Synthesis and Characterization of Modified Chitosan Microspheres: Effect of the Grafting Ratio on the Controlled Release of Nifedipine through Microspheres*

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ABSTRACT: The grafting of acrylamide onto a chitosan backbone was carried out at three acrylamide concentrations (polymer/monomer ratio = 1:1, 1:2, and 1:3). The synthesis of the grafted polymer was achieved by $K_2S_2O_8$-induced free-radical polymerization. Microspheres of polyacrylamide-*g*-chitosan crosslinked with glutaraldehyde were prepared to encapsulate nifedipine (NFD), a calcium channel blocker and an antihypertensive drug. The microspheres of polyacrylamide-*g*-chitosan were produced by a water-in-oil emulsion technique with three different concentrations of glutaraldehyde as the crosslinking agent. Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) were used to characterize the grafted copolymers, and the microspheres were prepared from them. FTIR and DSC were also used to analyze the extent of crosslinking. The microspheres were characterized by the particle size; the water transport into these microspheres, as well as the equilibrium water uptake, were studied. Scanning electron microscopy confirmed the spherical nature of the particles, which had a mean particle size of 450 μm. Individual particle dynamic swelling experiments suggested that with an increase in crosslinking, the transport became case II. The release of NFD depended on the crosslinking of the network and on the amount of drug loading. Calculating the drug diffusion coefficients with the initial time and later time approximation method further supported this. The drug release in all 27 formulations followed case II transport, and this suggested that the time dependence of the NFD release followed zero-order kinetics. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 2940–2949, 2003

Key words: nifedipine; microspheres; chitosan; acrylamide; grafting

INTRODUCTION

Polymeric drug-loaded microspheres have several inherent advantages over the conventional dosage forms in optimizing patient treatment regimes. In particular, swelling controlled-release (CR) systems have been studied extensively for the delivery of a variety of drugs at controlled rates.[1] These systems are capable of delivering drugs at constant rates over an extended period of time. In these systems, the rate of drug release is controlled by the balance between the drug diffusion across the concentration gradient and the polymer relaxation rate as a result of the diffusion-controlled process due to swelling.[2,3] Swelling CR systems can achieve zero-order release[2–5] by modifying the structure–property relationships of the device. For the effective control of drug release over an extended length of time, understanding different types of transport processes is important.[2–5]

In continuation of our ongoing efforts to develop swelling CR systems, here we report the synthesis of polyacrylamide (PAAm)-*g*-chitosan copolymers and the preparation of microspheres from them for the slow release of nifedipine (NFD), an antihypertensive drug. The main advantages of using such biocompatible polymers are that they are cost-effective and biodegradable and produce no systemic toxicity upon administration.[6,7] Microspheres were prepared by a water-in-oil emulsion method with glutaraldehyde (GA) as the crosslinking agent. NFD was loaded before the crosslinking of the matrix. However, short-acting formulations of NFD should be used with great caution because of their associated problems of increased dose-dependent death from myocardial infarction. Therefore, there is a need to develop CR devices of NFD for the effective management of hypertension. The microspheres prepared in this study were characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and particle size analyses. The microspheres were evaluated for their thermal behavior, water-transport properties, and *in vitro* drug-release characteristics.

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TABLE I
Synthetic Details of the Grafted Copolymers

Polymer	Polymer sample	Mass of chitosan (g)	Mass of AAm (g)	$K_2S_2O_8$ (mol/L)	% Grafting	Grafting efficiency	% Conversion of AAm
Chitosan	Neat	5.00	0	0	0	0	0
PAAm-*g*-Chitosan	Polymer I	5.00	5.00	0.012	49.67	100	94
PAAm-*g*-Chitosan	Polymer II	5.00	10.00	0.012	66.17	100	95
PAAm-*g*-Chitosan	Polymer III	5.00	15.00	0.012	75.17	100	95

EXPERIMENTAL

Materials

A chitosan (medium molecular weight = 65,850) solution of 0.002 g/dL with a viscosity of 15.524 mPa.s was purchased from Aldrich Chemical Co. (Milwaukee, WI). Acrylamide (AAm), acetic acid, potassium persulfate, polysorbate-80, a GA (25% w/w) solution, and liquid paraffin were all purchased from S.D. Fine Chemicals (Mumbai, India). NFD, a USP-grade drug, was obtained as a gift sample from Torrent Pharmaceuticals (Ahmedabad, India). The deacetylation degree of chitosan was 86%, as determined by IR spectroscopy.[8] All the other reagents and solvents were analytical-grade and were used without further purification.

Synthesis of the graft copolymer of chitosan and AAm

The grafting of AAm onto a chitosan backbone was carried out by persulfate-induced free-radical polymerization, as reported earlier.[9] Briefly, 5 g of chitosan was dissolved in 400 mL of a 1% aqueous acetic acid solution with constant stirring, and a clear solution was obtained. Later, 5 g of AAm, previously dissolved in 50 mL of deaerated and distilled water, was mixed with a chitosan solution with stirring. Then, a 0.001M solution of potassium persulfate was added dropwise for about of 5 min at a constant reaction temperature of 50°C. Nitrogen was purged into the solution during polymerization, and the reaction was continued for 6 h; adding hydroquinone terminated it. The copolymer formed was precipitated by the addition of an excess amount of acetone and was dried in a vacuum oven at 40°C. The resulting polymer was designated polymer I. The grafting percentage was estimated from the mass of the polymer before and after grafting as follows:

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

where W_g and W_0 represent the masses of the grafted copolymer and chitosan. The grafting percentage of AAm on chitosan and the grafting efficiency were calculated as follows:

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

$$\text{Grafting efficiency} = \left(\frac{\text{Mass of copolymer}}{\text{Mass of copolymer} + \text{Mass of PAAm homopolymer}}\right) \times 100 \quad (3)$$

In a similar manner, two other grafted copolymers were synthesized by the variation of the polymer/monomer ratio (1:2 and 1:3), with all other variables kept constant. The resultant copolymers were designated polymers II and III, respectively. The synthetic details are given in Table I.

Preparation of the PAAm-*g*-chitosan microspheres

The PAAm-*g*-chitosan microspheres containing NFD were prepared by the dissolution of 2.50 g of the polymer in 40 mL of 1% acetic acid in hot water at 50°C. To this solution, 1 mL of conc. HCl was added and mixed thoroughly. NFD was ground into a fine powder, passed through a 100-mesh sieve, added to the polymer solution, dispersed uniformly with a magnetic stirrer for about 10 min, and then sonicated with a sonicator (Ikasonic U50 model, IKA Labortechnik, Staufen, Germany) for 10 min. At this stage, GA was added as the crosslinking agent to the polymer solution with constant stirring. Then, the solid-in-water suspension was emulsified in 150 mL of liquid paraffin in the presence of 2% polysorbate-80 with a Eurostar digital stirrer (IKA Labortechnik) at a 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 any excess amount of GA. The microspheres were dried at 50°C for about 24 h and kept in a desiccator until needed.

Three crosslinked systems were prepared by treatment with 5, 7.5, and 10 mL of GA. The resulting nine formulations were prepared out of the three crosslinking variables, and the three copolymers were desig-

nated PI-1, PI-2, and PI-3 for polymer I, PII-1, PII-2, and PII-3 for polymer II, and PIII-1, PIII-2, and PIII-3 for polymer III, respectively.

Drug loading was done with 10, 20, and 40% (w/w) NFD based on the dry mass of the polymer. Twenty-seven formulations of the microspheres were prepared out of the three copolymers, three different amounts of the crosslinking agent, and three different drug loadings. These microspheres were designated PI–GA5–NFD10, PI–GA5–NFD20, PI–GA5–NFD40, PI–GA7.5–NFD10, PI–GA7.5–NFD20, PI–GA7.5–NFD40, PI–GA10–NFD10, PI–GA10–NFD20, and PI–GA10–NFD40 for polymer I; PII–GA5–NFD10, PII–GA5–NFD20, PII–GA5–NFD40, PII–GA7.5–NFD10, PII–GA7.5–NFD20, PII–GA7.5–NFD40, PII–GA10–NFD10, PII–GA10–NFD20, and PII–GA10–NFD40 for polymer II; and PIII–GA5–NFD10, PIII–GA5–NFD20, PIII–GA5–NFD40, PIII–GA7.5–NFD10, PIII–GA7.5–NFD20, PIII–GA7.5–NFD40, PIII–GA10–NFD10, PIII–GA10–NFD20, and PIII–GA10–NFD40 for polymer III. Because NFD is a photosensitive drug, extreme care was taken to prevent its degradation during the formulation and characterization of the microspheres. Most of the experiments were conducted in the absence of direct light.

FTIR measurements

FTIR spectral measurements were performed with an Impact 410 spectrophotometer (Nicolet, Madison, WI) to confirm the grafting reaction between chitosan and AAm and to investigate the crosslinking reaction. Empty microspheres (~2 mg) were finely ground with KBr, and pellets were made under a hydraulic pressure of 400 kg. Spectra were scanned in the range 4000–500 cm^{-1}.

Differential scanning calorimetry (DSC) measurements

DSC analyses were performed for chitosan, PAAm-*g*-chitosan (i.e., polymers I–III), crosslinked microspheres, and NFD-loaded microspheres with a PerkinElmer thermal analyzer at a heating rate of 10°C/min from 0 to 250°C under a constant flow of nitrogen gas. These measurements were done at NCL (Pune, India).

Microscopy studies

The particle size of the microspheres was measured with an optical microscope, with 100–200 particles examined at a time on a glass slide under regular polarized light. SEM photographs were obtained with a JOEL G-1 SEM (model JFM-5800; (JOEL, Ltd., Peabody, MA) at an accelerating voltage of 6 kV at a working distance of 25 mm. With no tilt angle, magnifications were varied between 160× and 220×. Scale bars for the particle ensembles represented 1 mm, whereas the scale bar for a single particle represented 100 μm. DSG digital image acquisition allowed contrast-enhanced post-image processing of the micrographs. All the SEM photographs were obtained at Southwestern Medical Center, Dallas, TX.

Estimation of the drug loading and encapsulation efficiency

The amount of NFD loaded into the microspheres was estimated by the extraction of the drug into a 7.4 pH phosphate buffer containing 0.05 (w/v) polysorbate-80. Samples were filtered and analyzed for the drug with an ultraviolet–visible spectrophotometer (Anthelie, Secomam, France) at λ_{max} = 239 nm. The drug loading percentage and encapsulation efficiency percentage were calculated as follows:

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in the microspheres}}{\text{Mass of microspheres}}\right) \times 100 \quad (4)$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical loading}}\right) \times 100 \quad (5)$$

Transport studies

To understand the molecular transport of water through crosslinked microspheres, we adopted the microscopic method reported by Robert et al.[10] In this method, a change in the diameter of the microspheres in the presence of distilled water was monitored at various time intervals. For this study, particles with almost identical diameters were chosen because transport in such microspheres may be influenced not only by the extent of crosslinking but also by the size of the microspheres. Size measurements were performed in triplicate, and the average normalized diameter of the particles was calculated. For this study, microspheres were prepared without the drug. For polymer I, three microspheres with increasing crosslinking (i.e., 5, 7.5, and 10 mL of GA) were prepared. These are designated as PI–GA5, PI–GA7.5, and PI–GA10, respectively. Similarly, for polymer II, we designate PII–GA5, PII–GA7.5, and PII–GA10. For polymer III, we designate PIII–GA5, PIII–GA7.5, and PIII–GA10.

In vitro drug release

The *in vitro* drug release from the microspheres was studied at 37°C with a Dissotest peddle-type dissolution tester (LabIndia, Mumbai, India) at a rotation

speed of 100 rpm. A weighed amount of the microspheres was placed in 900 mL of a 7.4 pH phosphate buffer containing 0.05 (w/v) polysorbate-80 to maintain perfect sink conditions; each time, a 10-mL aliquot was withdrawn at regular time intervals and was analyzed for NFD with an ultraviolet spectrophotometer at λ_{max} = 239 nm.

RESULTS AND DISCUSSION

Synthesis of the grafted copolymer of AAm and chitosan

The graft copolymerization of chitosan with AAm was achieved with potassium persulfate-catalyzed free-radical polymerization. The complex formed by the reaction between —NH_2 and —OH groups of chitosan decomposed to generate free-radical sites at 50°C, thereby facilitating the reaction site for the AAm monomer onto the chitosan backbone.[9] When grafting was carried out at temperatures higher than 50°C, there was a decrease in grafting due to the formation of the homopolymer.[11] The synthesis details are given in Table I. The grafting percentage increased from 49 to 75 from polymer I to polymer III with 100% grafting efficiency. In all cases, the conversion of AAm was about 95%.

FTIR spectroscopy study

FTIR spectral analyses were carried out to confirm the grafting and crosslinking of PAAm-*g*-chitosan microspheres. FTIR spectra of pure chitosan and PAAm-*g*-chitosan (polymers I–III) have been discussed elsewhere.[9] A broad band appearing at approximately 3400 cm^{-1} corresponded to associated —OH stretching vibrations of hydroxy groups, whereas the peak at 1647 cm^{-1} corresponded to —NH deformation of chitosan. In the spectra of the copolymer, a new peak appearing at 3200 cm^{-1} corresponded to bonded —NH stretching vibrations. An antisymmetric —NH bending observed at 1659 cm^{-1} was due to the primary amides. A relatively high intense peak seen at 2919 cm^{-1} corresponded to an aliphatic —CH stretching vibration in the grafted copolymers; this further confirmed the grafting reaction. The crosslinking of PAAm-*g*-chitosan led to the formation of an imine moiety,[12] which was due to the reaction between free —NH_2 groups of the copolymer and —CHO groups of GA. This appeared at 1665 cm^{-1}. With an increase in the GA concentration, the intensity of this peak increased because of the formation of more imine groups.

DSC study

DSC scans of the neat chitosan, polymer I, polymer II, and polymer III showed a systematic trend in their glass-transition temperatures (T_g's; see Fig. 1). The T_g values varied between approximately 53 and 65°C from neat chitosan and polymers I–III. The change in T_g to a higher value was attributed to an increase in the thermal stability of the polymer. This increase in T_g was due to the increase in the PAAm chain length of the polymer. DSC studies were also performed on neat NFD and NFD-loaded microspheres crosslinked with 5, 7.5, and 10 mL of GA. A sharp endothermic peak observed at 172.28°C corresponded to the melting point of NFD.

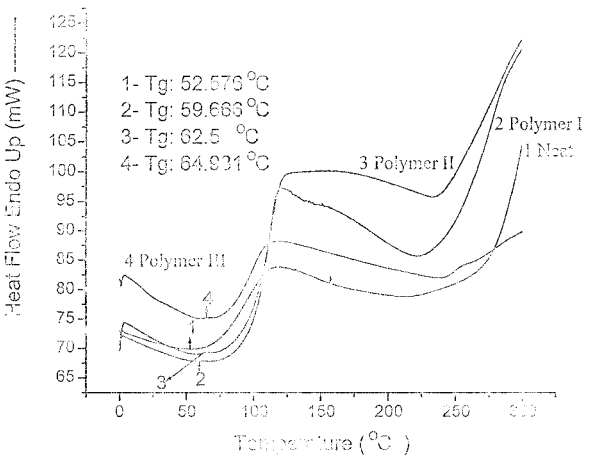


Figure 1 DSC thermograms of (1) neat chitosan, (2) polymer I, (2) polymer II, and (3) polymer III.

An increase in the crosslinking of the matrix resulted in an increased crosslinking density, giving a further shift of T_g to a higher temperature scale. However, we could not measure this temperature more precisely because of the complete crosslinking of the microspheres. In all these formulations, a characteristic sharp endothermic peak observed for NFD showed an increase in intensity with an increasing amount of drug loading, and this indicated the formation of small crystal domains inside the microspheres. However, the intensity of this peak was less in the microspheres compared with that of neat NFD, indicating the overall amorphous nature and molecular level distribution of NFD inside the microspheres.

Microscopy studies

The particle size of PAAm-$_g$-chitosan microspheres in both dry and swollen states was measured with optical microscopy; the average values are presented in Table II. Nine samples, that is, empty microspheres crosslinked with 5, 7.5, and 10 mL of GA (formulations PI–GA5 to PIII–GA10), were also analyzed for the particle size, and these were found to be in the size range of 385–354 μm. With an increase in crosslinking, the particle size decreased because of the formation of a more rigid polymeric network. Also, an increase in

TABLE II
Encapsulation Efficiency, Particle Size, and Equilibrium Water Uptake Values For Drug-Loaded Formulations

Formulation code	% Encapsulation efficiency	Average drug particle size (μm)	Average swollen particle size (μm)	% Equilibrium water uptake
Polymer I				
PI–GA5–NFD10	60.83	452	500	226.3
PI–GA5–NFD20	70.20	382	409	201.4
PI–GA5–NFD40	72.56	332	360	195.4
PI–GA7.5–NFD10	61.43	458	471	199.5
PI–GA7.5–NFD20	70.79	388	410	186.5
PI–GA7.5–NFD40	73.28	341	369	164.6
PI–GA10–NFD10	64.59	463	480	200.3
PI–GA10–NFD20	69.81	391	425	186.3
PI–GA10–NFD40	72.17	344	374	160.9
Polymer II				
PII–GA5–NFD10	67.67	438	461	230.0
PII–GA5–NFD20	73.26	360	394	206.7
PII–GA5–NFD40	74.17	329	341	190.8
PII–GA7.5–NFD10	68.03	444	464	220.7
PII–GA7.5–NFD20	71.27	371	398	204.9
PII–GA7.5–NFD40	74.38	336	350	183.2
PII–GA10–NFD10	68.97	454	480	210.8
PII–GA10–NFD20	72.09	379	425	193.7
PII–GA10–NFD40	72.43	341	374	180.3
Polymer III				
PIII–GA5–NFD10	66.78	418	440	232.3
PIII–GA5–NFD20	69.21	354	376	218.1
PIII–GA5–NFD40	72.43	323	341	205.7
PIII–GA7.5–NFD10	67.97	434	448	220.9
PIII–GA7.5–NFD20	71.64	366	372	208.0
PIII–GA7.5–NFD40	73.21	329	332	200.0
PIII–GA10–NFD10	71.78	443	452	230.1
PIII–GA10–NFD20	70.88	370	381	215.8
PIII–GA10–NFD40	72.34	334	355	198.3

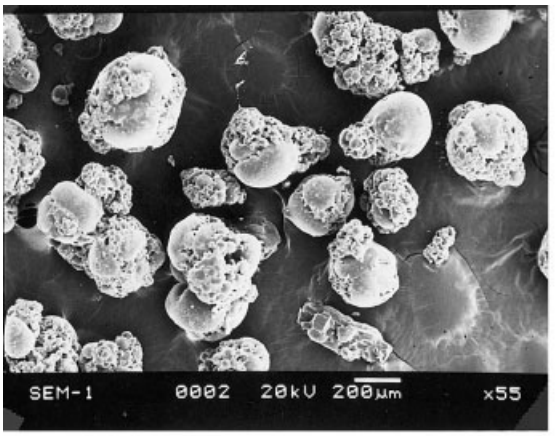
the drug loading percentage increased the size of the microspheres, which may be due to the filling of the free volume of the microspheres (see Table II).

The grafting ratio played an important role in the particle size. For example, formulation PI–GA5–NFD10 had an average particle size of 452 μm, whereas microspheres prepared with higher grafting ratios (PII–GA5–NFD10 and PII–GA10–NFD40) had particle sizes ranging between 438 and 341 μm for the same extent of crosslinking (5 mL) and the same drug loading (10%). However, the swollen microspheres exhibited a systematic trend, as shown in Table II. The larger the initial particle size was, the larger its swollen diameter was, and this may be due to the availability of a larger free volume inside the microspheres. For example, PI–GA5–NFD10 had an average swollen diameter of 500 μm, whereas for formulations PII–GA5–NFD10 and PII–GA10–NFD40, the particle sizes were 461 and 440 μm, respectively.

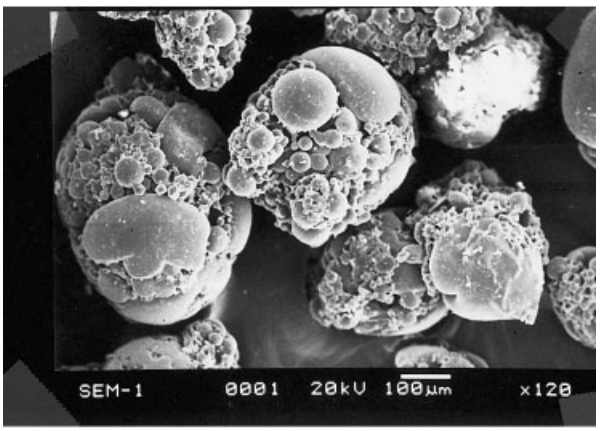
In all cases, the particles were almost spherical in nature and aggregated with rough surfaces, as evidenced by SEM photographs shown in Figures 2 and 3. Visibly porous structures with the presence of no drug crystals were present on the surfaces of the microspheres. Regular optical microscopy and SEM at lower magnifications failed to detect the presence of small intact microspheres, which could be seen by SEM at higher magnifications [see Fig. 3(B)].

Drug loading

Drug loading was performed during the crosslinking of the microspheres. The results for the encapsulation efficiency, presented in Table II, varied between 60 and 73%. These values were much better than our earlier results for indomethacin-loaded formulations.[9] However, lower encapsulation efficiencies in some cases were probably due to the loss of NFD in liquid paraffin during crosslinking in the presence of polysorbate-80. The encapsulation of NFD showed a systematic trend with the grafting ratio of the polymer, the extent of crosslinking and drug loading percentage. The encapsulation efficiency increased with increasing crosslinking and also drug loading. Also, with an increasing grafting ratio, the entrapment efficiency increased. In all cases, a higher entrapment efficiency may be attributed to the increased crosslink densities and poor solubility of NFD during encapsulation.



(a)



(b)

Figure 2 SEM photographs of (A) a group of microspheres at a lower magnification and (B) the surface morphology of the same group of microspheres at a higher magnification.

Swelling studies

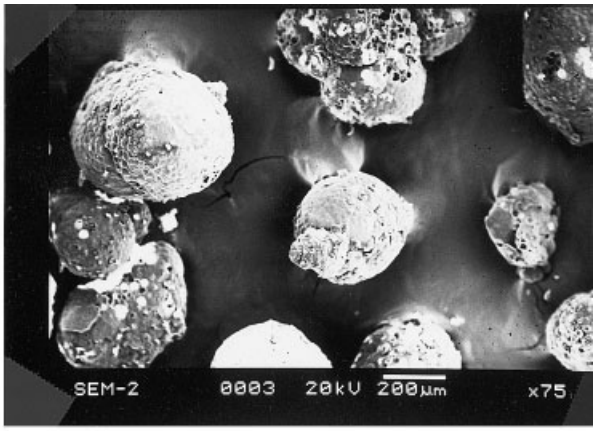
The dynamic swelling of PAAm-*g*-chitosan microspheres was determined from measured changes in the particle diameter, D_t, as a function of time with an optical microscope. The dynamic swelling results calculated by the ratio D_t/D_0 (where D_0 is initial diameter of the microsphere in the unswollen state) are plotted as a function of time in Figure 4 for three types of microspheres prepared from polymers I, II, and III with different amounts of GA as the crosslinking agent. Those microspheres crosslinked with larger amounts of GA showed the least swelling (and vice versa).

Polymer swelling has an effect on the molecular transport of liquids within polymeric matrices. A loose polymer network absorbs more liquid with higher swelling (and vice versa). Equilibrium swelling studies were performed in triplicate, but the average values are presented in Table II. The equilibrium water uptake percentage showed a systematic dependency; that is, it decreased with increasing crosslinking and increasing drug loading for those formulations with a particular grafting ratio. However, increasing the drug loading caused a decrease in the water uptake, probably because of the availability of a smaller free volume. Also, the water uptake increased with an increasing grafting ratio.

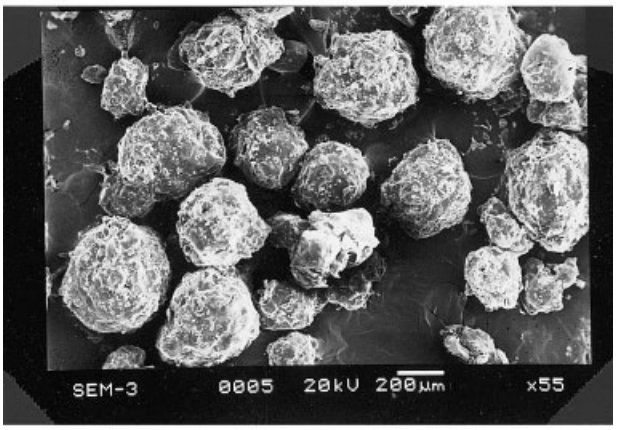
Because drug release from the swollen polymers (i.e., for empty formulations PI–GA5 to PIII–G10) was mainly controlled by swelling or polymer chain relaxation, the dynamic swelling results were analyzed with an empirical equation:[10]

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

where D_t is the change in the microsphere diameter at time t and D_∞ is the equilibrium diameter, that is, of the fully swollen microsphere. The exponent value of n indicates the type of transport. A least-squares method was used to calculate the values of n and k at the 95% confidence limit, and these data are presented



(a)



(b)

Figure 3 SEM photographs of (A) NFD-loaded microspheres crosslinked with 7.5 mL of GA and (B) the same microspheres crosslinked with 10 mL of GA.

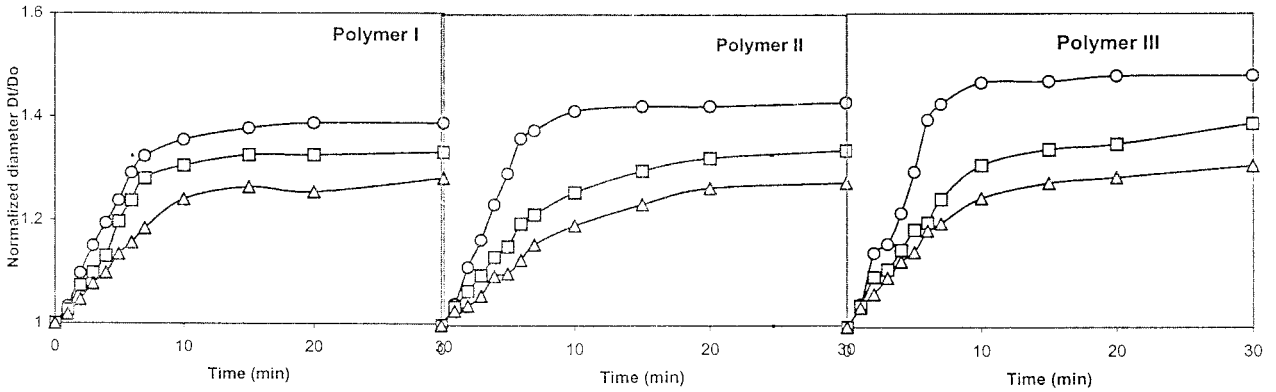


Figure 4 Dynamic swelling [i.e., normalized diameter (D_t/D_0)] of microspheres of polymers I–III as a function of time with (○) 5, (□) 7.5, and (△) 10 mL of GA.

in Table III. The values of n varied between 0.65 and 0.85, indicating that the transport was anomalous. The values of k systematically decreased with an increasing amount of GA in the matrix. The equilibrium swollen particle diameter, D_∞, normalized to the original diameter, D_0, decreased significantly (Table III), and this indicated rigidity of the crosslinked network because it did not expand in water in comparison with the loosely crosslinked matrix for all three grafting ratios.

In vitro release

To understand the release patterns of NFD from all 27 formulations prepared, we performed *in vitro* release studies in a pH 7.4 phosphate buffer containing 0.05 (w/v) polysorbate-80 at 37°C. These experiments were performed in triplicate, but the average values were used in the graphical presentations and in the treatment of the data. The standard deviations were less than 5% and are not displayed in Figures 5–7. The release profiles of formulations loaded with 10, 20, and 40% NFD as well as those matrices prepared with three levels of crosslinking for each type of polymer are presented. 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 drug diffusion from microspheres depends on the mesh size of the polymer network, which generally decreases with increasing crosslinking.

Crosslinking might also induce an increase in the polymer crystallinity and, therefore, the rigidity of the polymer chain; this was also observed from DSC studies and from equilibrium water uptake data. For instance, the PI–GA5–NFD10 formulation released 95%, whereas for the PI–GA5–NFD20 and PI–GA5–NFD40 formulations, the release was 89 and 79%, respectively, at 620 min. Such a decrease in the release rates followed a trend with the crosslink density of the matrix. Drug release decreased with an increase in the drug loading, and this was due to the formation of drug crystals within the microspheres; this was further supported by DSC data. For instance, PI–GA5–NFD10 released 95% at 620 min for a 10% drug loading, whereas the PI–GA7.5–NFD10 and PI–GA10–NFD40 formulations released 86 and 84%, respectively, for the same extent of crosslinking (i.e., 5 mL of GA) at 620 min for 20 and 40% drug loadings. This indicated the formation of an increased number of drug crystals inside the microspheres.

The extent of the grafting ratio played an important role in the release of NFD from the microspheres. For instance, an increase in grafting also increased the release rates. PI–GA5–NFD10 released 95%, whereas PII–GA5–NFD10 and PII–GA10–NFD40 formulations prepared from polymers II and polymer III, respectively, released 97 and 100% at 620 min for a 10% drug loading for the same amount of the crosslinking agent (i.e., 5 mL of GA). In all the formulations, regardless of the extent of grafting, release rates decreased with increasing crosslinking as well as increasing drug loading.

TABLE III
Transport Properties of Blank Microspheres Prepared from Polymer I, Polymer II, and Polymer III at Cross linking Extents of 5, 7.5, and 10% of GA for Each Polymer

Formulation	Equilibrium normalized diameter (D_∞/D_0)	n	$k \times 10^3$ (min^{-n})	Correlation coefficient
PI–GA5	1.39	0.69	1.58	0.905
PI–GA7.5	1.33	0.74	1.34	0.920
PI–GA10	1.28	0.83	0.97	0.944
PII–GA5	1.43	0.65	1.76	0.893
PII–GA7.5	1.34	0.74	1.17	0.975
PII–GA10	1.29	0.85	0.74	0.983
PIII–GA5	1.48	0.67	1.66	0.902
PIII–GA7.5	1.39	0.68	1.33	0.971
PIII–GA10	1.31	0.70	1.19	0.982

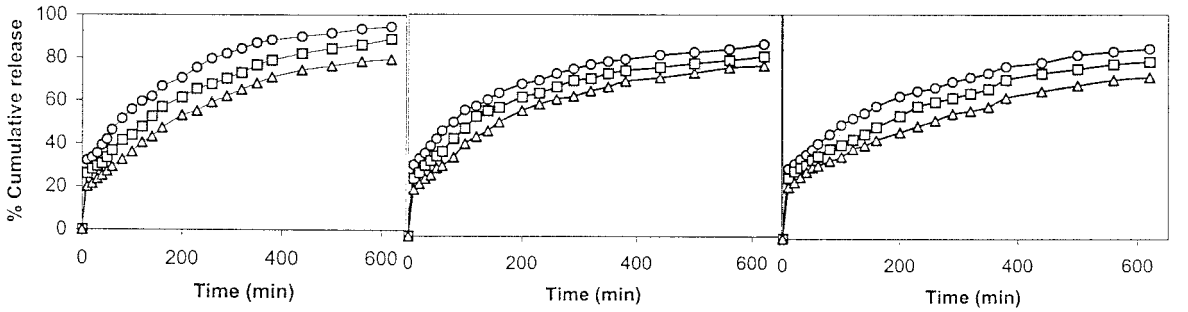


Figure 5 Cumulative release of NFD (%) from matrices prepared from polymer I for 10, 20, and 40% loadings from microspheres prepared by crosslinking with (○) 5, (□) 7.5, and (△) 10 mL of GA, respectively (i.e., for PI–GA5–NFD10 to PI–GA10–NFD40).

The *in vitro* release data were analyzed with a modified exponential relation, which relates the amount of drug released from microspheres as a function of time:[13]

$$\left(\frac{M_t}{M_\infty}\right) = \alpha + kt^n \tag{7}$$

where M_t is the amount of drug released at time t and M_∞ is the amount of the drug released at the equilibrium time; k is a constant characteristic of the drug–polymer system, and n is a diffusional exponent, which suggests that the nature of drug release depends on its value. If n is 0.5, polymer relaxation does not affect molecular transport, and diffusion follows Fickian. However, polymer chain relaxation becomes the rate-controlling factor for case II transport if n is 1. The intermediary values of n, ranging between 0.5 and 1.0, are indicative of anomalous transport.[14,15] In a majority of the formulations presented in Figures 5–7, within about 10–15 min, nearly 25–35% NFD was released, and this suggested that release patterns were somewhat difficult to predict.

To accurately predict the release mechanism and to account for the initial burst release, we introduced the parameter α in eq. (7). Least-squares estimations of the fractional release data along with the estimated correlation coefficient values, are presented in Table IV. From these data, we find that the values of n varied around 1 with a correlation coefficient of 0.99; this indicated that drug release followed case II transport. However, the time dependence of the NFD release followed zero-order in all the formulations; this effect was observed earlier also.[2,5] Even though the water uptake of the microspheres reached equilibrium very quickly (i.e., within 15–20 min), release was continued for several hours, and this indicated that polymer chain relaxation was less influenced by drug release, and so the release was governed by its molecular transport phenomenon.

The release data of NFD presented in Figures 5–7 indicated that the release became quite slow after 2 h, probably because of the lower solubility of NFD in water. To calculate the values of the apparent diffusion coefficients, D, of NFD from hydrogel microspheres, we analyzed the initial and later time portions of the release profiles (i.e., $0 < M_t/M_\infty < 0.4$ and $0.6 < M_t/M_\infty < 1.0$) with Fick's theory[16] in its most simplified form to calculate diffusion coefficients from the initial time and later time approximation:[17]

$$\frac{M_t}{M_\infty} = \left(\frac{36D_1t}{\pi r^2}\right)^{1/2} - \left(\frac{3D_1t}{r^2}\right) \tag{8}$$

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\exp\left[-\left(\frac{\pi^2 D_2 t}{r^2}\right)\right] \tag{9}$$

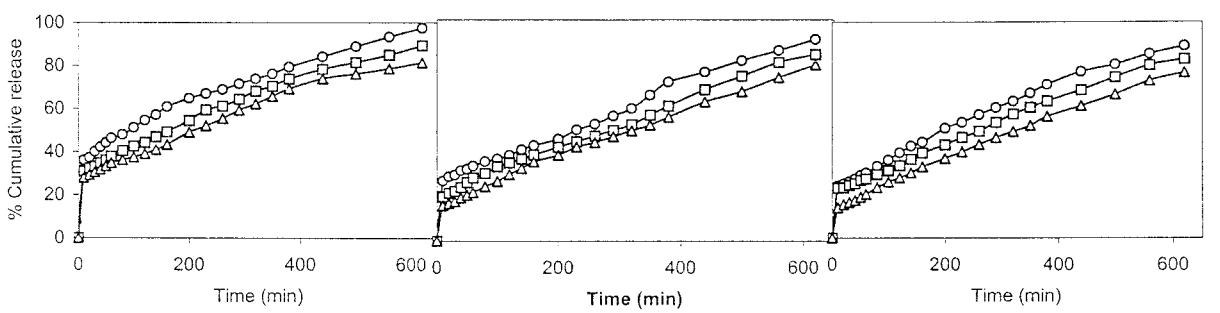


Figure 6 Cumulative release of NFD (%) from matrices prepared from polymer II for 10, 20, and 40% loadings from microspheres prepared by crosslinking with (○) 5, (□) 7.5, and (△) 10 mL of GA, respectively (i.e., for PII–GA5–NFD10 to PII–GA10–NFD40).

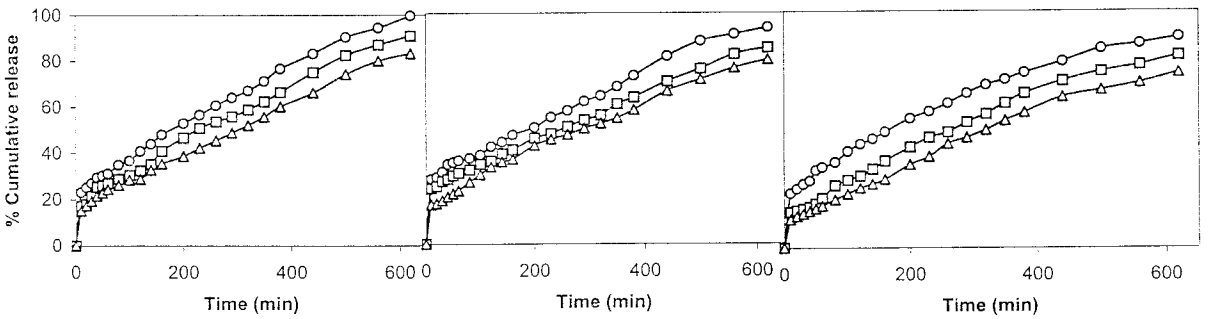


Figure 7 Cumulative release of NFD (%) from matrices prepared from polymer III for 10, 20, and 40% loadings from microspheres prepared by crosslinking with (○) 5, (□) 7.5, and (△) 10 mL of GA, respectively (i.e., for PIII–GA5–NFD10 to PIII–GA10–NFD40).

r is the average radius of the dry microspheres for eq. (8), whereas for eq. (9), r is the average diffusional distance, which corresponds to the radius of the fully swollen microsphere.

The data reported in Table IV show the relationship between the extent of crosslinking and drug loading. For instance, if D_1 was a diffusion coefficient calculated by the initial time approximation and D_2 was a diffusion coefficient calculated by the later time approximation, it decreased with an increasing amount of crosslinking. For instance, for PI–GA5–NFD10 to PI-GA10NFD10, D_1 decreased from 8.98×10^{-6} to 4.86×10^{-6} cm^2/s; similarly, at a later time, D_2 decreased from 8.98×10^{-7} to 3.21×10^{-7} cm^2/s. This was in concurrence with the increased crosslinking of microspheres. The apparent diffusion coefficients were low in both cases; such lower values were attributed to the poor solubility of NFD in water. Even though the

TABLE IV
Analysis of Drug Release from Microspheres Prepared from Different Polymers

Formulation	n	$k \times 10^3$ (min^{-n})	Correlation coefficient	Diffusion coefficient	
				Initial time $D_1 \times 10^6$ (cm^2/s)	Later time $D_2 \times 10^7$ (cm^2/s)
Polymer I					
PI–GA5–NFD10	1.06	1.88	0.993	8.98	8.98
PI–GA5–NFD20	1.00	2.01	0.998	6.35	5.84
PI–GA5–NFD40	0.99	1.75	0.997	4.92	3.72
PI–GA7.5–NFD10	0.92	3.43	0.988	9.31	5.30
PI–GA7.5–NFD20	0.99	2.35	0.995	6.66	3.58
PI–GA7.5–NFD40	0.97	2.26	0.993	5.03	3.21
PI–GA10–NFD10	1.13	1.12	0.952	4.86	6.04
PI–GA10–NFD20	0.97	1.61	0.995	6.66	4.68
PI–GA10–NFD40	0.90	3.16	0.993	6.12	6.36
Polymer II					
PII–GA5–NFD10	0.90	2.55	0.997	5.48	7.72
PII–GA5–NFD20	0.92	1.92	0.998	3.57	5.65
PII–GA5–NFD40	0.94	1.43	0.994	2.68	3.47
PII–GA7.5–NFD10	0.95	1.29	0.998	4.96	9.90
PII–GA7.5–NFD20	0.98	1.12	0.995	3.74	7.10
PII–GA7.5–NFD40	1.04	0.90	0.999	4.55	6.01
PII–GA10–NFD10	1.04	1.10	0.998	6.95	10.70
PII–GA10–NFD20	0.87	3.25	0.969	7.21	5.78
PII–GA10–NFD40	1.06	0.80	0.996	4.32	6.78
Polymer III					
PIII–GA5–NFD10	0.97	1.49	0.985	4.85	16.06
PIII–GA5–NFD20	0.87	2.76	0.989	4.25	10.34
PIII–GA5–NFD40	0.91	1.84	0.999	3.56	7.30
PIII–GA7.5–NFD10	0.98	1.12	0.995	5.05	18.73
PIII–GA7.5–NFD20	0.98	1.10	0.997	3.76	7.70
PIII–GA7.5–NFD40	0.96	1.48	0.991	4.90	5.25
PIII–GA10–NFD10	0.96	1.98	0.988	8.26	9.37
PIII–GA10–NFD20	1.03	1.18	0.994	4.39	6.87
PIII–GA10–NFD40	0.92	1.76	0.999	3.88	3.67

results of D_2 were somewhat lower than those of D_1, they were still comparable. Values of D_1 higher than those of D_2 may exist because the initial release of NFD was mainly influenced by polymer chain relaxation giving a quick release, but at a later time, the release of NFD was mainly controlled by diffusion, which was rather slow. As explained before, the diffusion coefficients decreased with higher drug loadings in all the formulations.

CONCLUSIONS

The grafting of AAm onto chitosan was carried out by the selection of three AAm concentrations. Microspheres of PAAm-*g*-chitosan crosslinked with GA were prepared and used to encapsulate NFD. The modification of chitosan by variations in the grafting ratio, extent of the crosslinking agent, and amount of drug loading was possible for optimizing the formulation conditions. The microspheres showed rough surfaces with a mean particle size of 450 μm. Dynamic swelling experiments on individual microspheres suggested that with an increase in crosslinking, drug transport shifted from non-Fickian to case II.

The release of NFD depended on the extent of crosslinking and the amount of drug loading. In all the formulations, drug release was delayed for more than 10 h. The release of NFD from the microspheres followed zero-order. The microspheres prepared in this study may be useful biomaterials that can be used to prepare once-a-day dosage forms for NFD-like drugs. More research work on this problem is being continued in our laboratory.

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References

1. Peppas, N. A.; Khare, A. R. Adv Drug Delivery Rev 1993, 11, 1.
2. Cory, B.; Martin, K.; Amanda, C.; Kyekyoon, K.; Daniel, W. P. J Controlled Release 2002, 82, 137.
3. Cadée, J. A.; de Groot, C. J.; Jiskoot, W.; den Otter, W.; Hennink, W. E. J Controlled Release 2002, 78, 1.
4. Ryoichi, M.; Honda, R.; Takahashi, Y. J Controlled Release 2000, 68, 115.
5. Pillay, V.; Fassihi, R. J Controlled Release 2000, 67, 67.
6. Pitt, L. Biodegradable Polymers; Marcel Dekker: New York, 1990; p 97.
7. Hamdi, G.; Ponchel, G.; Dunchene, D. J Controlled Release 1998, 55, 193.
8. Roberts, G. A. F. Chitin Chemistry; Houndmills: McMillan, United Kingdom, 1992.
9. Kumbar, S. G.; Soppimath, K. S.; Aminabhavi, T. M. J Appl Polym Sci, 2003, 82, 1525.
10. Robert, C. C. R.; Buri, P. A.; Peppas, N. A. J Appl Polym Sci 1985, 30, 301.
11. Najjar, A. M. K.; Yunus, W. M. Z. W.; Ahmad, M. B.; Rahaman, M. Z. A. B. J Appl Polym Sci 2000, 77, 2314.
12. Kumbar, S. G.; Kulkarni, A. R.; Aminabhavi, T. M. J Microencapsulation 2002, 19, 173.
13. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 37.
14. Aminabhavi, T. M.; Naik, H. G. J Hazard Mater 1998, 60, 175.
15. Aminabhavi, T. M.; Naik, H. G. Polym Compos 1998, 6, 205.
16. Crank, J. The Mathematics of Diffusion, 2nd ed.; Clarendon: Oxford, 1974.
17. Baker, R. W.; Lonsdale, H. K. In Controlled Release of Biologically Active Agents; Tanquarry, A. C.; Lacey, R. E., Eds.; Plenum: New York, 1974; pp 15.