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Carbohydrate Polymers 71 (2008) 208–217
Carbohydrate Polymers
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Preparation and in-vitro release of chlorothiazide novel pH-sensitive chitosan-*N*,*N*′-dimethylacrylamide semi-interpenetrating network microspheres ☆

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Received 6 January 2007; received in revised form 22 May 2007; accepted 24 May 2007
Available online 10 June 2007

Abstract

pH-sensitive novel semi-interpenetrating networks (semi-IPN) of *N*,*N*′-dimethylacrylamide (NNDMA) and chitosan (CS) were prepared in the form of microspheres by water-in-oil (w/o) emulsion technique. Microspheres were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and X-ray diffractometry (X-RD) technique to confirm the cross-linking as well as polymorphism of chlorothiazide (CLT) drug. X-RD and DSC techniques indicated molecular level dispersion of CLT in the IPN matrix. Scanning electron micrographs (SEM) of the microspheres indicated smooth surfaces of the spherical microspheres. Cumulative release characteristics of the matrices for CLT, anti-hypertensive drug, were investigated in pH 1.2 and 7.4 media. Particle size and size distribution of the microspheres was measured by dynamic laser light diffraction technique. It was possible to release CLT in a controlled manner up to 12 h.

Keywords: Chitosan; *N*,*N*′-Dimethylacrylamide; Semi-IPN microspheres; Drug delivery

1. Introduction

Evolution of pharmaceutical technology has lead to the development of newer methods of drug administration as well as the design and application of controlled release (CR) formulations for the effective targeting of certain drugs to the site of action. In particular, the use of novel types of polymers provide newer approaches in the development of CR dosage formulations with an ultimate goal to achieve desired therapeutic results as well as optimize the CR of drug to obtain the maximum dose regimen with minimum of side effects (Garcia, Blanco, Martin, & Teijon, 2000). The release of any drug from the polymeric matrix occurs due to transport of solute molecules (drug) to the surrounding medium by molecular diffusion through the walls of the polymeric microspheres. Among many polymeric systems investigated in CR applications, the prime attention has been focused on natural, synthetic as well as combination of both types of polymers (Lou, Munro, & Wang, 2004; Palmieri, Lovato, & Martelli, 1999; Ramesh Babu et al., 2006c; Ramesh Babu, Sairam, Hosamani, & Aminabhavi, 2006b; Ramesh Babu, Sairam, Hosamani, & Aminabhavi, in press; Yuk, Cho, & Lee, 1995). Natural polymers like sodium alginate, chitosan and methyl cellulose have been the preferred polymers because of their biocompatibility and biodegradability. However, there are some synthetic polymers that exhibit biocompatibility under the physiological conditions used in CR studies. A combination of judicially selected natural and synthetic polymers has been found to be useful in enhancing the release of short half-lived drugs under physiological conditions. In order achieve this, the properties of natural (Gurdag, Yaar, & Gurkaynak, 1997; Shukla & Sharma, 1987; Trimmnel, Fanta, & Salch, 1996) and synthetic

☆ This paper is CEPS communication #172.
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doi:10.1016/j.carbpol.2007.05.039

(Nho & Jin, 1997; Sacak & Celik, 1996) polymers have been modified by grafting, blending and other means. Grafting of vinyl monomers onto natural polymers such as cellulose has been widely accepted.

Chitosan, obtained from deacetylation of chitin, is one of the most facile polymers that can be altered structurally to give useful matrices (Binder & Gruber, 2000; Park, You, Park, Haam, & Kim, 2001). Chitosan is quite useful in biomedical applications due to easy degradation in aqueous solutions as well as the hydroxyl and amino groups of chitosan can be easily modified (Kang, Choi, & Kweon, 1999; Peniche et al., 1999). However, the key properties of chitosan has biocompatibility, nonantigenicity, nontoxicity (its degradation products are the well known natural metabolites) (Mi, Tan, Liang, & Sung, 2002; Risbud, Hardikar, Bhat, & Bhonde, 2000; Thomas & Sharma, 1990), the ability to improve wound healing, blood clotting, ability to absorb liquids, its easy to form protective films and coatings, selective binding of liquids, all these have been used to lower the serum cholesterol levels (Thomas & Sharma, 1990). Chitosan is a copolymer of D-glucosamine and *N*-acetyl glucosamine derived from chitin. For drug delivery applications, chitosan needs to be crosslinked. Various crosslinking agents such as formaldehyde and glutaraldehyde have been used for this purpose (Illum, 1998; Nakatsuka & Andrady, 1992; Ramesh Babu, Sairam, Hosamani, & Aminabhavi, 2007a; Thacharodi & Rao, 1993). The crosslinked chitosan can be used as a pH-sensitive hydrogel that swells in acidic solutions due to protonation of free amino groups and chitosan hydrogels have been widely used in CR of drugs in stomach via oral route (Illum, 1998; Nakatsuka & Andrady, 1992; Thacharodi & Rao, 1993).

Chlorothiazide (CLT) is a diuretic and anti-hypertensive drug with a limited water solubility (0.2 and 0.4 g/l at pH 4 and 7, respectively) having a plasma half-life of 45–120 min (Katti & Krishnamurti, 1999; Satpathy & Rosenberg, 2003) and a biological half-life of nearly 10 h. The relatively short half-life time makes also this drug a suitable candidate for microencapsulation, which would release the drug in a controlled manner. Specific objectives of this study are to investigate the effects of wall-to-core ratio (WTCR) and crosslinking conditions (CL) of the encapsulated matrices on drug retention, size and structure of microcapsules as well as in-vitro core release profiles. After oral doses, 10–15% of the dose is excreted unchanged in the urine. The microspheres produced from semi-IPN matrices of chitosan and *N*,*N*′-dimethylacrylamide have been well characterized using a variety of techniques. The release patterns of the drug through the prepared matrices have been investigated in pH 7.4 media at 37 °C.

2. Experimental

2.1. Materials

Chitosan, St. Louis, MS, USA, *N*,*N*′-dimethylacrylamide, span 80, chlorothiazide were purchased from Sigma Aldrich Chemicals, Milwaukee, WI, USA. Potassium persulfate, light liquid paraffin oil and glutaraldehyde (25% solution) were purchased from s.d. fine chemicals, Mumbai, India.

2.2. Preparation of chitosan-N,N′-dimethylacrylamide semi-IPN microspheres

Varying amounts of chitosan were weighed and dissolved under constant magnetic stirring in 2% acetic acid solution for overnight. To this solution, a required amount of *N*,*N*′-dimethacrylamide and potassium persulfate were added and stirred well for 2 h. This reaction mixture was polymerized under the nitrogen inert atmosphere for 6 h at 70 °C. The polymer product was extracted by precipitating the polymer in acetone and precipitated polymer was dried under nitrogen atmosphere for 24 h. A 0.5 g of dried polymer was weighed and dissolved in 2% acetic acid solution and to this, a required amount of drug was added and stirred well to mix homogeneously.

The final polymer containing the drug solution was emulsified into liquid paraffin to form a water-in-oil (w/o) emulsion at 400 rpm using a Eurostar (IKA Labortechnik, Germany) high-speed stirrer for 30 min in a separate 500 mL beaker containing 100 mL of light liquid paraffin oil, 2% (w/v) of Tween-80, 1 mL of 0.1 M HCl and a required amount of GA. The microspheres formed were filtered, washed repeatedly with hexane and water to remove the oil as well as excess of surfactant and the unreacted glutaraldehyde (GA). Microspheres were dried under vacuum at 40 °C and stored in a desiccator before further testing. First, microspheres with different extent of crosslinking were prepared by taking 2.5, 5.0 and 7.5 mL of GA with 10% (wt/wt) of NNDMA and 10 (wt/wt)% drug. These are designated, respectively as CS-NNDMA-4, CS-NNDMA-1 and CS-NNDMA-5. Secondly, the microspheres were prepared by varying the amount of NNDMA (i.e., 10, 20 and 30 wt/wt%) and plain chitosan with 5 mL of GA and 10 (wt/wt)% of drug, which are designated as CS-NNDMA-1, CS-NNDMA-6, CS-NNDMA-7 and CS-NNDMA-8, respectively. In the third set of experiments, microspheres were prepared by varying the amount of drug, i.e., 10, 20 and 30 wt/wt% with wt/wt 10% of NNDMA and 5 mL of GA; these are designated as CS-NNDMA-1, CS-NNDMA-2 and CS-NNDMA-3, respectively. All the nine formulations were prepared by varying three parameters viz., amount of NNDMA, extent of drug loading and extent of crosslinking.

2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectral measurements were performed using Nicolet spectrophotometer (Model Impact 410, Milwaukee, WI, USA) to confirm the presence of crosslinking in CS-NNDMA matrix. The microspheres were finely ground with KBr to prepare the pellets under a hydraulic pressure

of 400 kg and spectra were scanned between 400 and 4000 cm^{-1}.

2.4. Differential scanning calorimetry (DSC)

DSC curves of the placebo CS-NNDMA microspheres, plain drug and drug-loaded microspheres were recorded using Rheometric Scientific differential scanning calorimetry, (Model-DSC SP, UK). The analysis was performed by heating the samples at the rate of 10 °C/min in an inert atmosphere.

2.5. X-ray diffractometry (X-RD)

The X-RD patterns of placebo beads, plain chlorothiazide (CLT), plain CS-NNDMA microspheres, CLT-loaded microspheres were recorded using a Rigaku Geigerflex diffractometer equipped with a Ni-filtered CuKα radiation ($\lambda = 1.5418$ Å). Dried microspheres of uniform size were mounted on a sample holder and X-RD patterns were recorded in the range of 0–50° at the speed of 5°/min.

2.6. Scanning electron microscopy (SEM)

SEM images of the microspheres were recorded using a JOEL MODEL JSM 840A scanning electron microscope, equipped with Phoenix energy dispersive analysis of X-rays (EDAX) at the required magnification. A working distance of 33.5 mm was maintained and the acceleration voltage used was 10 kV with the secondary electron image (SEI) as a detector. Samples were gold coated with thickness in the range of 10–15 nm.

2.7. Particle size analysis

Particle size of the microspheres was measured using a particle size analyzer (Mastersizer 2000, Malvern Instruments, UK) by dry powder form. About 500 mg of microspheres were transferred to dry sample holder and stirred vigorously to avoid agglomeration of particles during measurements. For the measurement of sizes of different formulations/batches, the sample holder was cleaned by vacuum. The particle size was also measured using an optical microscopy.

2.8. Estimation of drug loading and encapsulation efficiency

Chlorothiazide content from microspheres was estimated according to the method reported in the literature (Ritger & Peppas, 1987). Microspheres ~10 mg were ground to powder using an agate mortar, extracted for 18 h at 25 °C with 50 mL of 7.4 pH buffer solution and sonicated for 1 h (UP 400s, Dr. Hielscher, GmbH, Germany). The entire solution was centrifuged (Jouan, MR23i, France) to remove polymeric debris and washed twice to extract the drug completely. The clear supernatant solution was analyzed by UV spectrophotometer (Secomam, model Anthelie, France) at λ_{max} of 279 nm. The % drug loading and encapsulation efficiency were calculated using Eqs. (1) and (2), respectively. These data are compiled in Table 1.

$$\%\ \text{Drug loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100 \quad (2)$$

2.9. In-vitro release study

Dissolution was carried out using the automatic tablet dissolution tester with UV-system (Logon, D800, USA) equipped with eight baskets. Dissolution rates were measured at 37 °C under 100 rpm paddle speed. Drug release from the microspheres was measured in 1.2 and 7.4 pH phosphate solutions. At regular intervals of time, aliquot samples were withdrawn and analyzed using a UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 279 nm.

2.10. Swelling studies

Dynamic swelling of CS-NNDMA microspheres prepared by using three different crosslink densities as well as three different drug loadings was studied in water by weight 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 (Korsmeyer & Peppas, 1981). To perform the swelling experiments, microspheres were soaked in water; several of them were removed from the swelling bottles at different time intervals and blotted carefully (without pressing hard) to remove the surface-adhered water. The microspheres were then weighed (w_1) on an electronic microbalance (Mettler, AT 120, Switzerland) accurate to ±0.00001 g. The microspheres were 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 the average values were used in data analysis. The standard deviations (SD) in all cases were <3%. The weight % water uptake was calculated as:

$$\%\ \text{Water uptake} = \left(\frac{\text{Weight of swollen microspheres}(w_1) - \text{Weight of dry microspheres}(w_2)}{\text{Weight of dry microspheres}(w_2)}\right) \times 100 \quad (3)$$

3. Results and discussion

3.1. Fourier transform infrared spectroscopy (FTIR)

Fig. 1 compares the FTIR spectra of (A) CS-NNDMA crosslinked matrix with GA with that of (B) uncrosslinked CS-NNDMA microspheres. The spectrum of plain chitosan powder (curve A) has shown two peaks around 894

Table 1
Results of % encapsulation efficiency, mean size and water uptake of different formulations

Formulation codes	% NNDMA (wt/wt)	% CLT (wt/wt)	GA (mL)	% Encapsulation efficiency	Mean particle size (μm) ± SD	% Water uptake
CS-NNDMA-1	10	10	5	59.9 ± 1.6	95 ± 5	182
CS-NNDMA-2	10	20	5	68.1 ± 1.4	101 ± 5	245
CS-NNDMA-3	10	30	5	82.7 ± 1.9	128 ± 7	289
CS-NNDMA-4	10	10	2.5	72.2 ± 1.8	143 ± 6	290
CS-NNDMA-5	10	10	7.5	50.5 ± 1.4	88 ± 4	168
CS-NNDMA-6	20	10	5	65.9 ± 2.4	146 ± 5	145
CS-NNDMA-7	30	10	5	72.7 ± 1.8	185 ± 5	112
CS-NNDMA-8	00	10	5	55.0 ± 2.5	75 ± 5	393

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. 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.

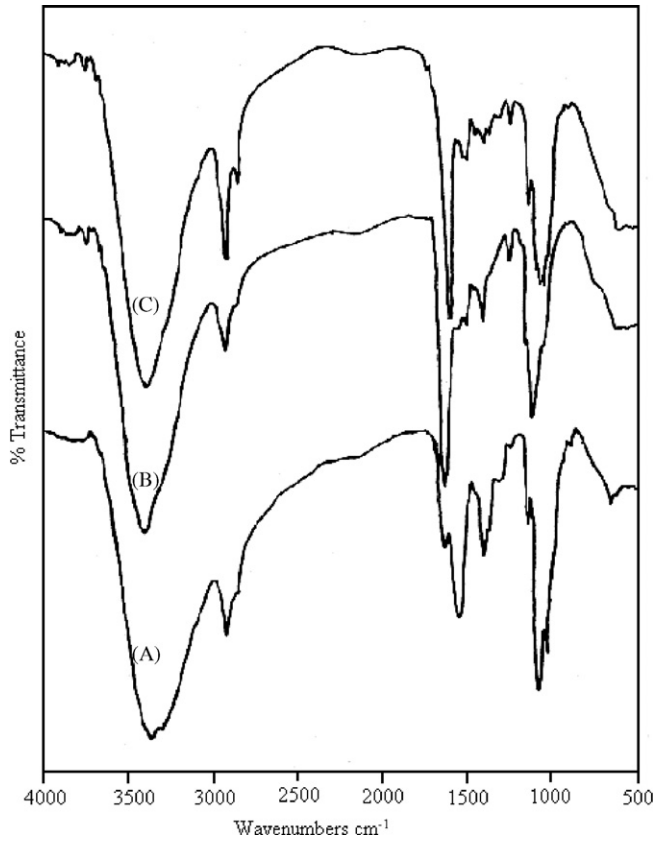
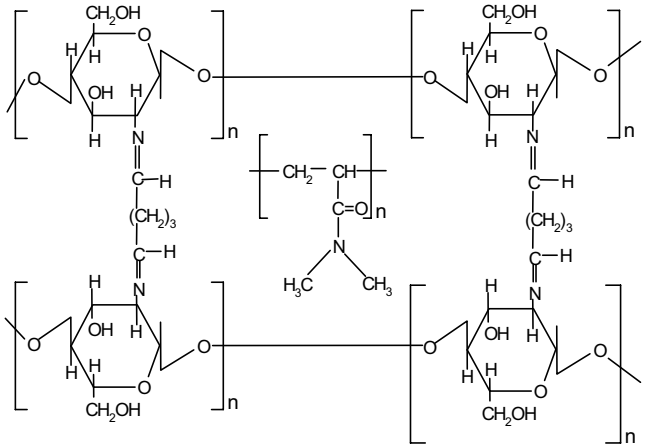


Fig. 1. FTIR spectra of plain chitosan (A), CS-NNDMA uncross linked (B) and CS-NNDMA crosslinked (C).

Peaks observed at ~2845 and ~2919 cm^{-1} are typical of C—H stretching vibrations. The peak at 1133 cm^{-1} is attributed to C—O stretching vibration in NNDMA. 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) (see curve C). A reaction leading to the formation of crosslinks is depicted in Scheme 1. 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.

3.2. Differential scanning calorimetry (DSC)

DSC tracings of plain chlorothiazide, drug-loaded CS-NNDMA microspheres and plain CS-NNDMA microspheres are displayed in Fig. 2. Melting peak of chlorothiazide was observed at 360 °C. However, there is no characteristic peak of chlorothiazide in the drug-loaded microspheres, suggesting that drug is molecularly dispersed in the polymer matrix.



Scheme 1. Crosslinking of chitosan with glutaraldehyde in presence of NNDMA.

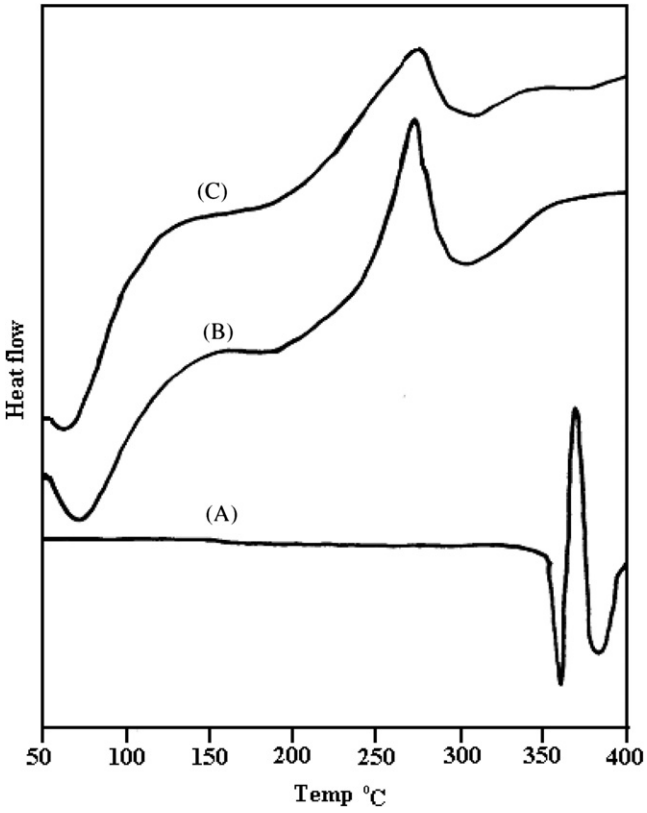


Fig. 2. DSC thermograms of (A) plain CLT, (B) CLT loaded CS-NNDMA microspheres and (C) plain CS-NNDMA microspheres.

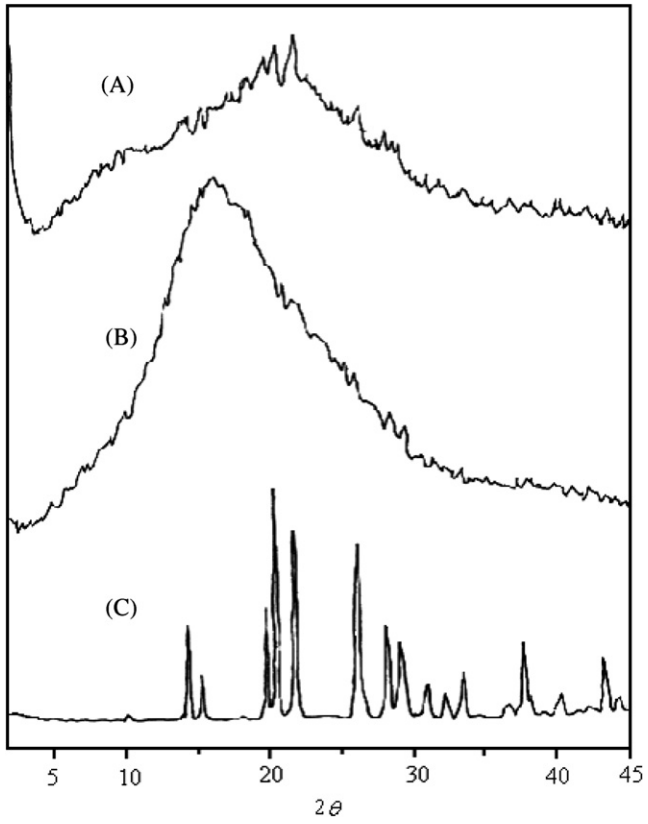


Fig. 3. X-RD spectra of CLT-loaded CS-NNDMA microspheres (A), plain CS-NNDMA microspheres (B) and plain CLT (C).

3.3. X-ray diffraction (X-RD)

X-ray diffractograms of (A) placebo microspheres, (B) drug-loaded microspheres and (C) plain CLT are displayed in Fig. 3. These studies are useful to investigate the crystallinity of the drug in crosslinked microspheres. CLT has shown the characteristic intense peaks at 2θ of 14°, 20°, 22° and 26° due to its crystalline nature. However, these peaks have disappeared in the CLT-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 loadings, the characteristic peaks of CLT overlapped with the noise of the coated polymer itself. Further, loaded drug is amorphous, which is difficult to measure at the detection limit of the crystal size in the present case. This further confirms that drug is molecularly dispersed at a molecular level in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

3.4. Scanning electron microscopy (SEM)

SEM images of single microspheres taken at 300 magnifications are shown in Fig. 4. Microspheres are spherical without agglomerations and their surfaces are smooth. However, polymeric debris seen around some particles could be due to the method of particle production (i.e., simultaneous particle production and formation of semi-IPN matrix).

3.5. Laser particle size analyzer

Results of mean particle size with standard errors are presented in Table 1, while the size distribution curve for a typical formulation containing 10% NNDMA, 30% CLT and 5 mL of CS-NNDMA-3 is displayed in Fig. 5. It is obvious that size distribution is narrow and volume

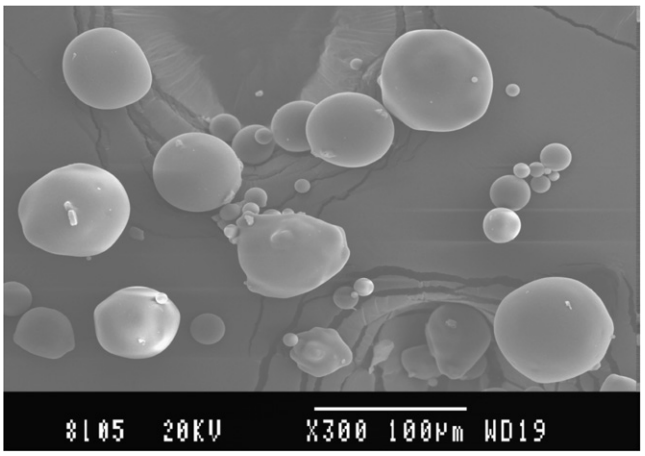


Fig. 4. Scanning electron micrograph of CS-NNDMA microspheres.

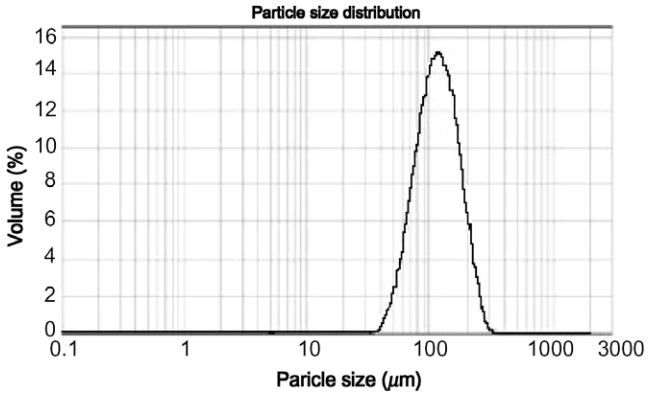


Fig. 5. Particle size distribution curve for CS-NNDMA microspheres.

mean diameter of the microspheres is found to be 115 μm. Particle size of different formulations containing different amount of drug, GA and different amount of NNDMA are presented in Table 1.

3.6. Microscopic study

Particle size was also measured alternatively by optical microscopy. These results along with % encapsulation efficiency, % drug loading and mean particle sizes for different formulations are presented in Table 1. The size of particles depends on the amount of drug present, % NNDMA content and extent of GA employed for crosslinking. Particles are generally spherical in shape with sizes ranging from 80 to 190 μm. Particle size of the plain chitosan is higher than those of CS-NNDMA microspheres. By increasing the NNDMA content of the microspheres, size of the microspheres increased from 100 to 190 μm for 10% CLT-loaded microspheres. This can be explained on the basis of hydrodynamic viscosity concept, i.e., as the amount of NNDMA in microspheres increases, the interfacial viscosity of polymer droplets in emulsion also will increase. On the other hand, with increasing amount of NNDMA, the numbers of free sites available for crosslinking are less such that the size of the microspheres will also increase with increasing NNDMA content. For instance, as the amount of NNDMA increases from 10% to 30%, particle size has increased from 100 to 190 μm.

For all formulations, with increasing amount of drug in the microspheres, particle size also has increased. For formulations containing 10% NNDMA and microspheres loaded with different amounts of drug, the particle size has increased from 100 to 135 μm; a similar trend was observed for all other formulations (see Table 1). This is attributed to the fact that drug molecules might have occupied the free volume spaces within the matrix, thereby hindering the inward shrinkage of the polymer matrix, (Soppimath, Kulkarni, & Aminabhavi, 2002). However, the extent of crosslinking has shown a significant effect on particle size (see data in Table 1). For microspheres containing 10 wt% NNDMA and 10 wt% CLT with increasing amount of GA from 2.5 to 7.5 mL, the particle size decreased from 149 to 92 μm. This is attributed to the fact that with increasing amount of GA in the semi-IPN matrix, the shrinkage of particles has taken place, thereby reducing their sizes.

3.7. Encapsulation efficiency

Three different concentrations of CLT, i.e., 10, 20 and 30 wt% were loaded during the crosslinking of microspheres. Results of % encapsulation efficiency included in Table 1 show increasing trends with increasing drug loading. Encapsulation efficiency of 57.5% was observed for plain chitosan microspheres, but for the remaining formulations, it ranged from 61.5% to 84.6%. Such smaller values are due to a lesser soluble drug in the polymer solution, thus incorporating a lesser amount of CLT into microspheres. Notice that % encapsulation efficiency increased with increasing amount of NNDMA in the microspheres. For microspheres containing 10, 20 and 30 wt% NNDMA and 5 wt% CLT with 5 mL GA, encapsulation efficiencies were 61.5%, 68.3% and 74.5%, respectively. For 10% NNDMA in the matrix, the results of extent of crosslinking on the size and encapsulation efficiency have increased, but the % encapsulation efficiency decreased (see Table 1). For microspheres crosslinked with 2.5, 5 and 7.5 mL of GA, encapsulation efficiencies are, respectively, 74%, 61.5% and 51.9%. Such a decreasing trend is due to an increase in crosslink density, because the microspheres will become rigid, thereby reducing the free volume spaces within the polymer matrix and hence, a reduction in encapsulation efficiency is observed.

3.8. Swelling studies

The extent of crosslinking depends upon the amount of crosslinking agent (GA) used. In the present study, different amounts of GA were added as the crosslinking agent to the semi-IPN microspheres of CS-NNDMA containing 10 wt% of CLT and these data are also included in Table 1. Extent of crosslinking is dependent upon the equilibrium swelling. For instance, % equilibrium swelling decreased from 290 to 168 with increasing amount of GA from 2.5 to 7.5 mL. This is due to increased crosslink density and decreased pore volume of the semi-IPN matrix (Patel, Patel, & Kansara, 1994)with increasing amount of GA in the matrix. By increasing the drug loading of the matrix, % water uptake also increased, i.e., as the drug wt% loading increased from 10 to 30, the % equilibrium swelling were 182, 245 and 289, respectively. Such an increase in swelling of the matrix is due to the incorporation of hydrophilic chitosan along with NNDMA chains to form a semi-IPN matrix. The % water uptake or % dynamic swelling of the formulated semi-IPN matrix has decreased with an increasing amount of NNDMA in the semi-IPN matrix. However, % water uptake or % dynamic swelling of the semi-IPN matrix containing 10%, 20% and 30% of

NNDMA are 182, 145 and 112, respectively. The % water uptake has decreased with an increasing amount of NNDMA in the semi-IPN matrix. This is due to the fact that as the amount of NNDMA increases in the matrix, hydrophobicity of the matrix could increase slightly due to the presence of methyl groups in NNDMA.

3.9. Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data vs time by fitting to an exponential equation of the type (Ritger & Peppas, 1987):

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{4}$$

Here, M_t/M_∞ represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is an empirical parameter characterizing the release mechanism. Using the least squares procedure, we have estimated the values of n and k for all the nine formulations and these data are given in Table 2. If $n = 0.5$, drug diffuses and releases out of the polymer matrix following the Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian type drug diffusion occurs. If $n = 1$, a completely non-Fickian or Case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type of diffusive transport (Aminabhavi & Naik, 1998; Ritger & Peppas, 1987).

The values of k and n have shown a dependence on the extent of crosslinking, % drug loading and NNDMA content of the matrix. Values of n for microspheres prepared by varying the amounts of NNDMA in semi-IPN microspheres containing 10, 20 and 30 wt% and keeping CLT (10%) and GA (5 mL) constant, ranged from 0.88 to 0.57 suggesting shift of drug transport from Fickian to anomalous type. However, the CLT-loaded microspheres exhibited n values ranging from 0.88 to 0.61 (see Table 2), indicating a shift from erosion type release to a swelling-controlled, non-Fickian type mechanism. This may be due to a reduction in the regions of low microviscosity inside the matrix and closure of microcavities during the swollen state of the polymer. Similar findings have been found elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was investigated (Lyu, Sparer, Hobot, & Dang, 2005). On the other hand, values of k are quite smaller for drug-loaded microspheres, suggesting lesser interactions as compared to microspheres containing varying amounts of NNDMA. In order to study the effect of composition on k of NNDMA in the matrix, we have plotted the results of k vs % NNDMA of the matrix composition as shown in Fig. 6. A perfect linear increase of k with increasing amount of % NNDMA is observed, suggesting a systematically varying interaction of the drug solution with NNDMA content of the matrix.

The diffusion coefficient, (D) of drug containing fluid media in the microspheres was calculated using the equation (Kulkarni, Soppimath, Aminabhavi, Dave, & Mehta, 2000):

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

where θ is slope of the linear portion of the plot of M_t/M_∞ vs $t^{1/2}$, r is the radius of spherical particles and M_∞ is the maximum sorption value. Diffusion coefficients were estimated by assuming Fickian diffusion transport. The D (see Table 2) values calculated are in the range of $(1.01–7.23) \times 10^{-6}$ cm^2/s, which are found to be dependent upon the extent of crosslinking extent. For instance, the D values have shown a systematic decrease with increasing crosslinking of the matrix in all the formulations. This is obvious because of the increased rigidity of the chain due to increased crosslinking, thereby prohibiting the transport of more drug molecules.

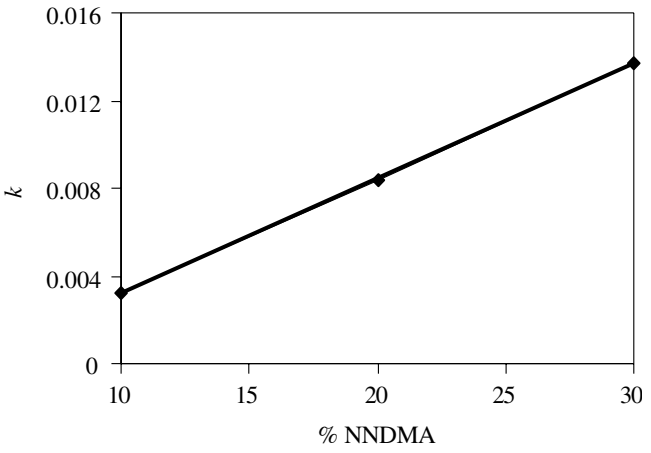


Fig. 6. Values of k *vs* composition of NNDMA in microspheres.

Table 2
Release kinetics parameters of different formulations

Formulation code	k	n	$D \times 10^{-6}$ (cm^2 s^{-1})	Correlation coefficient, r
CS-NNDMA-1	0.0032	0.88	1.71	0.976
CS-NNDMA-2	0.0184	0.55	3.23	0.942
CS-NNDMA-3	0.0183	0.61	7.24	0.972
CS-NNDMA-4	0.0115	0.68	5.18	0.930
CS-NNDMA-5	0.0142	0.56	1.01	0.983
CS-NNDMA-6	0.00839	0.68	4.69	0.988
CS-NNDMA-7	0.0137	0.57	3.88	0.965
CS-NNDMA-8	0.106	0.35	1.35	0.991

3.10. Effect of N,N′-dimethylacrylamide content

Effect of NNDMA content was studied at constant loading of 10 wt% CLT. It was found that chitosan produced almost 100% cumulative drug release in about 10 h, whereas CS-NNDMA semi-IPN microspheres produced up to 80% of cumulative release in 12 h. The release trends of CS-NNDMA microspheres prepared with different amounts of NNDMA are displayed in Fig. 7. Notice that during dissolution experiments, the microspheres have shown systematic swollen trends with decreasing amount of NNDMA, probably due to the formation of loosely crosslinked network chains of NNDMA. As the amount of NNDMA increases, cumulative release decreased due to lesser swelling of the NNDMA chains than chitosan. This could be because as the amount of NNDMA increases in semi-IPN matrix, the hydrophobicity of the overall matrix decreases due to the presence of methyl groups of NNDMA as well as the more number of residual $-CH_3$ groups available, which increases the hydrophobicity of the matrix, thereby decreasing the release rates for CLT. Thus, a regaining-type response of polymeric chains is possible due to the stresses induced by the surrounding solvent media during the dissolution step, resulting in a decrease of chain dimension (radius of gyration) of the semi-IPN polymer; this will further decrease the molecular volume of the hydrated polymer due to decreased swelling of NNDMA component of the semi-IPN matrix, thereby reducing the free volume spaces of the matrix. Notice that the nature of release profiles remains almost identical in all the formulations containing different amounts of GA, indicating a linear relationship with the drug release profiles.

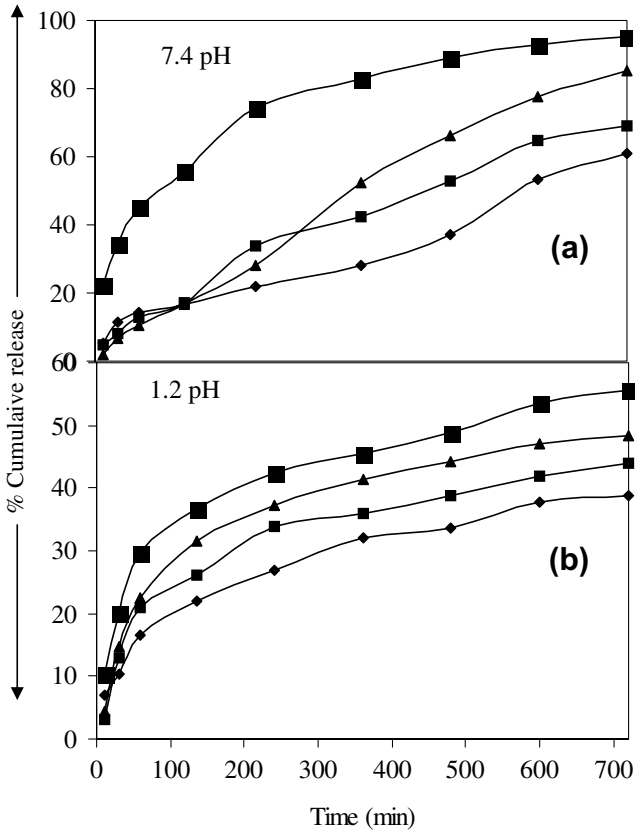


Fig. 7. Cumulative % release of CLT through CS-NNDMA microspheres containing different of amounts of NNDMA in 1.2 and 7.4 pH. Symbols: (■) plain Chitosan, (●) 10 wt% NNDMA, (■) 20 wt% NNDMA and (▲) 30 wt% of NNDMA.

3.11. Effect of pH

In order to investigate the effect of pH and ionic strength of the external medium on the swelling of microspheres, we have measured the % cumulative release in both pH 1.2 and 7.4 media. Cumulative release data presented in Fig. 7 indicate that by increasing the pH from 1.2 to 7.4, a considerable increase in the cumulative release is observed for all microspheres. From Fig. 7, it is seen that the microspheres have shown longer drug release rates than the plain chitosan microspheres. But, there is a drastic difference in the release rates of the formulated blend microspheres in pH 1.2 and pH 7.4 media. Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. For instance, only 50% drug was released at 12 h (see Fig. 7b) for microspheres when compared to 95% drug release in pH 7.4 media (see Fig. 7a) for the same time. In the case of hydrolyzed blend polymer, 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 ($NH_3^+COO\sim$) formed.

3.12. Effect of crosslinking agent

The % cumulative release vs time curves for varying amounts of GA, i.e., 2.5, 5.0 and 7.5 mL at a fixed amount of drug (5 wt%) are displayed in Fig. 8. The % cumulative

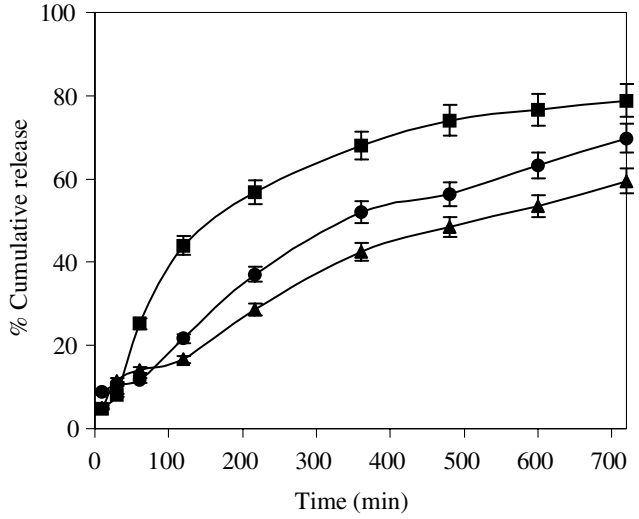


Fig. 8. Cumulative % release of CLT through CS-NNDMA microspheres containing different of amounts of crosslinking agent. Symbols: (■) 2.5 mL, (●) 5 mL and (▲) 7.5 mL.

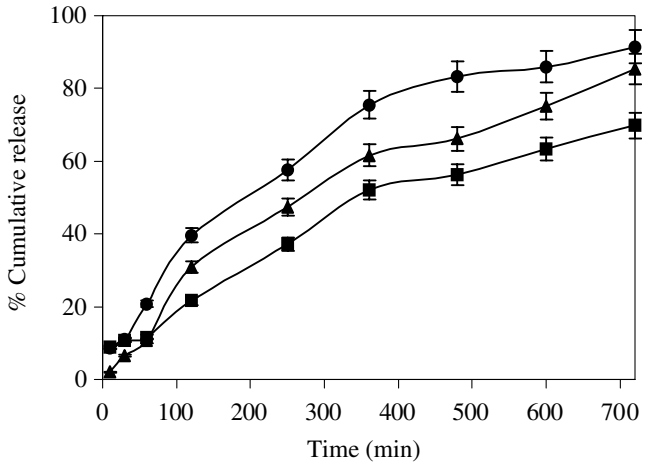


Fig. 9. Cumulative % release of CLT through Cs-NNDMA microspheres containing different amount of CLT. Symbols: (●) 30 wt%, (▲) 20 wt% (■) and 10 wt%.

release is quite fast and large at a lower amount of GA (i.e., 2.5 mL), whereas the release becomes quite slow at higher amount of GA (i.e., 7.5 mL). The cumulative release is somewhat less when the lower amount of GA was used, probably because at higher concentration of GA, polymeric chains would become rigid due to the contraction of microvoids, thus decreasing the % cumulative release of CLT through polymeric matrices. As expected, the release becomes slower at the higher amount of GA, but becomes faster at the lower amount of GA.

3.13. Effect of drug loading

Fig. 9. displays the release profiles of CLT-loaded CS-NNDMA semi-IPN microspheres at different amounts of drug loadings. Release data showed that formulations containing highest amount of drug (30 wt%) displayed fast and higher release rates than those formulations containing a small amount of CLT. The prolonged drug release was observed for formulation containing lower amount of CLT. Notice that the release rate becomes quite slower at the lower amount of drug in the matrix, due to the availability of more free void spaces through which a lesser number of drug molecules could transport. For all the CLT-loaded formulations, 70% of CLT release has occurred in about 600 min, but 85% of the drug release was observed around 700 min.

4. Conclusions

Novel CS-NNDMA microspheres of chitosan and *N*,*N*′-dimethylacrylamide were prepared and characterized by differential scanning calorimetry, X-ray diffractometry, scanning electron microscopy and particle size analyzer. DSC thermograms have confirmed the uniform molecular distribution of the drug molecules in the microspheres. SEM micrographs exhibited a spherical morphology of the prepared microspheres. The drug was released in a controlled manner. Swelling studies of the microspheres have shown that with an increasing amount of NNDMA in the microspheres, drug containing water uptake has decreased. This effect is correlated with the release rates of drug though the microspheres containing different amounts of NNDMA. Microspheres have exhibited lower densities and hence, these could be retained for more than 12 h.

Acknowledgements

The authors thank University Grants Commission (UGC), New Delhi, India for a major funding (Grant No. F1-41/2001/CPP-II) to establish Center of Excellence in Polymer Science at Karnatak University, Dharwad.

References

Aminabhavi, T. M., & Naik, H. G. (1998). Chemical compatibility study of geomembranes-sorption/desorption, diffusion and swelling phenomena. *Journal of Hazardous Materials, 60*, 175–203.

Bellamy, L. J. (1980). *The infrared spectra of complex molecules*. New York: Chapman & Hall.

Binder, W. H., & Gruber, H. (2000). Block copolymers derived from photoreactive 2-oxazolines, 1. Synthesis and micellization behavior. *Journal of Macromolecular Chemistry Physics, 201*, 949–957.

Garcia, M. O., Blanco, D., Martin, J. A., & Teijon, J. M. (2000). 5-Fluorouracil trapping in poly(2-hydroxyethyl methacrylate) hydrogels: In vitro drug delivery studies. *European Polymer Journal, 36*, 111–122.

Gurdag, G., Yaar, M., & Gurkaynak, M. A. (1997). Graft copolymerization of acrylic acid on cellulose: Reaction kinetics of copolymerization. *Journal of Applied Polymer Science, 66*, 929–934.

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

Kang, D. W., Choi, H. R., & Kweon, D. K. (1999). Stability constants of amidoximated chitosan-*g*-poly(acrylonitrile) copolymer for heavy metal ions. *Journal of Applied Polymer Science, 73*, 469–476.

Katti, D., & Krishnamurti, N. (1999). Preparation of albumin microspheres by an improved process. *Journal of Microencapsulation, 16*, 231–242.

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.

Kulkarni, A. R., Soppimath, K. S., Aminabhavi, T. M., Dave, A. M., & Mehta, M. H. (2000). Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application. *Journal of Controlled Release, 63*, 97–105.

Lou, X., Munro, S., & Wang, S. (2004). Drug release characteristics of phase separation pHEMA sponge materials. *Biomaterials, 25*, 5071–5080.

Lyu, S. P., Sparer, R., Hobot, C., & Dang, K. (2005). Adjusting drug diffusivity using miscible polymer blends. *Journal of Controlled Release, 102*, 679–687.

Mi, F. L., Tan, Y. C., Liang, H. F., & Sung, H. W. (2002). In vivo biocompatibility and degradability of a novel injectable chitosan bead implant. *Biomaterials, 23*, 181–191.

Nakatsuka, S., & Andrady, A. L. (1992). Permeability of vitamin B-12 in chitosan membranes; effect of crosslinking and blending with poly vinyl alcohol on permeability. *Journal of Applied Polymer Science, 44*, 17–28.

Nho, Y. C., & Jin, J. H. (1997). Graft polymerization of acrylic acid and methacrylic acid onto radiation-peroxidized polyethylene film in presence of metallic salt and sulfuric acid. *Journal of Applied Polymer Science, 63*, 1101–1106.

Palmieri, G. F., Lovato, D., & Martelli, S. (1999). New controlled release ibuprofen tablets. *Drug Development and Industrial Pharmacy, 25*, 671–677.

Park, S. B., You, J. O., Park, H. Y., Haam, S. J., & Kim, W. S. (2001). A novel pH-sensitive membrane from chitosan-TEOS IPN; preparation and its drug permeation characteristics. *Biomaterials, 22*, 323–330.

Patel, G. M., Patel, N. K., & Kansara, S. (1994). Dilute solution properties of poly(2-methoxycyanurate) of bisphenol F and bisphenol A. *Polymer International, 35*, 83–88.

Peng, T., Yao, K. D., Chen, Y., & Goosen, M. F. (1994). Structural changes of pH-sensitive chitosan/polyether hydrogels in different pH solution. *Journal of Polymer Science: Polymer Chemistry Edition, 32*, 591–596.

Peniche, C., Monal, W. A., Davidenkom, N., Sastre, R., Gallardo, A., & Roman, J. S. (1999). Self-curing membranes of chitosan/PAA IPNs obtained by radical polymerization: Preparation, characterization and interpolymer complexation. *Biomaterials, 20*, 1869–1878.

Ramesh Babu, V., Krishna Rao, K. S. V., Sairam, M., Naidu, B. V. K., Hosamani, K. M., & Aminabhavi, T. M. (2006c). pH-Sensitive interpenetrating network microgels of sodium alginate-acrylic acid for the controlled release of ibuprofen. *Journal of Applied Polymer Science, 99*, 2671–2678.

Ramesh Babu, V., Sairam, M., Hosamani, K. M., & Aminabhavi, T. M. (2006b). Development of 5-fluorouracil loaded poly(acrylamide-co-methyl methacrylate) novel core-shell microspheres: In vitro release studies. *International Journal of Pharmaceutics, 325*, 55–62.

Ramesh Babu, V., Sairam, M., Hosamani, K. M., & Aminabhavi, T. M. (2007a). Preparation of sodium alginate-methyl cellulose blend microspheres for controlled release of nifedipine. *Carbohydrate Polymers, 69*, 141–150.

Ramesh Babu, V., Sairam, M., Hosamani, K. M., Aminabhavi, T. M. (in press). Preparation and characterization of novel semi- interpenetrating 2-hydroxyethyl methacrylate-*g*-chitosan copolymeric microspheres for sustained release of indomethacin. *Journal of Applied Polymer Science*.

Risbud, M. V., Hardikar, A. A., Bhat, S. V., & Bhonde, R. R. (2000). pH sensitive freeze-dried chitosan-poly vinylpyrrolidone hydrogels as controlled release systems for antibiotic delivery. *Journal of Controlled Release, 68*, 23–30.

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.

Sacak, M., & Celik, M. M. (1996). Hydrogen peroxide initiated grafting of acrylamide onto poly(ethylene terephthalate) fibers in benzyl alcohol. *Journal of Applied Polymer Science, 59*, 1191–1194.

Sannan, T., Kurita, K., Ogura, K., & Iwakura, Y. (1978). Studies on chitin: 7. IR spectroscopic determination of degree of deacetylation. *Polymer, 19*, 458–459.

Satpathy, G., & Rosenberg, M. (2003). Encapsulation of chlorothiazide in whey proteins: Effects of wall-to-core ratio and cross-linking conditions on microcapsule properties and drug release. *Journal of Microencapsulation, 20*, 227–245.

Shukla, J. S., & Sharma, G. K. (1987). Graft copolymerization of methyl methacrylate onto wool initiated by ceric ammonium nitrate-thioglycolic acid redox couple in presence of air. IV. *Journal of Polymer Science. Part A. Polymer Chemistry, 25*, 595–605.

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

Thacharodi, D., & Rao, K. P. (1993). Propranol hydrochloride release behaviour of crosslinked chitosan membranes. *Journal of Chemical Technology Biotechnology, 58*, 177–181.

Thomas, C., & Sharma, P. (1990). Chitosan as biomaterial. *Biomaterials Artificial Cells and Artificial Organs, 18*, 1–24.

Trimmnel, D., Fanta, G. F., & Salch, J. H. (1996). Graft polymerization of methyl acrylate onto granular starch: Comparison of the Fe^{+2}/H_2O_2 and ceric initiating systems. *Journal of Applied Polymer Science, 60*, 285–292.

Yoshioka, T., Hirano, R., Shioya, T., & Kako, M. (1990). Encapsulation of mammalian cell with chitosan-CMC capsule. *Biotechnology and Bioengineering, 35*, 66–72.

Yuk, S. H., Cho, S. H., & Lee, H. B. (1995). pH-sensitive drug delivery system using O/W emulsion. *Journal of Controlled Release, 37*, 69–74.