Synthesis and Characterization of Polyacrylamide-Grafted Chitosan Hydrogel Microspheres for the Controlled Release of Indomethacin

Sangamesh G. Kumbar, Kumaresh S. Soppimath, Tejraj M. Aminabhavi

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

Received 27 December 2001; accepted 9 May 2002

ABSTRACT: Microspheres of polyacrylamide-*grafted*-chitosan crosslinked with glutaraldehyde were prepared and used to encapsulate indomethacin, a nonsteroidal anti-inflammatory drug. The microspheres were produced by the water/oil emulsion technique and encapsulation of indomethacin was carried out before crosslinking of the matrix. The extent of crosslinking was analyzed by Fourier transform infrared spectroscopy and differential scanning calorimetry. Microspheres were characterized for drug-entrapment efficiency, particle size, and water transport into the polymeric matrix as well as for drug-release kinetics. Scanning electron microscopy confirmed the spherical nature and surface morphology of the particles with a mean particle size of 525 μm. Dynamic swelling experiments suggested that, with an increase in crosslinking, the transport mechanism changed from Fickian to non-Fickian. The release of indomethacin depends upon the crosslinking of the network and also on the amount of drug loading. This was further supported by the calculation of drug-diffusion coefficients using the initial time approximation. The drug release in all the formulations followed a non-Fickian trend and the diffusion was relaxation-controlled. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 87: 1525–1536, 2003

Key words: indomethacin; hydrogel; microspheres; polyacrylamide-grafted chitosan

INTRODUCTION

Polymeric microsphere hydrogels have been extensively used in the delivery of several drugs.[1–6] The preparation of such microspheres is generally based on the water/oil (w/o) emulsion technique.[7] It was found in our previous articles[8–13] that a number of modified forms of natural polymers can be used for the delivery of drugs. The main advantages of using such natural polymers are that they can be biocompatible and biodegradable and produce no systemic toxicity upon administration.[14,15] Several biopolymers belonging to the class of polysaccharides have some inherent disadvantages such as poor mechanical strength, uncontrolled water uptake, and microbial contamination. To overcome these problems, efforts have been made to develop chemically modified matrices by combining them with synthetic monomers.[16–18] In our earlier research efforts,[4,11] we developed hydrogels of polyacrylamide (PAAm) grafted with natural polymers. In pursuance of this goal, we continue to develop newer polymeric systems and present here the procedure to modify chitosan by grafting with PAAm.

The microspheres were prepared by the w/o emulsion method using glutaraldehyde (GA) as the crosslinking agent. Indomethacin (IM), a nonsteroidal anti-inflammatory drug (NSAID), was used as a model drug and loaded before crosslinking of the matrix. The advantages of such controlled release (CR) formulations containing NSAID over the conventional-dosage forms were reported earlier[12,13,19]; such formulations help to minimize the serious gastric irritation side effects of the conventional-dosage NSAID formulations. The microspheres prepared were characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and laser-beam particle-size analysis. Microspheres were evaluated for their thermal behavior, water-transport properties, as well as *in vitro* drug-release kinetics.

EXPERIMENTAL

Materials

Chitosan (medium molecular weight solution of 0.002 g/dL with a viscosity of 15.524 mPa s) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Acrylamide (AAm), acetic acid, potassium persulfate, a polysorbate-80, GA (25% w/w) solution, and liquid paraffin were all purchased from S.D. Fine Chemicals (Mumbai, India). Indomethacin, USP grade (Sigma–

CEPS Communication No. 14.

Correspondence to: T. M. Aminabhavi (aminabhavi@yahoo.com; cc rrist@bgl.vsnl.net.in).

Contract grant sponsor: GSFC Science Foundation.

Aldrich Chemie Gmbh, Steinheim, Germany), was used as received. The deacetylation degree of chitosan was 86% as determined by IR spectroscopy.[20] All the other reagents/solvents were of analytical grade, which were used without further purification.

Synthesis of graft copolymer of chitosan and AAm

The grafting of AAm onto the chitosan backbone was carried out by a persulfate-induced free-radical reaction as reported in the earlier literature.[1]. Briefly, 4.1 g of chitosan was dissolved in 250 mL of a 2% aqueous acetic acid solution with constant stirring to obtain a clear solution. Later, 0.16 mol of AAm dissolved in 50 mL of deareated-distilled water was mixed with the chitosan solution by stirring. Then, a 0.001*M* solution of potassium persulfate was added dropwise over a period of 5 min by maintaining the reaction temperature at 50°C. Nitrogen was purged into the solution during the polymerization reaction. The reaction was continued for 6 h and then terminated by adding hydroquinone. The copolymer was precipitated by adding an excess amount of acetone and dried in a vacuum oven at 40°C. The percentage grafting was estimated from the mass of the polymer before and after grafting using the relationship

$$\% \text{ Grafting} = \frac{W_g - W_0}{W_0} \times 100 \quad (1)$$

where W_g and W_0 are the masses of the graft copolymer and of the chitosan backbone, respectively. The percent grafting of AAm onto chitosan and the grafting efficiency were calculated as

$$\text{AAm \% grafting} = \left(\frac{\text{mass of copolymer} - \text{mass of chitosan}}{\text{mass of chitosan}}\right) \times 100 \quad (2)$$

Viscometric measurements

The viscosities of the solutions of chitosan and PAAm-*g*-chitosan in 2% aqueous acetic acid were determined using an automated Ubbelohde viscometer (Schott Gerate, AVS 350, Germany) thermostatically maintained at 30°C. The unit automatically performs the measurements of flow-through times in the capillary viscometer. The efflux times were determined on a digital display within an accuracy of ±0.01 s.

Elemental analysis

Elemental analysis data on chitosan and PAAm-*g*-chitosan were obtained using an EA1110 CHN analyzer (Thermoquest, CE Instruments, Italy) and the percentages of nitrogen, carbon, and hydrogen were estimated.

Preparation of chitosan microspheres

PAAm-*g*-chitosan microspheres containing IM were prepared by dissolving 2 g of the polymer in 35 mL of 2% acetic acid in hot water at 50°C. The solution was concentrated to about 25 mL. To this solution, 1 mL of HCl was added and mixed thoroughly. Indomethacin was ground, passed through a sieve of 100 mesh, added to the above polymer solution, and dispersed uniformly using a magnetic stirrer for about 10 min and then sonicated using an Ikasonic U50 Model, (IKA Labortechnik, Germany) for 5 min. At this stage, GA was added as a crosslinking agent to the polymer solution with constant stirring. This solid in water (s/w) suspension was emulsified into 150 mL of liquid paraffin in the presence of 2% polysorbate-80 using an Eurostar digital stirrer (IKA Labortechnik, Germany) at the rotation speed of 600 rpm. The exposure time of the emulsion to GA was kept constant (3 h) for all the batches. The hardened microspheres were separated by filtration and washed with hexane to remove liquid paraffin and also with distilled water to wash off any excess amount of GA. The microspheres were dried at 50°C for 24 h and kept in a desiccator until further use.

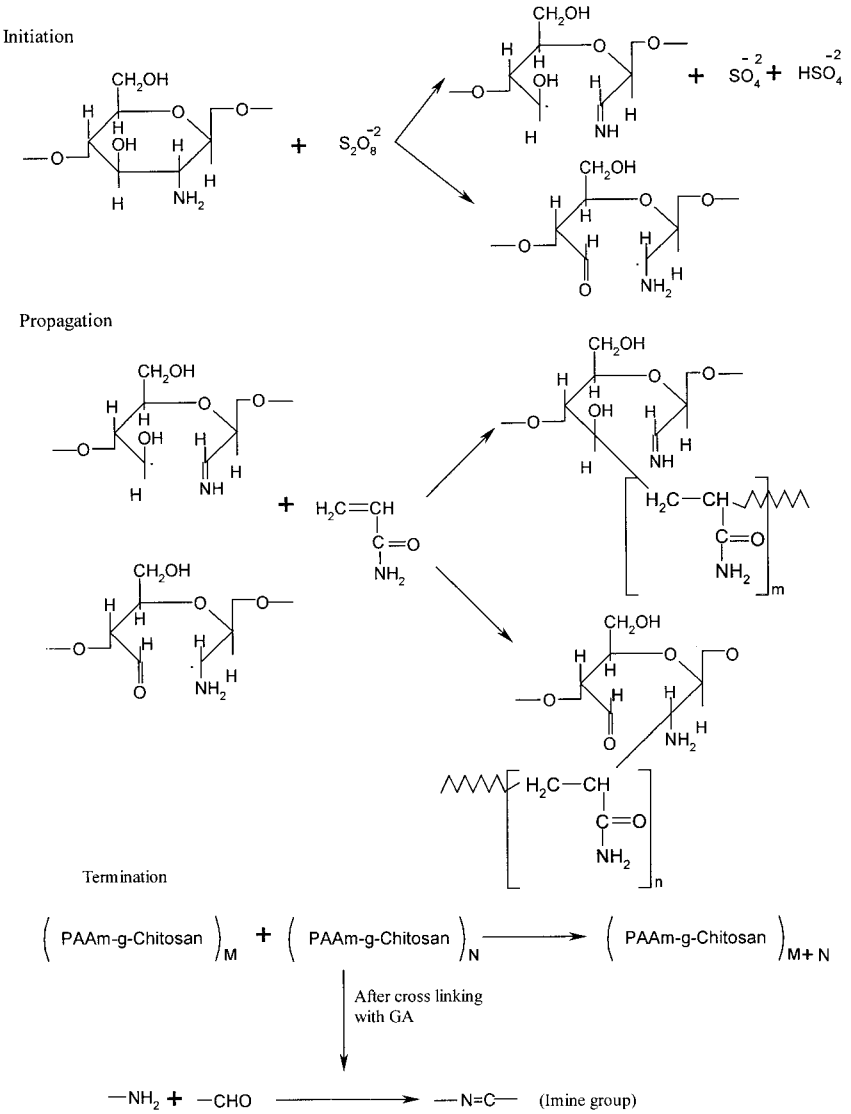
Three different crosslinked systems were prepared by treating with 5, 7.5, and 10 mL of GA. Drug loading was done by using 10, 20, and 30 % (w/w) of IM based on the dry mass of the polymer. The resulting nine formulations of the microspheres were designated from IM-1 to IM-9 with increasing crosslinking and with increasing drug loading. Since IM is a photosensitive drug, extreme care was taken to avoid its possible degradation during the formulation and characterizing of the microspheres.

FTIR measurements

FTIR spectral measurements were performed using a Nicolet (Model, Impact 410, USA) spectrophotometer to confirm the grafting reaction between chitosan and acrylamide as well as to study the crosslinking reaction of the microspheres. Empty microspheres (~2 mg) were finely ground with KBr and pellets were made.

Differential scanning calorimetric study (DSC)

DSC analyses were performed for chitosan, PAAm-*g*-chitosan, the crosslinked microspheres, as well as the IM-loaded microspheres using a DuPont-2000 microcalorimeter at the heating rate of 10°C/min from −50



Scheme 1 Grafting and crosslinking reactions of AAm onto chitosan.

to 250°C under the constant flow of argon gas. These measurements were done at IEIS at Southwest Texas State University, San Marcos, TX (courtesy of Ms. Tracy Mayer).

Microscopic studies

The particle size of the microspheres was measured using an optical microscope by taking 100–200 particles on a glass slide under regular polarized light. Few of the samples, namely, 7.5- and 10-mL GA crosslinked empty microspheres and microspheres of 30% IM-loaded crosslinked with 10 mL GA (IM-9), were also analyzed by a HELOS laser light-induced particle size analyzer (Sympatec GmbH, Germany, courtesy of Mr. Art).

SEM photographs were obtained by using a JOEL SEM scope, Model JSM-840A (JOEL Ltd., Peabody, MA), at an accelerating voltage of 6 kV, a working distance of 25 mm, no tilt angle, and magnifications varying between 160× and 220×. Scale bars for the particle ensembles represent 1 mm, while the scale bar for a single particle represents 100 μm. DSG digital image acquisition allowed for contrast-enhanced post-image processing of the micrographs. All the SEM photographs were obtained at the UT Southwestern Medical Center in Dallas, TX (Molecular and Cellular Imaging Facility by the courtesy of Professor P. V. Kulkarni).

Estimation of drug-loading and encapsulation efficiency

Indomethacin loaded in the microspheres was estimated by extracting the drug into 7.4 pH phosphate buffer. The samples were then filtered and analyzed using a UV-visible spectrophotometer (Model Anthe-

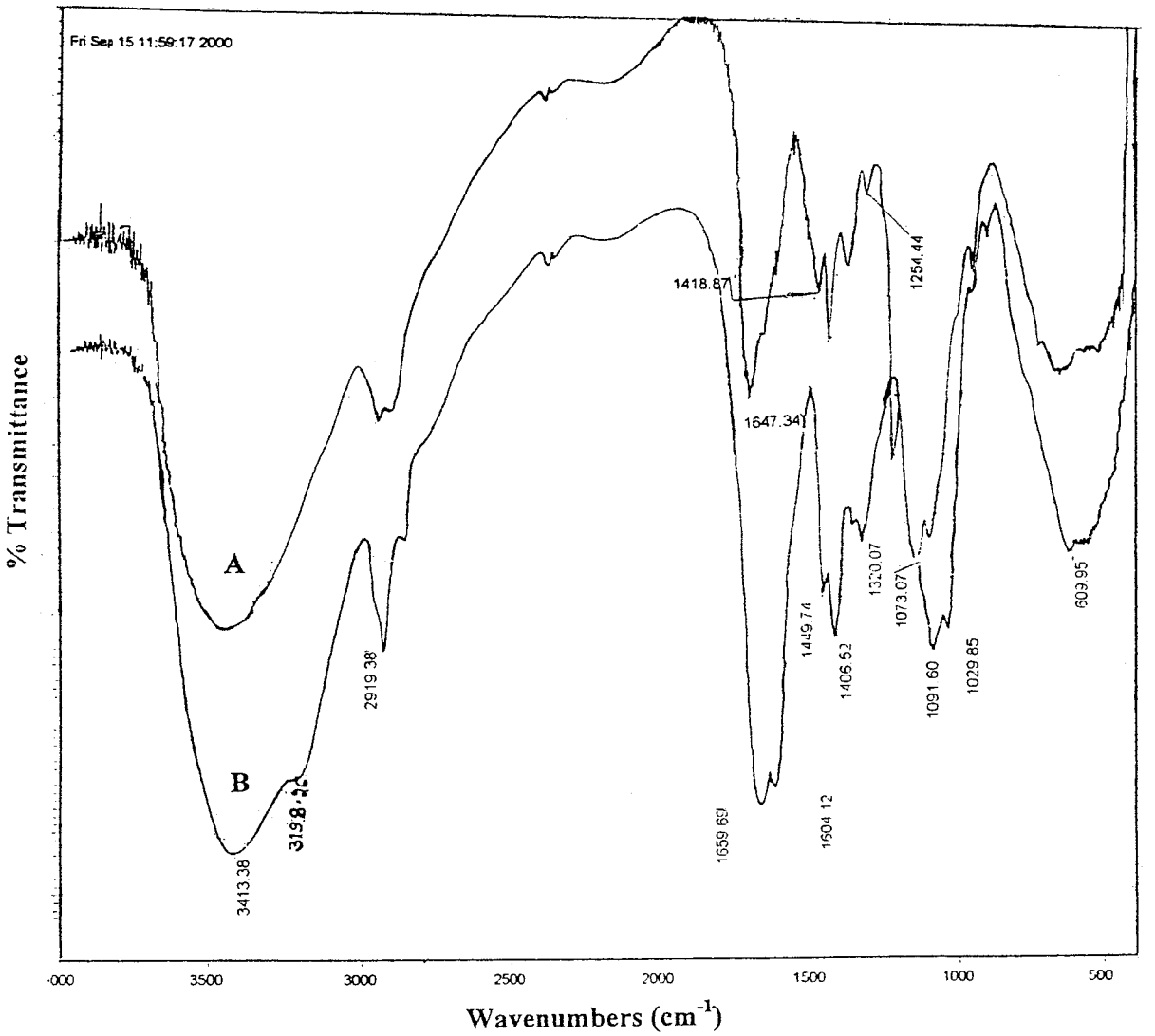


Figure 1 FTIR spectra of (A) neat chitosan and (B) PAAm-*g*-chitosan.

lie, Secomam, France) at λ_{max} = 265 nm. The percentage of drug loading was then calculated as

$$\% \text{ Drug loading} = \frac{\text{amount of drug in the microspheres}}{\text{mass of microspheres}} \times 100 \quad (3)$$

$$\% \text{ Encapsulation efficiency} = \frac{\text{amount drug loading}}{\text{theoretical loading}} \times 100 \quad (4)$$

Transport studies

To understand the molecular transport of water through the crosslinked microspheres, the microscopic method reported by Robert et al.[21] was adopted. In this method, the change in the diameter of the microspheres in the presence of distilled water was monitored at various time intervals. For this study, particles having almost an identical diameter were chosen, because transport in such microspheres may not be influenced only by the extent of crosslinking, but also by the size of the particles. The size measurements were performed in triplicate and the average normalized diameter of the particles was calculated.

In vitro drug release

The *in vitro* drug release from the microspheres was studied at 37°C using a Dissotest (Lab India, Mumbai, India) peddle-type dissolution tester at the rotation speed of 100 rpm. A weighed amount of microspheres was put in 900 mL of a 7.4 pH phosphate buffer, and,

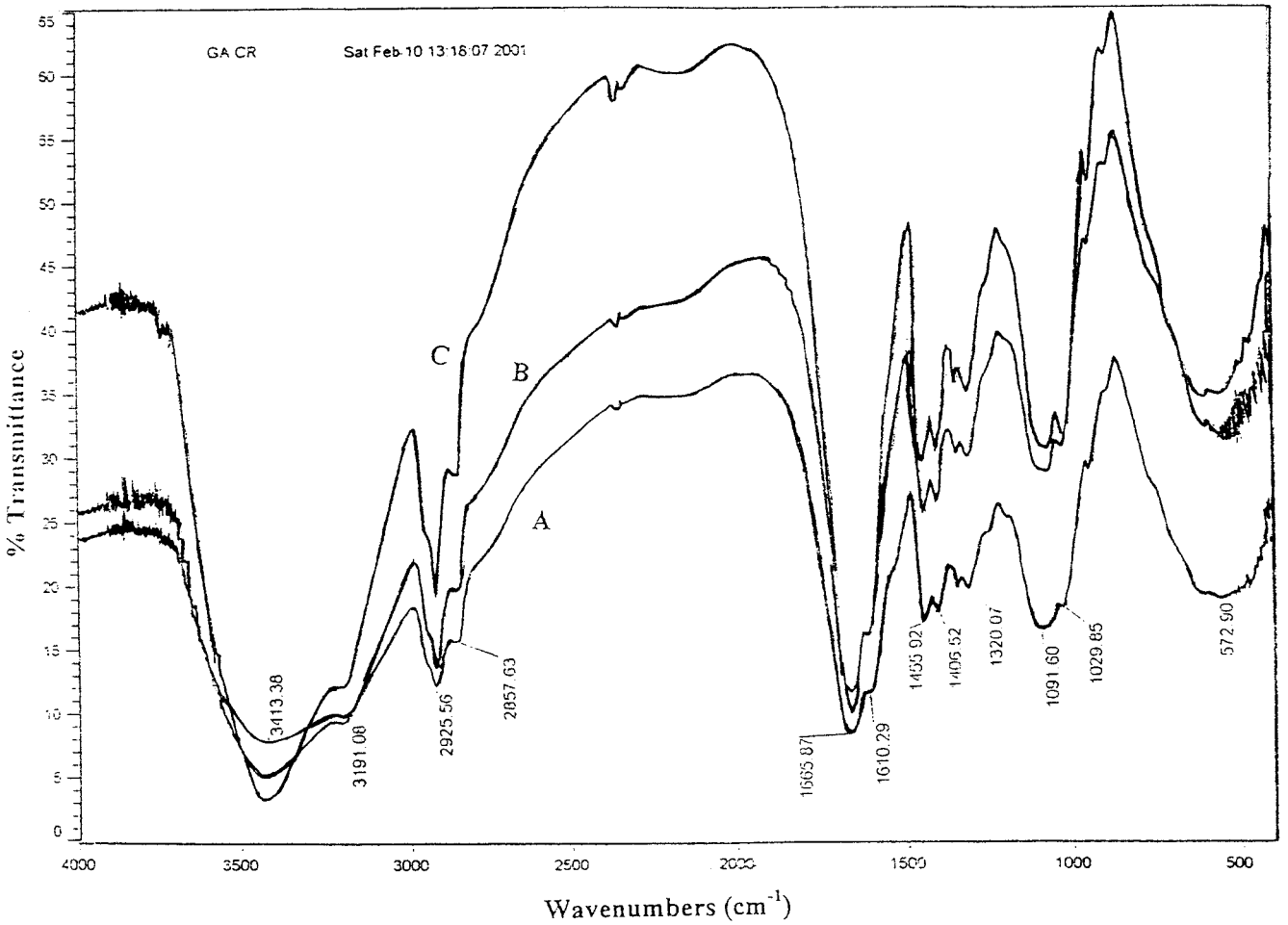


Figure 2 FTIR spectra of (A) GA crosslinked PAAm-*g*-chitosan with 5 mL, (B) 7.5 mL, and (C) 10 mL of GA.

each time, a 10-mL aliquot was withdrawn at regular time intervals. This solution was then analyzed for indomethacin using a UV spectrophotometer.

RESULTS AND DISCUSSION

Synthesis of grafted copolymer of chitosan and AAm

Graft copolymerization of chitosan with AAm was achieved in the presence of potassium persulfate-catalyzed free-radical polymerization. The complex formed by the reaction between —NH_2 and —OH groups of chitosan decomposed to generate the free-radical sites at 50°C, facilitating the reaction site for the AAm monomer on the chitosan backbone, as shown in the reaction (Scheme 1). The grafting reaction carried out at a temperature higher than 50°C resulted in a decreased grafting possibility due to the formation of the homopolymer.[17] At the monomer concentration of 0.16*M* used in the present study, no homopolymer was formed and the grafting efficiency was up to 100% with 92% grafting. A considerable decrease in the viscosity of the PAAm-*g*-chitosan polymer was observed when compared to the neat chitosan polymer solution. The PAAm-*g*-chitosan solution of a 0.002-g/dL concentration had a viscosity of 1.342 mPa s.

Elemental analyses data

A nitrogen content of 6.90% was observed for chitosan; after grafting with AAm, the amount of nitrogen was increased to 13.72%. Similarly, there was an increase in the number of carbon and hydrogen atoms in the grafted polymer. These results confirm the grafting reaction.

FTIR spectroscopic study

FTIR spectral analyses were carried out to confirm grafting as well as crosslinking of the PAAm-*g*-chitosan microspheres. The FTIR spectra of pure chitosan and PAAm-*g*-chitosan are shown in Figure 1. A broad band appearing at ~3400 cm^{-1} corresponds to the associated —OH stretching vibrations of the hydroxy groups, and the peak at 1647 cm^{-1} corresponds to the

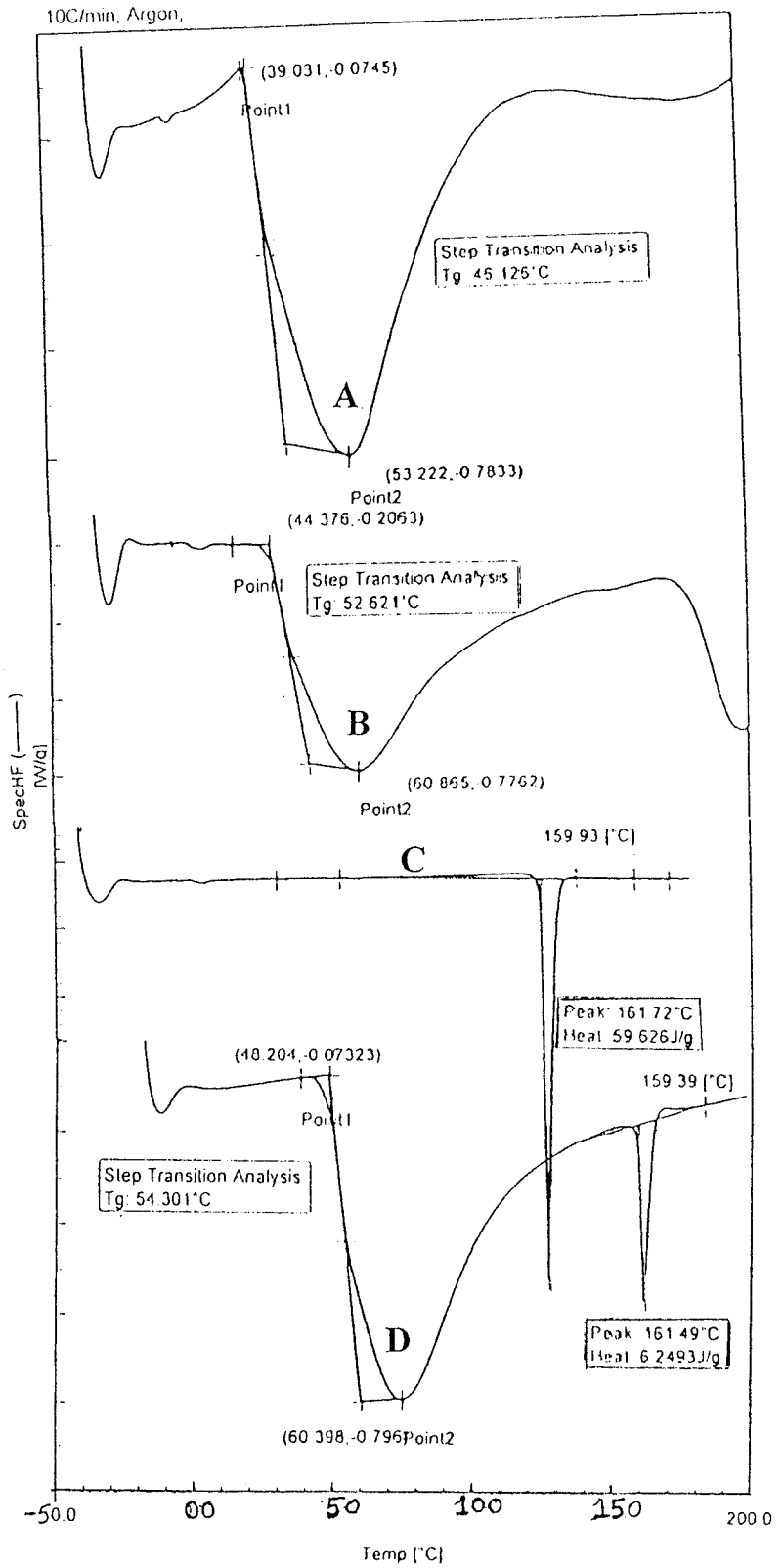


Figure 3 DSC thermograms of (A) chitosan, (B) PAAm-*g*-chitosan, (C) indomethacin, and (D) indomethacin-loaded PAAm-*g*-chitosan microspheres with 10 mL of GA crosslinked.

N—H deformation of chitosan. In the spectra of the copolymer, a new peak that appeared at 3200 cm^{-1} corresponds to the bonded —NH stretching vibrations and antisymmetric —N—H bending at 1659 cm^{-1} due to the primary amides. A relatively high intense peak at 2919 cm^{-1} corresponds to the aliphatic —C—H

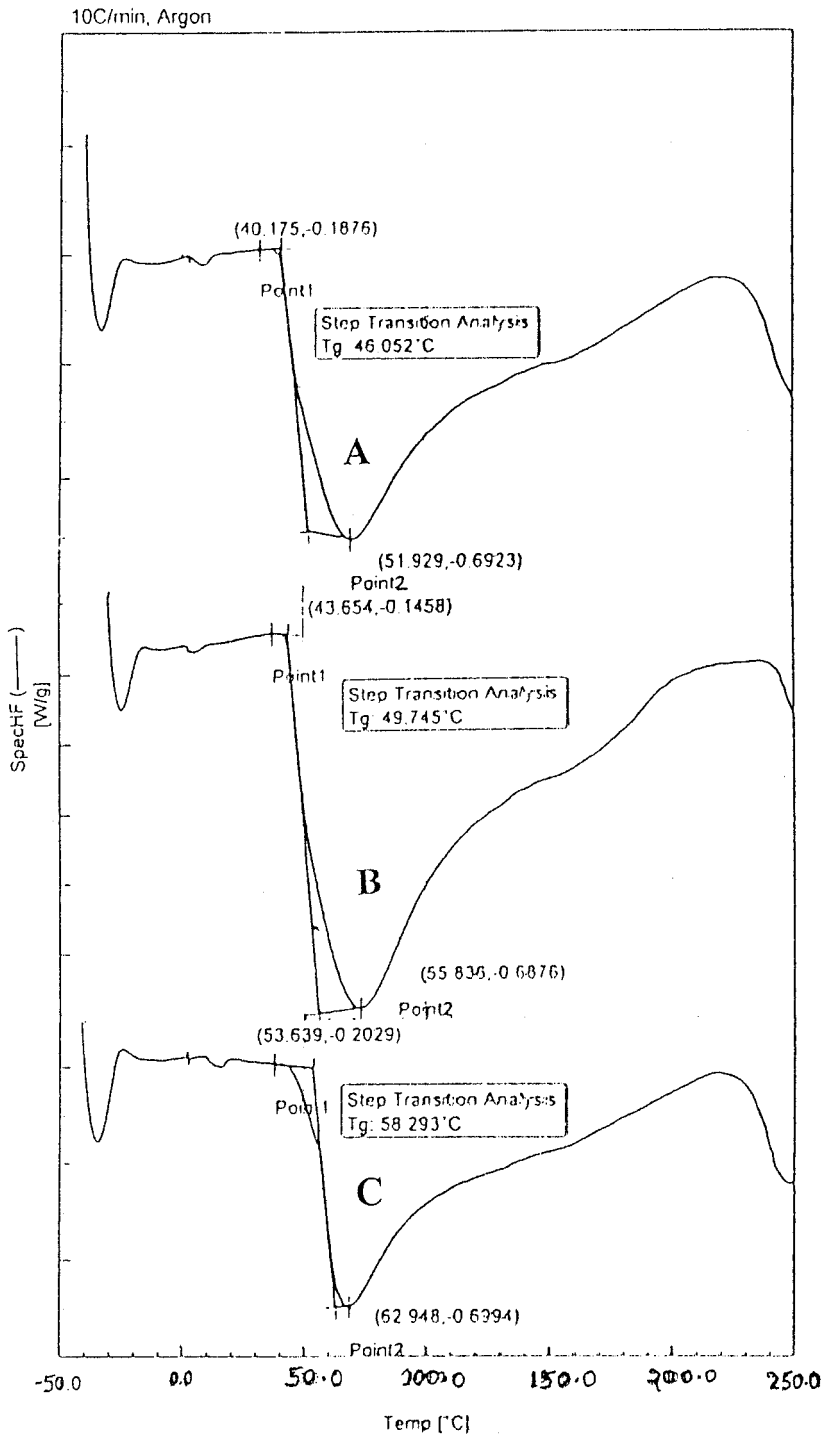


Figure 4 DSC thermograms of PAAm-*g*-chitosan empty microspheres crosslinked with (A) 5 mL, (B) 7.5 mL, and (C) 10 mL of GA.

stretching in the graft copolymer, further confirming the grafting reaction.

Crosslinking of PAAm-*g*-chitosan leads to the formation of an imine moiety 8, which is due to the reaction between free —NH_2 groups of the copolymer and the —CHO groups of GA, and this appears at 1665 cm^{-1} as shown in the FTIR spectra given in Figure 2. With an increase in the GA concentration, the intensity of this peak increased due to the formation of more imine groups.

DSC study

DSC performed on the plain chitosan and PAAm-*g*-chitosan shows the T_g at ~46 and ~52°C, respectively. This supports that modification of chitosan by grafting

TABLE I
Initial Drug Loading, Percentage Encapsulation Efficiency, and Particle Size of Crosslinked PAAm-*g*-Chitosan Microspheres

Formulation code[a]	Amount of crosslinking agent (mL)	Initial % drug loading (w/w)	Encapsulation efficiency (%)	Mean particle size (μm)
IM-1	5	10	53.05 ± 2.21	651
IM-2	7.5	10	53.57 ± 2.92	536
IM-3	10	10	64.65 ± 3.12	464
IM-4	5	20	47.21 ± 3.41	672
IM-5	7.5	20	54.15 ± 3.25	558
IM-6	10	20	53.44 ± 3.62	480
IM-7	5	30	48.89 ± 3.92	682
IM-8	7.5	30	41.91 ± 3.78	641
IM-9	10	30	50.65 ± 2.98	520

[a] Formulation codes are as follows: IM-1, IM-2, and IM-3 refer to 10% IM-loaded microspheres crosslinked with 5, 7.5, and 10-mL GA; IM-4, IM-5, and IM-6 refer to 20% IM-loaded cross-linked microspheres with 5, 7.5, and 10 mL GA; IM-7, IM-8, and IM-9 refer to 30% IM-loaded crosslinked microspheres with 5, 7.5, and 10 mL GA.

with AAm makes the chitosan polymer thermally more stable (see Fig. 3). DSC performed on the IM-loaded microspheres as well as neat IM are also presented in Figure 3. For IM, a sharp endothermic peak is observed at 161°C; this peak was also observed in the IM-loaded microspheres and its intensity is smaller when compared to the neat IM, suggesting the less crystalline nature of IM in the microspheres.

The DSC scans of the crosslinked PAAm-*g*-chitosan empty microspheres presented in Figure 4 exhibit a (endothermic peaks) step transition at T_g around 46–58°C. A shift in T_g at higher temperature is attributed to an increase in the crosslinking density of the matrix. This increase in T_g is due to the high energy required to break the highly crosslinked polymer network, thereby confirming the formation of highly crystalline microspheres with increasing crosslinking.

Microscopic study

The particle size of PAAm-*g*-chitosan microspheres was measured using optical microscopy and these results are given in Table I. Three samples, that is, empty microspheres crosslinked with 7.5 and 10 mL GA as well as the drug-loaded microspheres (formulation IM-9) were analyzed by a HELOS laser-light-induced particle-size analyzer. These results indicated a size range of 459–641 μm with a narrow size distribution as shown in Figure 5 for the empty microspheres. With increase in the crosslinking, the particle size decreases and this may be due to the formation of a more rigid polymer network. Also, an increase in percent drug loading increases the size of the microspheres, which may be due to the filling of the free volume of the microspheres. For example, 7.5-mL crosslinked empty microspheres have a mean volume diameter of 617 μm, whereas the 10-mL crosslinked empty microspheres have a diameter of 459 μm. In all the cases, particles are spherical and aggregated with rough surfaces as evidenced by the SEM photographs shown in Figures 6 and 7 and the photographs did not

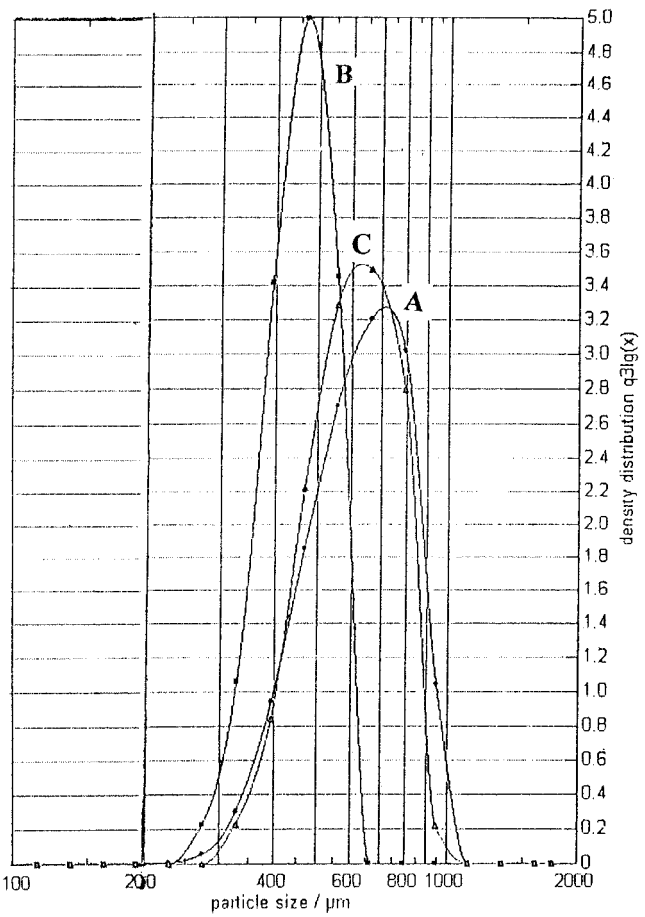
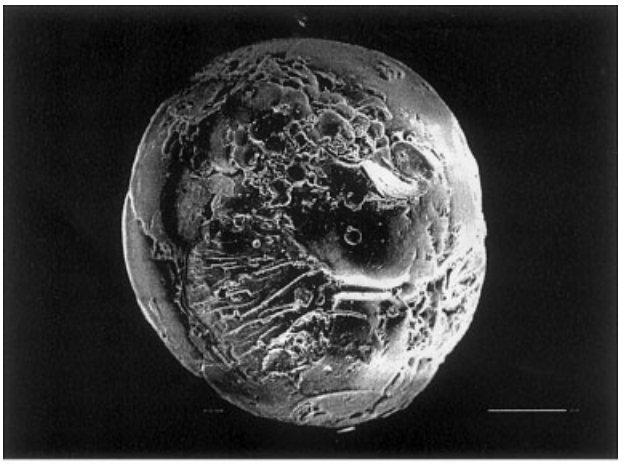
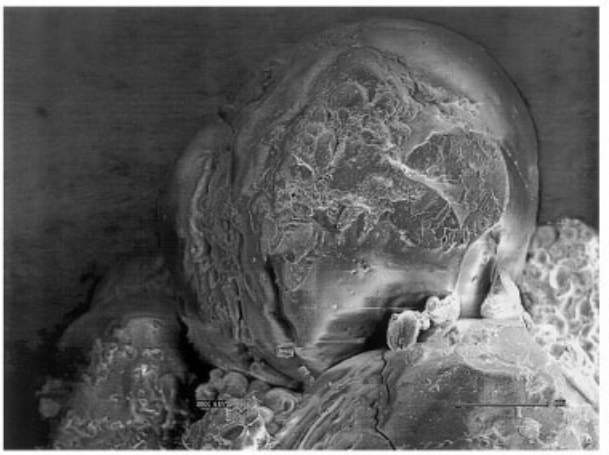


Figure 5 Particle-size distribution for (A) 7.5-mL GA-crosslinked empty microspheres, (B) 10-mL GA-crosslinked empty microspheres, and (▲) 40% IM-loaded microspheres crosslinked with 10 mL GA.



A



B

Figure 6 SEM photographs of (A) a single IM-loaded PAAm-*g*-chitosan microsphere and (B) surface photographs of the same.

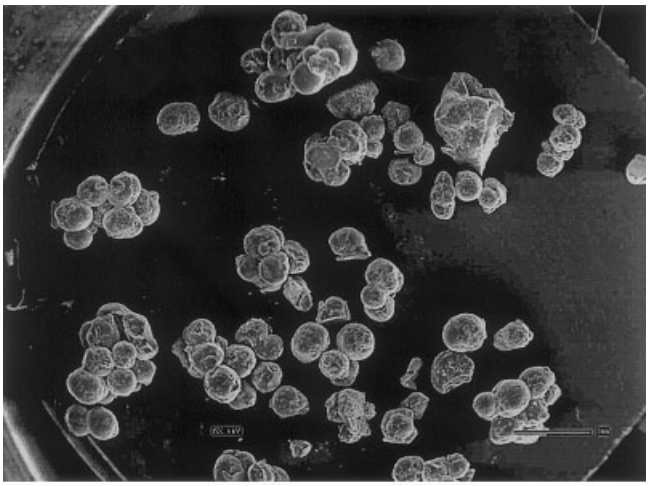
show any porous structure and no crystals of the drug were present on the surface.

Drug loading

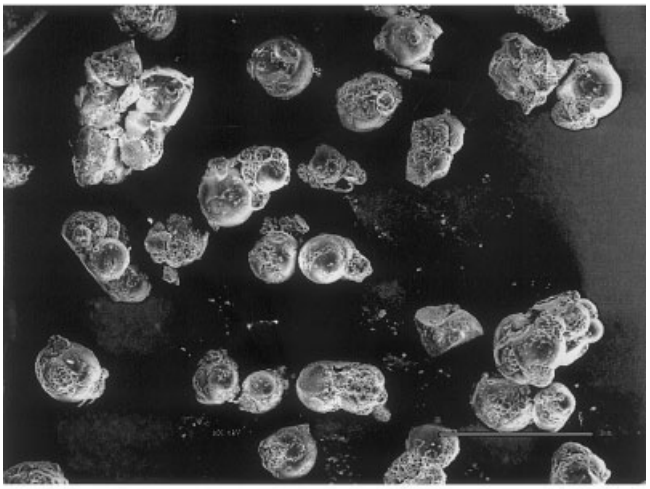
Drug loading was done at the time of crosslinking of the microspheres. The results of encapsulation efficiency are given in Table I and these values range between 41 and 64%. The lower encapsulation values are probably due to the loss of IM in liquid paraffin during the process of crosslinking in the presence of polysorbate-80. However, the extent of drug loading and crosslinking did not show any systematic effect on the encapsulation efficiency.

Transport studies

Dynamic swelling of the PAAm-*g*-chitosan microspheres was studied by measuring the change in the particle diameter, D_t, as a function of time using an optical microscope. Figure 8 represents the dynamic swelling data, wherein an increase in crosslinking showed a decrease in the water-uptake characteristics. These results indicated that a dense three-dimensional network structure might have been formed by increasing the amount of GA (crosslinking agent) and these results are also supported by the FTIR and DSC data. The molecular transport of liquids within the polymeric matrices are influenced by polymer swelling. The loose polymer network generally absorbs a higher quantity of liquid, thereby giving higher swelling. Equilibrium swelling (Q) studies were performed in triplicate, but the average values are presented in Table II. The 5-mL GA crosslinked microspheres show a 368% equilibrium water uptake, whereas the 7.5- and 10-mL GA crosslinked microspheres show 314 and



A



B

Figure 7 SEM photographs of (A) IM-loaded PAAm-*g*-chitosan microspheres crosslinked with 10 mL GA and (B) 7.5-mL GA crosslinked photographs of the same.

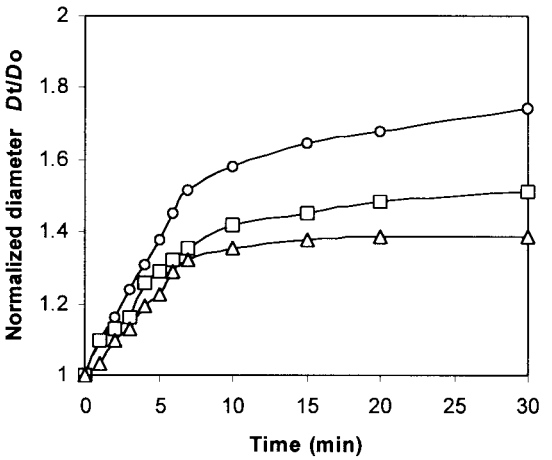


Figure 8 Normalized diameter of PAAm-*g*-chitosan crosslinked microspheres as a function of swelling time for GA: (○) 5 mL; (□) 7.5 mL; (△) 10 mL.

233%, respectively; this also supports the above explanations.

Since the drug release from the swollen polymers is controlled mainly both by the swelling or relaxation of the chain, the dynamic swelling data were analyzed using an empirical equation[21]:

$$\frac{D_t}{D_\infty} = kt^n \tag{5}$$

where D_t is the change in microsphere diameter at time t and D_∞ is the equilibrium diameter of the swollen microsphere. The exponent value of n indicates the type of transport mechanism. The least-squares method was used to estimate the values of n and k at the 95 % confidence limit, but only the n values are presented in Table II. It is observed that n increases with an increasing extent of the crosslinking agent, suggesting the deviation of transport from Fickian to non-Fickian. The equilibrium swollen particle diameter, D_∞, normalized with respect to the original diameter, D, decreased significantly (Table II), indicating that a more rigid the crosslinked matrix network since it does not expand in water as much as does the loosely crosslinked matrix.

In vitro release study

To understand the release of IM from the crosslinked PAAm-*g*-chitosan microspheres, the *in vitro* release study was carried out in a pH 7.4 phosphate buffer at 37°C. These experiments were performed in triplicate, but the average values are presented graphically and used in the data treatment. The standard deviations in all the cases were less than 5%. Figures 9–11 present the release profiles of PAAm-*g*-chitosan microspheres loaded with 10, 20, and 30 % of IM as well as those matrices prepared with three levels of crosslinking. These results showed a systematic effect of the extent of crosslinking on the drug-release profiles in all the formulations. This may be due to the fact that diffusion of the drug from the hydrogel depends upon the mesh size of the polymer network, which will decrease with increasing crosslinking. The crosslinking also induces an increase in the crystallinity of the polymer and, hence, the rigidity of the polymeric chain, which is evident from the DSC analyses. For instance, 10% drug-loaded microspheres when crosslinked with 5 mL of GA showed a release of 89%, whereas when 10 mL of GA was used, the crosslinked microspheres exhibited drug release to 73%.

The drug release also showed a dependence on the extent of drug loading. In the case of formulations containing 10 % IM, the release was fast when compared to the formulations containing 20% of the drug. This indicates that the drug release at lower loadings (<10%) is somewhat quicker. This may be due to that at lower drug loadings there is a possibility of the formation of a large pore volume, which, in turn, might enhance the drug release. Any further increase in drug loading increases the drug release.

The *in vitro* release data were analyzed by using an empirical equation to estimate the values of n and k (refs. 22 and 23):

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{6}$$

Here, M_t is the amount of drug released at time t and M_∞ is the drug released at equilibrium time; k, a constant characteristic of the drug–polymer system; and n, the diffusional exponent, which suggests the nature of the release mechanism. A value of $n = 0.5$ indicates

TABLE II
Transport Data on Empty Microspheres at 37°C

GA (mL)	(D_∞/D_0)	Upper limit	n lower limit	Mean	r	Q[a]
5	1.74	0.56	0.46	0.50 ± 0.07	0.997	368 ± 82
7.5	1.51	0.69	0.62	0.66 ± 0.03	0.989	314 ± 58
10	1.39	0.94	0.85	0.90 ± 0.04	0.998	233 ± 55

[a] % Equilibrium water uptake.

TABLE III
Analysis of Release Kinetics from Eq. (6) of the IM-Loaded Microspheres from PAAm-*g*-Chitosan

Formulations	k (min^{-n}) $\times 10^2$	n	r	$D \times 10^7$ ($\text{cm}^2/\text{s}^{-1}$)
IM-1	1.22	0.41	0.992	6.90
IM-2	0.95	0.38	0.994	2.58
IM-3	0.37	0.58	0.988	2.02
IM-4	0.69	0.49	0.997	4.13
IM-5	0.43	0.54	0.991	2.25
IM-6	0.21	0.66	0.993	1.97
IM-7	0.99	0.47	0.999	3.35
IM-8	0.43	0.62	0.993	2.02
IM-9	0.58	0.52	0.997	1.73

Fickian transport, while $n = 1$ is of Case II transport. The intermediary values ranging between 0.5 and 1.0 are indicative of the anomalous transport.[24,25] The least-squares estimations of the fractional release data along with the estimated correlation coefficient values, r, are presented in Table III. From these data, we notice that the n value ranged between 0.38 and 0.66, with correlation coefficient values of 0.99, indicating the drug release deviating slightly from the Fickian transport.[7,17,19] Even though water uptake by the microspheres reaches equilibrium very fast (i.e., 10–20 min), converting the glassy polymer into the rubbery polymer, the release is continued for several hours, indicating that the polymer chain relaxation has less influence on the drug release, but is governed by the molecular diffusion. In an earlier study[9] on the water-transport characteristics of the PVA–GG crosslinked matrix loaded with nifedipine, it was found that the release is not only governed by drug diffusion through the polymeric network, but also by the polymer chain relaxation process.

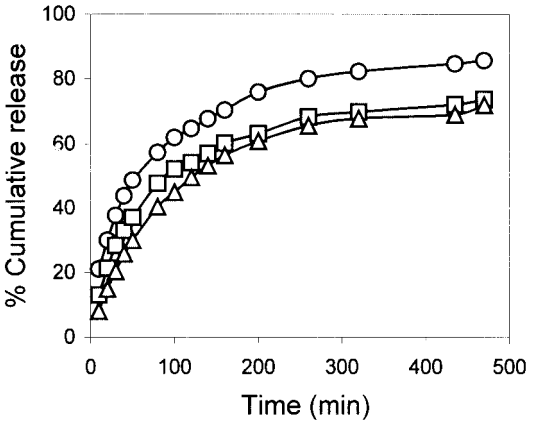


Figure 10 *In vitro* percent cumulative release versus time for IM-loaded PAAm-*g*-chitosan microspheres of (○) IM-4, (□) IM-5, and (△) IM-6.

To calculate the values of the apparent diffusion coefficients, the D of IM from the hydrogel microspheres, the initial portions of the release profiles (i.e., $0 < M_t/M_\infty < 0.4$) as shown in Figures 9–11 were analyzed by the Fickian theory.[26] This equation, given in the most simplified form, calculates the diffusion coefficients from the initial time approximation[27]:

$$\frac{M_t}{M_\infty} = \left(\frac{36Dt}{\pi r^2}\right)^{1/2} - \left(\frac{3Dt}{r^2}\right) \tag{7}$$

where r is the average radius of the microspheres. The data reported in Table III show a relationship between the extent of crosslinking and drug loading. An increase in the amount of GA from 5 to 10 mL decreased the values of the diffusion coefficients from 6.90×10^{-7} to 2.02×10^{-7} cm^2/s for the 10% IM-loaded

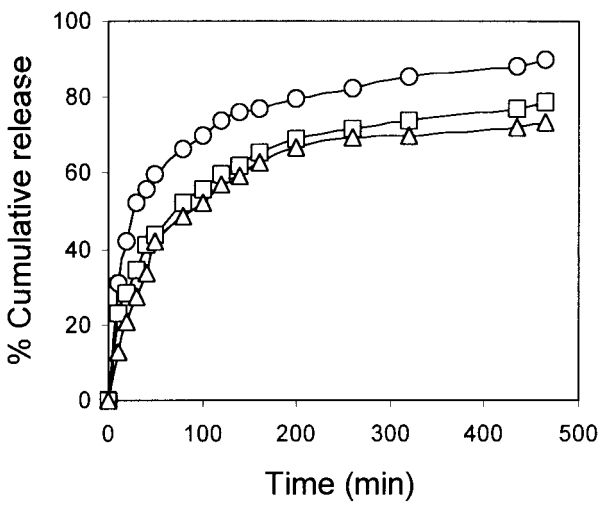


Figure 9 *In vitro* percent cumulative release versus time for IM-loaded PAAm-*g*-chitosan microspheres of (○) IM-1, (□) IM-2, and (△) IM-3.

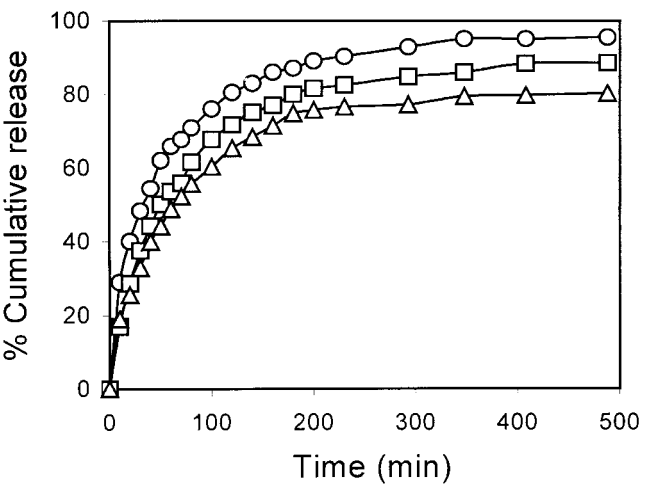


Figure 11 *In vitro* percent cumulative release versus time for IM-loaded PAAm-*g*-chitosan microspheres of (○) IM-7, (□) IM-8, and (△) IM-9.

microspheres. As explained before, the diffusion coefficients tend to decrease with higher drug loadings.

CONCLUSIONS

PAAm-*g*-chitosan microspheres were successfully prepared by chemical crosslinking and used for the delivery of indomethacin. The microspheres may be good biomaterials for the controlled release of NSAID. By varying the extent of crosslinking and drug loading, it is possible to easily monitor the physicochemical properties of the crosslinked microspheres. At higher crosslinking, it is possible to prolong the indomethacin release for longer periods of time. The initial release of IM from these microspheres is due to the polymer chain relaxation process, but at longer times, the release occurs from the fully swollen polymer and is controlled mainly by the molecular diffusion phenomenon. This study suggests that the hydrogel microspheres prepared from chitosan-based polymers can be useful in the delivery of indomethacin-like drugs.

The authors are thankful to GSFC Science Foundation, Vadodara, for sponsoring this project. We also thank the University Grants Commission, New Delhi, for major financial support to establish the Center of Excellence in Polymer Science. Mr. Andrew Abbott helped us in conducting the SEM studies at UT Southwestern Medical Center, Dallas, TX.

References

1. Peppas, N. A.; Korsmeyer, D. W. In Hydrogels in Medicine and Pharmacy; Peppas, N. A., Ed.; CRC: Boca Raton, FL, 1987; Vol. 3, p 109.
2. Park, K.; Shalaby, W. S. W.; Park, H. Biodegradable Hydrogels for Drug Delivery; Technomic: New York, 1993.
3. Graham, N. B.; Mc Neill, M. E. Biometerials 1984, 5, 27.
4. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. J Control Rel 2001, 75, 331.
5. Xu, H.; Ding, P.; Wec, G; Zhang, J. Drug Dev Ind Pharm 2001, 27, 171.
6. Hoffman, A. S. In Polymer Gels; DeRossi, D.; Kijiwara, K.; Osad, Y.; Yamauchi, A., Eds; Plenum: New York, 1991.
7. Kriwet, B.; Walter, E.; Kissel, T. J Control Rel 1998, 56 149.
8. Kumbar, S. G.; Kulkarni, A. R.; Aminabhavi, T. M. J Microencapsulation 2002, 19, 173.
9. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. J Biomater Sci Polym Ed 2000, 11, 27.
10. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. J Microencapsul 2001, 18, 397.
11. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. Int Symp Control Rel Bioact Mater 2000, 27, 572.
12. Kulkarni, A. R.; Soppimath, K. S.; Aminabhavi, T. M. Pharma Acta Helv 2000, 74, 29.
13. Kulkarni, A. R.; Soppimath, K. S.; Aminabhavi, T. M. Eur J Pharm Biopharm 2001, 511, 27.
14. Pitt, L. Biodegradable Polymers; Marcel Dekker: New York, 1990; p 97.
15. Hamdi, G.; Ponchel, G.; Dunchene, D. J Control Rel 1998, 55, 193.
16. Castellano, I.; Goni, I.; Ferrero, M. C.; Munoz, A.; Jimenez-Castellanos, R.; Gurruchaga, M. Drug Dev Ind Pharm 1999, 25, 1249.
17. Najjar, A. M. K.; Yunus, W. M. Z. W.; Ahmad, M. B.; Rahaman, M. Z. A. B. J Appl Polym Sci 2000, 77, 2314.
18. Singh, D. K.; Ray, A. R. JMS-Rev Macromol Chem Phys C 2000, 40, 69.
19. Kulkarni, A. R.; Soppimath, K. S.; Aminabhavi, T. M. J Microencapsul 2000, 14, 449.
20. Roberts, G. A. F. Chitin Chemistry; MacMillan: Houndmills, UK, 1992.
21. Robert, C. C. R.; Buri, P. A.; Peppas, N. A. J Appl Polym Sci 1985, 30, 301.
22. Ritger, P. L., G.; Peppas, N. A. J Control Rel 1987, 5, 37.
23. Peppas, N. A. Pharm Acta Helv 1985, 60, 110.
24. Aminabhavi, T. M.; Naik, H. G. J Haz Mater 1998, 60, 175.
25. Aminabhavi, T. M.; Naik, H. G. Polym Polym Comp 1998, 6, 205.
26. Crank, J. The Mathematics of Diffusion, 2nd ed.; Clarendon: Oxford, 1974.
27. Baker, R. W.; Lonsdale, H. K. In Controlled Release of Biologically Active Agents; Tanquarry, A. C.; Lacey, R. E., Eds.; Plenum: New York, 1974; p 15.