the help of an optical microscope. Fig. 7 displays the plot of normalized diameter, D_t/D_o (D_o is initial diameter of the microsphere) as a function of time, t for formulations containing different amounts of GA. It is evident that normalized diameter values decrease with an increasing amount of GA, which could be due to the rigid network structure formed at higher amount of GA. Fig. 8 displays the plot of D_t/D_o vs t for different amounts of NaCMC. It is observed that the normalized diameter increases with increasing amount of NaCMC, due to the hydrophilic nature of NaCMC, which will enhance the water transport rate as well as water uptake capacity of the microspheres. The results of equilibrium swelling diameter, D_∞, normalized to original diameter, D_o, are presented in Table 4.

Dimensional changes of the microspheres due to swelling (i.e., volume change ΔV_t with time with respect to initial volume, V_o) have been analyzed to compute diffusion

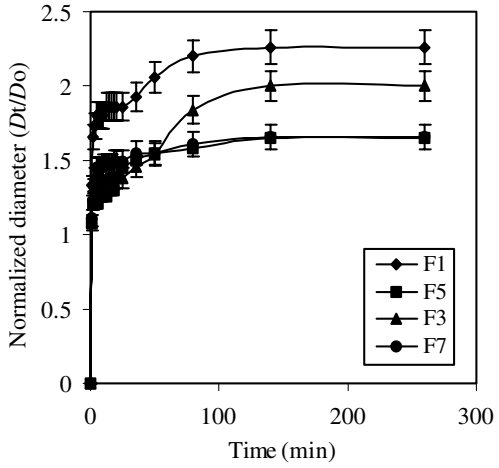

Fig. 7. Plot of D_t/D_o vs time, t: effect of extent of crosslinking.

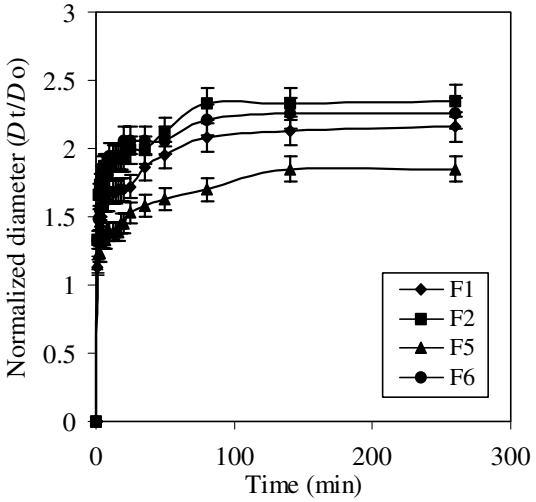

Fig. 8. Plot of D_t/D_o vs time, t: effect of amount of NaCMC.

coefficient, D_v, of the water molecules (Harogoppad & Aminabhavi, 1992) as:

$$\left(\frac{\Delta V_t}{V_0}\right) = \left(\frac{4\left(\frac{\Delta V_\infty}{V_0}\right)}{D_0}\right)\left(\frac{D_v}{\pi}\right)^{1/2} t^{1/2}. \quad (3)$$

Thus, D_v can be calculated as:

$$D_v = \left[(1.773 \times \text{Slope})\frac{V_0 D_0}{4\Delta V_\infty}\right]^2. \quad (4)$$

Here, ΔV_∞ represents the change in volume at equilibrium condition. Eq. (4) is used to calculate D_v from the slope of the initial linear plots of $\Delta V_t/V_o$ vs $t^{1/2}$. These data are also included in Table 4.

The solvent front velocity, u, of the advancing boundary for spherical microspheres was calculated using the equation:

$$u = \left(\frac{dv}{dt}\right)\frac{1}{A}. \quad (5)$$

Table 4
Transport data of water in semi-IPN microspheres

Formulation code	Equilibrium normalized diameter (D_∞/D_o)	n Eq. (6)	r	$D_v \times 10^5$ (cm^2/s) Eq. (4)	$u \times 10^3$ (cm/s) Eq. (5)
F1	2.17	0.199	0.927	6.33	6.21
F2	2.23	0.278	0.984	7.19	6.26
F3	2.00	0.284	0.957	5.36	4.41
F4	2.10	0.302	0.973	6.24	5.64
F5	1.93	0.333	0.948	2.32	3.10
F6	1.95	0.359	0.962	4.03	3.92
F7	1.84	0.395	0.961	1.70	1.04
F8	1.92	0.429	0.943	2.26	3.04

Here, dv/dt is change in volume of the microsphere per unit time and A is area of the microsphere. The results of u are also included in Table 4.

The values of diffusion coefficients and solvent front velocity decrease with increasing amount of crosslinking agent of the membrane matrix. For instance, by increasing the amount of GA from 5 to 10 mL, diffusion coefficients decrease from 7.19×10^{-5} to 1.70×10^{-5} cm^2/s; similarly, the solvent front velocity decrease from 6.260 to 1.039 cm/s. However, with increasing concentration of NaCMC, diffusion coefficients and solvent front velocities also increased, which is attributed to hydrophilic nature of NaCMC. Molecular transport into microspheres has thus shown dependence on the extent of crosslinking. Furthermore, these results support that a tighter crosslinked matrix does not expand greatly as compared to the loosely crosslinked matrix. At lower amounts of GA, the network is somewhat loose and has a high hydrodynamic free volume to accommodate more of solvent molecules, thereby causing the matrix swelling. The water uptake in hydrogels depends upon the extent of hydrodynamic free volume and the availability of hydrophilic functional groups for water to establish hydrogen bonds to swell more. Higher water uptake values observed at lower levels of crosslinking and vice versa observed in the present systems confirm the formation of semi-IPN due to crosslinking.

Dynamic swelling data of all the formulations have been fitted to an empirical equation (Robert et al., 1985; Soppimath et al., 2000).

$$\frac{D_t}{D_\infty} = kt^n. \tag{6}$$

Here, k is the rate constant and the exponent, n, is a parameter that represents the type of transport mode. The least-squares estimated values of n from the dynamic swelling data after fitting to Eq. (6) are presented in Table 4 along with correlation coefficients, r. Values of n range between 0.199 and 0.429 with increasing amount of GA from 5 to 10 mL. These values are in agreement as reported before (Agnihotri & Aminabhavi, 2005; Korsmeyer & Peppas, 1981; Soppimath & Aminabhavi, 2002; Soppimath et al., 2000), indicating the non-Fickian transport mode.

3.5. In vitro release study

To understand the drug release from KT-loaded semi-IPN microspheres of gelatin and NaCMC, in vitro release experiments were carried out in gastric and intestinal pH conditions. Results of % cumulative release vs time for drug-loaded microspheres for formulations F1, F5, F3, and F7 are compared in Fig. 9 to investigate the extent of crosslinking on in vitro release profiles. The F1 formulation shows higher release rates than F5. This is attributed to an increase in the extent of crosslinking, leading to the formation of a rigid network structure. Similarly, F3 shows a higher release rate than F7; this could be due to the formation of a more tightly crosslinked network structure.

Effects of NaCMC content in formulations F1, F2, F5, and F6 on release rates are presented in Fig. 10. The % cumulative release is higher in case of F2 than F1. This is because, as the NaCMC content in the polymer matrix increases, swelling of the matrix also increases due to the extremely hydrophilic nature of NaCMC. Similarly, F6 shows higher release rates than F5. The effect of drug loading on in vitro release profiles for formulations F1, F3, F5, and F7 is displayed in Fig. 11. The F3 exhibits higher release rates than F1. Similarly, F7 shows higher release

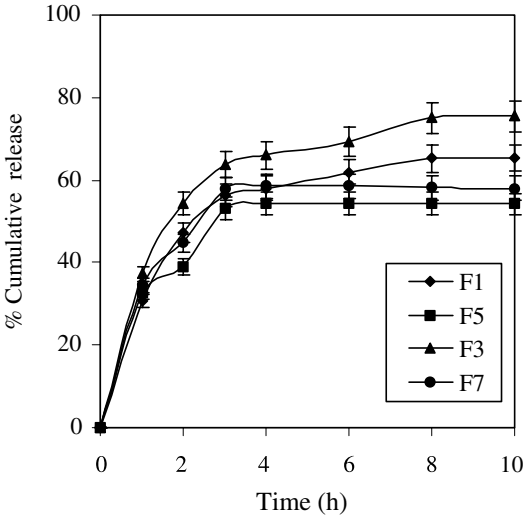

Fig. 9. Extent of crosslinking on in vitro release profile.

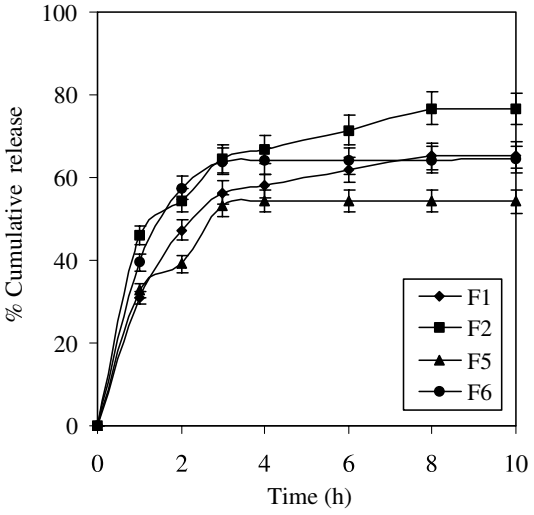

Fig. 10. Effect of NaCMC content on in vitro release profile.

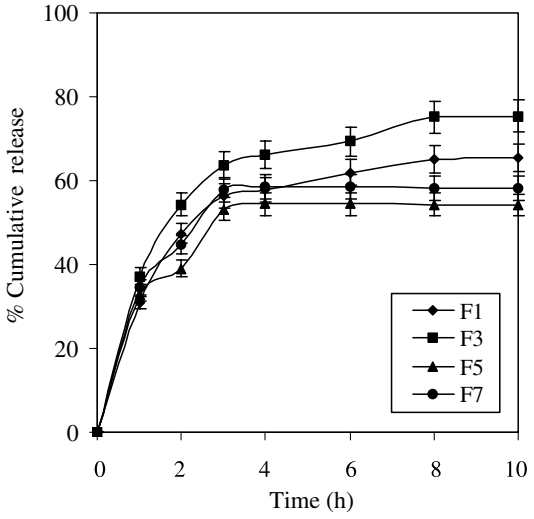

Fig. 11. Effect of % drug loading on in vitro release profile.

rates than F5. This indicates that release rates vary depending upon the amount of drug present in the matrices, i.e., release is slower for those formulations having lower amount of drug. During the first 2 h of release, dissolution was performed in 0.1 N HCl, wherein we could observe the burst release effect. Such a burst effect is observed for all the formulations, but the release of drug was extended up to 10 h. However, none of the formulations showed 100% drug release.

In order to establish a link between drug release rates and molecular transport parameters, we have fitted the release data to an empirical equation (Ritger & Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n. \tag{7}$$

Here, k and n have the same meanings as indicated in Eq. (6). The n values calculated by Eq. (7) and included

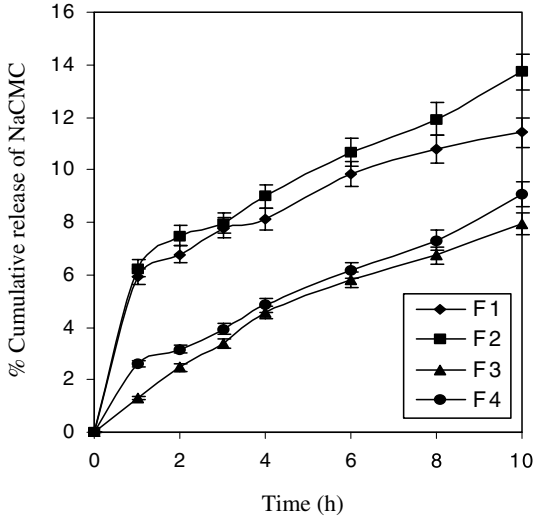

Fig. 12. Release data of NaCMC from microspheres.

in Table 2, for the present microspheres, ranging from 0.185 to 0.481 indicate non-Fickian transport trends as suggested earlier (Agnihotri & Aminabhavi, 2004a; Korsmeyer & Peppas, 1981; Soppimath et al., 2000). The n values for microspheres crosslinked with 5 mL of GA are smaller than those found for 10 mL GA containing microspheres. This could be the result of loosely crosslinked polymer network, which leads to increased swelling. The values of n being higher for microspheres crosslinked with 10 mL of GA could be due to the formation of a rigid IPN matrix.

The release of NaCMC from IPN microspheres is shown in Fig. 12. It is prevalent that formulations containing higher amount of NaCMC show a greater release rate than formulations containing a less amount of NaCMC. Formulations F2 and F4 exhibited greater release rates than F1 and F3. This could be due to extremely hydrophilic nature of NaCMC. Furthermore, formulations crosslinked with 5 mL GA show higher release rates than those containing 10 mL of GA. In this case, F1 and F2 formulations show higher release rates than F3 and F4. This could be due to the formation of a more rigid gelatin network structure at higher amount of GA in the matrix.

4. Conclusions

Two naturally available carbohydrate polymers, viz., gelatin and NaCMC have been chosen to develop semi-IPN microspheres for the effective encapsulation (by emulsification method) and controlled release of KT. FTIR was used to confirm the formation of interpenetrating network polymer. Microspheres with spherical shapes having somewhat rough surfaces were produced with a narrow size distribution of sizes ranging from 247 to 535 μm. Swelling kinetics was investigated in terms of the extent of crosslinking agent and the amount of NaCMC used. The amount of matrix crosslinking, drug loading, and NaCMC content of the matrix influ-

enced the release of KT. Release mechanism followed a non-Fickian type behavior. It is demonstrated that microspheres of this study are useful as CR devices to control the release rates of KT through the polymeric matrices developed.

Acknowledgments

Authors appreciate (T.M. Aminabhavi, S.A. Patil, and A.P. Rokhade) the financial support received from University Grants Commission (UGC), New Delhi, India (F1-41/2001/CPP-II), to establish Center of Excellence in Polymer Science.

References

Agnihotri, S. A., & Aminabhavi, T. M. (2004). Controlled release of clozapine through chitosan microparticles prepared by a novel method. *Journal of Controlled Release, 96*, 245–259.

Agnihotri, S. A., & Aminabhavi, T. M. (2004a). Formulation and evaluation of novel tableted chitosan microparticles for the controlled release of clozapine. *Journal of Microencapsulation, 21*, 709–718.

Agnihotri, S. A., & Aminabhavi, T. M. (2005). Development of novel interpenetrating network gellan gum–poly(vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. *Drug Development and Industrial Pharmacy, 31*, 491–503.

Agnihotri, S. A., Mallikarjuna, N. N., & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *Journal of Controlled Release, 100*, 5–28.

Bhaskaran, S., & Suresh, S. (2004). Biodegradable microspheres of ketorolac tromethamine for parenteral administration. *Journal of Microencapsulation, 21*, 743–750.

Burugapalli, K., Bhatia, D., Koul, V., & Choudhary, V. (2001). Interpenetrating polymer networks based on poly(acrylic acid) and gelatin. I: Swelling and thermal behavior. *Journal of Applied Polymer Science, 82*, 217–227.

Changez, M., Koul, V., Burugapalli, K., & Dinda, A. K. (2004). Studies on biodegradation and release of gentamicin sulphate from interpenetrating network hydrogels based on poly(acrylic acid) and gelatin: In vitro and in vivo. *Biomaterials, 25*, 139–146.

Changez, M., Koul, V., & Dinda, A. K. (2005). Efficacy of antibiotics-loaded interpenetrating network (IPNs) hydrogel based on poly(acrylic acid) and gelatin for treatment of experimental osteomyelitis: In vivo study. *Biomaterials, 26*, 2095–2104.

Dave, A. M., Mehta, M. H., Aminabhavi, T. M., Kulkarni, A. R., & Soppimath, K. S. (1999). A review on controlled release of nitrogen fertilizers through polymeric membrane devices. *Polymer Plastics Technology and Engineering, 38*, 675–711.

Davis, T. P., & Huglin, M. B. (1990). Effect of composition on properties of copolymeric *N*-vinyl-2-pyrrolidone/methyl methacrylate hydrogels and organogels. *Polymer, 31*, 513–519.

Desai, N. P., & Hubbell, J. A. (1992). Surface physical interpenetrating networks of poly(ethylene-terephthalate) and poly(ethylene oxide) with biomedical applications. *Macromolecules, 25*, 226–232.

Dong, L. C., & Hoffman, A. S. (1991). A novel approach for preparation of pH-sensitive hydrogels for enteric drug delivery. *Journal of Controlled Release, 15*, 141–152.

Ferdinando, J. C. (2000). Formulation solutions – softgels. Pharmaceutical Manufacturing and Packing, March, 69–73.

Harogoppad, S. B., & Aminabhavi, T. M. (1992). Diffusion and sorption of organic liquids through polymer membranes VIII. Elastomers versus monocyclic aromatic liquids. *Journal of Applied Polymer Science, 46*, 725–732.

Khare, A. R., & Peppas, N. A. (1993). Investigation of hydrogel water in polyelectrolyte gels using differential scanning calorimetry. *Polymer, 34*, 4595–4800.

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.

Kosmala, J. D., Henthorn, D. B, & Peppas, L. B. (2000). Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. *Biomaterials, 21*, 2019–2023.

Kurkuri, M. D., & Aminabhavi, T. M. (2004). Poly(vinyl alcohol) and poly(acrylic acid) sequential interpenetrating network pH sensitive microspheres for the delivery of diclofenac sodium to the intestine. *Journal of Controlled Release, 96*, 9–20.

Liang, H. C., Chang, W. H., Liang, H. F., Lee, M. H., & Sung, H. W. (2004). Crosslinking structures of gelatin hydrogels crosslinked with genipin or a water-soluble carbodiimide. *Journal of Applied Polymer Science, 91*, 4017–4026.

Peppas, N. A., Burns, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics, 50*, 27–46.

Peppas, N. A., & Korsmeyer, R. W. (1987). *Hydrogels in medicine and pharmacology*. Boca Raton, FL: CRC Press.

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.

Robert, C. C. R., Bun, P. A., & Peppas, N. A. (1985). Effect of degree of crosslinking on water transport in polymeric microparticles. *Journal of Applied Polymer Science, 30*, 301–306.

Roney, C., Kulkarni, V. P., Arora, V., Bennett, M., Antich, P., Bonte, F., et al. (2005). Targeted nanoparticles for drug delivery through the blood–brain barrier for Alzheimer's disease. *Journal of Controlled Release, 108*, 193–214.

Seigel, R. A., & Firestone, B. A. (1990). Mechanochemical to self-regulating insulin pump design. *Journal of Controlled Release, 11*, 181–192.

Soppimath, K. S., & Aminabhavi, T. M. (2002). Water transport and drug release study from crosslinked polyacrylamide-*grafted*-guar gum hydrogel microspheres for the controlled release application. *European Journal of Pharmaceutics and Biopharmaceutics, 53*, 87–98.

Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release, 70*, 1–20.

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.

Sperling, L. H. (1981). *Interpenetrating polymer networks and related materials*. Plenum Press: New York, p 1..

Tabata, Y., & Ikada, Y. (1989). Synthesis of gelatin microspheres containing interferon. *Pharmaceutical Research, 6*, 422–427.

Tabata, Y., & Ikada, Y. (1998). Protein release from gelatin matrices. *Advanced Drug Delivery Reviews, 31*, 287–301.