

Novel Methyl Cellulose-Grafted-Acrylamide/Gelatin Microspheres for Controlled Release of Nifedipine

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ABSTRACT: Naturally available carbohydrate polymers such as methylcellulose (MC) and gelatin (Ge) have been widely studied in the previous literature for controlled release (CR) applications. In this study, methyl cellulose-g-acrylamide/gelatin (MC-g-AAm/Ge) microspheres were prepared by water-in-oil (W/O) emulsion method and crosslinked with glutaraldehyde to encapsulate with nifedipine (NFD), an antihypertensive drug. The microspheres prepared were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and laser particle size analyzer. DSC thermograms of NFD-loaded AA-mc/Gel microspheres confirmed the molecular level distribution of NFD in the matrix. SEM indicated the formation of spherical particles.

Swelling experiments supported the drug diffusion characteristics and release data of the matrices. Cumulative release data were analyzed using an empirical equation to understand the nature of transport of drug through the matrices. Controlled release characteristics of the matrices for NFD were investigated in pH 7.4 media. Drug was released in a controlled manner up to 12 h. Particle size and size distribution of the microspheres as studied by laser light diffraction particle size analyzer indicated their sizes to be around 120 μm . © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 115: 3542–3549, 2010

Key words: carbohydrate polymers; methyl cellulose; gelatin; nifedipine; controlled release

INTRODUCTION

Polysaccharides, a class of naturally available carbohydrate polymers, have been widely used in food industry as gelling agents and for encapsulation of living cells as well as drugs.^{1–3} In recent years, such naturally available carbohydrate polymers are being widely used in controlled release (CR) of a wide variety of bioactive molecules.^{4–7} Such natural polymers are biocompatible and biodegradable and can be easily depleted from the body system after their consumption. Some synthetic polymers, i.e., polyacrylamide (PAAm) are also biocompatible. Combination of such synthetic polymers with natural polymers would enhance the overall properties of the matrix. One of the ways to increase the properties of natural^{8–10} and synthetic^{11,12} polymers to derive new properties is by graft copolymerization, i.e., by grafting vinyl monomers onto natural polymers, such as cellulose or its derivatives.

Methylcellulose (MC) is soluble in water and hence, it demonstrates a unique property of forming reversi-

ble physical gels because of hydrophobic interactions when heated above a particular temperature.¹³ MC is used as a binder or thickener in pharmaceutical, food, and ceramic processing applications. It is known to undergo thermo-reversible gelation in aqueous solution upon heating^{14–17} and is hydrophilic in nature. However, cellulose fibers contain crystalline ordered regions formed by intra and intermolecular hydrogen bonds; consequently, cellulose does not dissolve in water, but the crystalline fraction depends on its source. When methoxyl groups are substituted with a certain number of hydroxyl groups, some hydrogen bonds are broken such that MC becomes more water soluble. Being a polyhydroxy polymer, MC can be also chemically crosslinked with a dialdehyde in the presence of a strong acid to generate a hydrogel.^{18–20}

Gelatin, another carbohydrate polymer, is derived from collagen, a natural protein, which is a fibrous material that occurs in skin, bones, and connective tissues of animals. It is insoluble in water, but it can be solubilized by hydrolysis. The raw materials used for its manufacturing process are obtained from bovine bones or porcine skins. The reaction can be carried out at an acid pH level, yielding Type A gelatin (which is primarily produced from skins) and at the basic pH level giving Type B gelatin (primarily produced from bovine bones). Gelatin is a heterogeneous product that is a mixture of molecular species, such as α -, β -, and γ -peptides. The proportions and molecular weights are dependent on the nature of

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the chemical process. Gelatin is biocompatible, biodegradable, edible, and soluble at body temperature but undergoes gelation process at temperatures just above ambient,²¹ which makes it an ideal material in pharmaceutical applications.

Nifedipine (NFD) is a prototype 1,4-dihydropyridine calcium channel blocker. By allosteric interference with the gating mechanism of L-type voltage-activated calcium channels in smooth muscle, the drug prevents the influx of extracellular calcium required in activating the contractile machinery of the cell.^{22,23} NFD exerts its clinical effects due to vasodilation of arterial smooth muscle, leading to reduced peripheral resistance and improved coronary flow, but it has little effect on the cardiac tissue. NFD is indicated for the prophylaxis of angina pectoris and in peripheral circulatory disorders such as Raynaud's syndrome.²²

Our earlier research²⁴⁻²⁷ addresses the utilization of different types of carbohydrate polymers in developing into microspheres for the controlled release (CR) of a variety of drugs, including NFD. In continuation of these efforts, the present article addresses the development of novel type of grafted polymers of acrylamide with methylcellulose and gelatin by varying the blend ratio. The release of NFD from these microspheres was studied by varying the blend ratio, NFD content, and amount of crosslinking agent. The microspheres formed were characterized by scanning electron microscope (SEM) and differential scanning calorimetry (DSC) to investigate the shape and dispersion of drug particles in the blend microspheres. Swelling experiments were performed on the blend microspheres to evaluate the diffusion properties of NFD through the microspheres.

EXPERIMENTAL

Materials and methods

Methylcellulose, acrylamide, gelatin, light paraffin oil, and glutaraldehyde (25% aqueous solution) (GA) were purchased from s.d. fine Chemicals, Mumbai, India. Tween-80 was purchased from Sigma Chemical Co., St. Louis, MO. NFD was purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India.

Preparation of methyl cellulose-g-acrylamide/gelatin blend microspheres

A 5 g of MC was weighed and dissolved in water overnight under constant stirring. To this solution, different amounts of acrylamide and potassium persulfate were added and stirred well. This reaction mixture was polymerized under nitrogen atmosphere for 6 h at 70°C. This polymerized product was cooled and the polymer was extracted by precipitat-

ing it in acetone. The precipitated polymer was dried under vacuum for 24 h in an oven at 40°C.

A different weight ratio (10 : 90, 20 : 80, and 30 : 70) of gelatin and methyl cellulose-g-acrylamide was dissolved in water for an overnight. The two polymer solutions were stirred well for proper mixing, which lead to a miscible the polymer solution. Different amount of NFD (5, 10, and 15 %) was dissolved in 1 mL of methanol, which was then added to the blend polymer solution. The drug-loaded blend polymer solution was emulsified into liquid paraffin to form water-in-oil (w/o) emulsion at a 400-rpm speed using 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 0.1M HCl and different amounts of GA (i.e., 2.5, 5, and 7.5 mL). The microspheres formed were filtered, washed repeatedly with hexane and water to remove oil as well as excess amount of surfactant as well as the unreacted GA. These microspheres were dried under vacuum at 40°C and stored in a desiccator before further analysis.

Differential scanning calorimetry studies

DSC curves of the plain NFD, MC-g-AAm/G microspheres, and NFD-loaded MC-AAm/G microspheres were recorded using Rheometric Scientific differential scanning calorimeter (Model-DSC SP, UK). The analysis was performed by heating the samples at the rate of 10°C/min under inert atmosphere.

Scanning electron microscopic studies

SEM images of the microspheres were recorded using a Hitachi S520 scanning electron microscope (Japan) 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 as a detector.

Particle size analysis

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

Estimation of drug loading and encapsulation efficiency

Specific amount of dry microspheres were vigorously stirred in a beaker containing 10 mL of

TABLE I
Results of % Encapsulation Efficiency, Mean Particle Size and % Water Uptake of Different Formulations

Formulation codes	Ratio Ge : MC in microspheres	Amount of acrylamide added	% NFD loaded	Amount of GA added (mL)	% Encapsulation efficiency \pm SD	Mean particle size (μm) \pm SD	% Water uptake
AAM-g-MC/Ge-1	10 : 90	10	5	2.5	68.2 \pm 0.8	168 \pm 5	495
AAM-g-MC/Ge2	10 : 90	10	5	5	66.4 \pm 1.1	156 \pm 6	458
AAM-g-MC/Ge3	10 : 90	10	5	7.5	61.5 \pm 0.9	112 \pm 8	436
AAM-g-MC/Ge4	20 : 80	10	5	7.5	72.6 \pm 0.8	160 \pm 7	395
AAM-g-MC/Ge5	30 : 70	10	5	7.5	79.8 \pm 1.2	145 \pm 9	342
AAM-g-MC/Ge6	10 : 90	10	10	7.5	68.5 \pm 1.1	198 \pm 5	464
AAM-g-MC/Ge7	10 : 90	10	15	7.5	70.9 \pm 1.5	205 \pm 6	486
AAM-g-MC/Ge8	00 : 100	10	5	7.5	61.8 \pm 0.6	118 \pm 5	518
AAM-g-MC/Ge9	10 : 90	20	5	7.5	58.2 \pm 0.4	135 \pm 4	495
AAM-g-MC/Ge10	10 : 90	30	5	7.5	49.5 \pm 0.6	158 \pm 9	512

SD, standard deviation.

dichloromethane to extract drug from the microspheres. A 10 mL of 7.4 pH phosphate buffer containing 0.02% Tween-80 was added to the above solution to make the drug soluble. Dichloromethane was evaporated with gentle heating and continuous shaking. The aqueous solution was filtered and assayed using UV spectrophotometer (model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 238 nm. The results of % NFD loading and encapsulation efficiency were calculated using eqs. (1) and (2). These data are compiled in Table I.

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

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

Swelling studies

Dynamic swelling of MC-g-AAM/Ge microspheres prepared with three different crosslink densities and three different drug loadings were subjected to weight uptake measurements in water as a function of time. Swelling experiments performed in 7.4 pH buffer solutions produced no significant changes, and hence, we studied the swelling of microspheres in water.²⁸ To perform swelling experiments, microspheres were soaked in water; several of them were removed from the bottles at different time intervals and blotted carefully (without pressing hard) to remove the surface-adhered water droplets. Microspheres were then weighed (w_1) on an electronic microbalance (Mettler, AT 120, Switzerland) accurate to ± 0.00001 g. The microspheres were then 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 average values were used in data analysis. Standard deviations (SD) in all cases were < 5%. The weight % water uptake was calculated as follows:

$$\% \text{ Water uptake} = \left(\frac{\text{Weight of swollen MGs } (w_1) - \text{Weight of dry MGs } (w_2)}{\text{Weight of dry MGs } (w_3)} \right) \times 100 \quad (3)$$

where MGs represents microspheres.

In vitro release

In vitro release studies have been carried out by dissolution experiments using the tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37°C under 100 rpm speed. Drug release from the microspheres was studied in an intestinal (7.4 pH phosphate buffer) fluid. At regular intervals of time, sample aliquots were withdrawn and analyzed by a

UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 238 nm.

RESULTS AND DISCUSSION

Differential scanning calorimetry

DSC thermograms of pure NFD, NFD-loaded MC-g-AAM/Ge microspheres, and plain MC-g-AAM/Ge microspheres are displayed in Figure 1. Nipeditine shows a sharp peak at 177°C due to polymorphism and melting, but in case of NFD-loaded

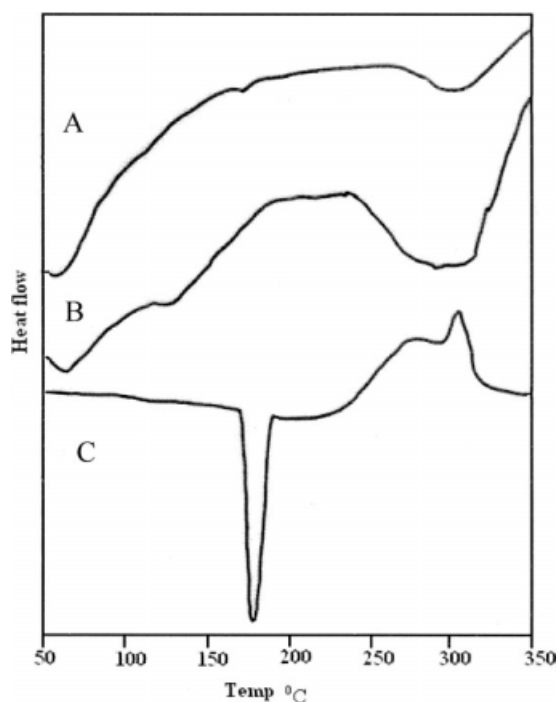


Figure 1 DSC thermograms of (a) pristine MC-g-AAM/Ge microspheres, (b) NFD-loaded MC-g-AAM/Ge microspheres, and (c) pristine NFD.

microspheres, no characteristic peak was observed at 177°C, suggesting that NFD is molecularly dispersed in the matrix.

Scanning electron microscopy

SEM images of the single microspheres taken at 350x magnifications are shown in Figure 2. Microspheres are spherical without forming agglomeration and their surfaces are somewhat rough. However, polymeric debris seen around some particles could

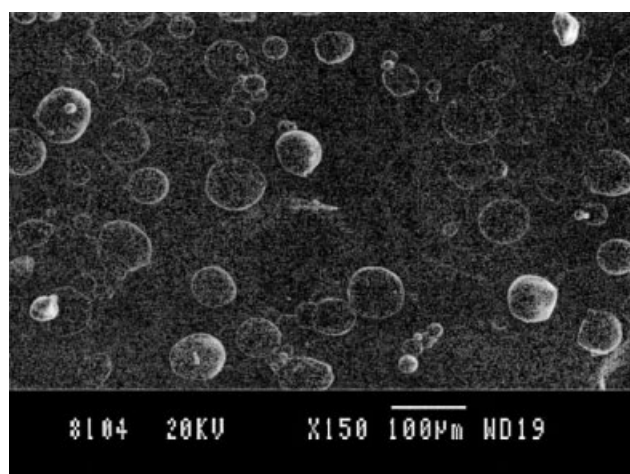


Figure 2 Scanning electron micrograph of MC-g-AAM/Ge microspheres.

be due to the method of particle production (i.e., simultaneous particle production and formation of blend matrix). Microspheres produced by blending different polymers did not show any effect on their surface properties.

Laser particle size analyzer

The results of mean particle size with standard errors are presented in Table I, whereas the size distribution curve for a typical formulation containing MC-g-AAM/Ge is displayed in Figure 3. It is obvious that size distribution is narrow and volume mean diameter of microspheres is around 120 µm. The particle size was small compared with our earlier literature²⁴ on blend microspheres of sodium alginate-methyl cellulose loaded with NFD.

Microscopic study

Particle size was also measured alternatively by optical microscopy. These results along with % encapsulation efficiency, % drug loading, and mean particle size for different formulations is presented in Table I. The size of particles depends on the amount of drug present, % gelatin content, and extent of GA employed. Particles are generally spherical in shape with sizes ranging from 112 to 205 µm. Particle size of the pristine MC is higher than those of the MC-g-AAM/Ge microspheres.

For all formulations, with increasing amount of drug in the microspheres, particle size also increased. For formulations containing 10% MC and microspheres loaded with different amounts of drug, particle size has increased from 112 to 205 µm; a similar trend was also observed for all other formulations (Table I). 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.²⁷ However, the extent of crosslinking has shown an effect on

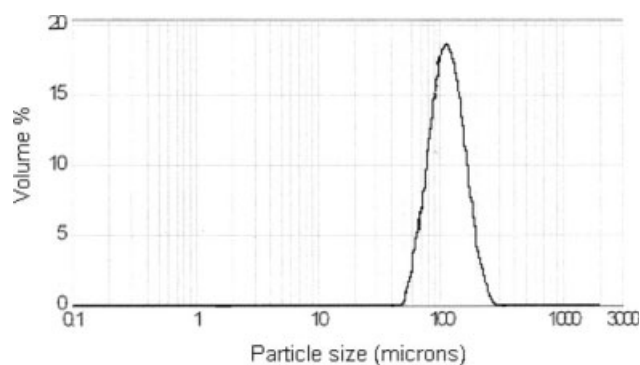


Figure 3 Particle size distribution curve for MC-g-AAM/Ge microspheres.

particle size (see data in Table I). For microspheres containing 10 wt % MC and 5 wt % NFD with increasing amount of GA from 2.5 to 7.5 mL, particle size decreased from 168 to 112 μm . This is attributed to the fact that with increasing amount of GA in the semi-interpenetrating polymer network (IPN) matrix, the shrinkage of particles has taken place, thereby reducing their sizes.^{27,28}

Encapsulation efficiency

Three different concentrations of NFD, i.e., 5, 10, and 15 wt % were loaded during crosslinking of the microspheres. Results of % encapsulation efficiency included in Table I show increasing trends with increasing drug loading. Encapsulation efficiency of 61.8% was observed for pristine MC microspheres, but for remaining formulations, it ranged from 66.4 to 70.9%. Such smaller values are due to less soluble drug in the polymer solution, thus making a small amount of NFD incorporated into microspheres. Notice that % encapsulation efficiency increased with increasing amount of gelatin in the polymer matrix. For microspheres containing 10, 20, and 30 wt % of gelatin and 5 wt % of NFD with 7.5 mL of GA, encapsulation efficiencies were 66.4, 72.6, and 79.8%, respectively. For microspheres crosslinked with 2.5, 5, and 7.5 mL of GA, encapsulation efficiencies, respectively, 68.2, 66.4, and 61.5%. Such a decreasing trend is due to the increase in crosslink density of the matrix, because microspheres would become rigid with a reduction in free volume spaces of the matrix giving lower encapsulation efficiency.

Swelling studies

Extent of crosslinking depends upon the amount of crosslinking agent used. In this study, different amounts of GA were added as the crosslinking agent to blend microspheres of MC-g-AAm/Ge containing 5 wt % NFD; these data are also included in Table I. Extent of crosslinking controls equilibrium swelling. For instance, % equilibrium swelling decreased from 495 to 436 with an increasing amount of GA from 2.5 to 7.5 mL, probably due to increased crosslink density and decreased pore volume of the blend matrix.²⁹ Alternatively, with increasing drug loading in the matrix, % equilibrium water uptake also increased from 436 to 486. However, the % swelling of microspheres decreased with increasing amount of gelatin in the polymer matrix. For instance % swelling containing 10, 20, and 30% of gelatin were 436, 395, and 342, respectively; this suggests that % water uptake also decreased with increasing gelatin content of the matrix, probably due to induced hydrophobicity of the matrix with increasing gelatin content of the matrix.

Drug release kinetics

Drug release kinetics was analyzed by plotting cumulative release data vs. time and by fitting these data to an exponential equation of the type³⁰:

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

Here, M_t/M_∞ represents the fractional drug release at time, t , k is a constant that is 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 formulations, and these data are given in Table II. If $n = 0.5$, drug diffuses and releases from the polymer matrix after a Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian type drug diffusion occurs. If $n = 1$, completely non-Fickian or Case II release kinetics is operative. Intermediary values ranging between 0.5 and 1.0 have been attributed to anomalous type transport.^{30,31}

In this study, the values of k and n showed a clear-cut dependence on extent of crosslinking, % drug loading, and AAm content of the matrix. Values of n for microspheres prepared by varying the amount of gelatin (10, 20, and 30 %) by keeping NFD (5%) and GA (7.5 mL GA) constant, ranged from 0.278 to 0.727, leading to a shift of transport from Fickian to anomalous type. The NFD-loaded particles have shown the n values ranging from 0.278 to 0.513 (Table II), indicating the shift from erosion type release to a swelling controlled, non-Fickian mechanism. This could possibly be due to a reduction in the regions of low microviscosity and closure of microcavities in the swollen state. Similar findings have been observed elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was studied. On the other hand, values of k are quite smaller for drug-loaded microspheres, suggesting their lesser interactions with the surrounding media compared with microspheres containing varying amounts of gelatin.

TABLE II
Release Kinetics Parameters of Different Formulations

Formulation code	k	n	Correlation coefficient, r
AAm-g-MC/Ge1	0.024	0.51	0.9769
AAm-g-MC/Ge2	0.010	0.73	0.961
AAm-g-MC/Ge3	0.032	0.59	0.959
AAm-g-MC/Ge4	0.055	0.46	0.963
AAm-g-MC/Ge5	0.025	0.51	0.968
AAm-g-MC/Ge6	0.111	0.32	0.971
AAm-g-MC/Ge7	0.162	0.28	0.957
AAm-g-MC/Ge8	0.065	0.42	0.974
AAm-g-MC/Ge9	0.012	0.57	0.995
AAm-g-MC/Ge10	0.027	0.51	0.983

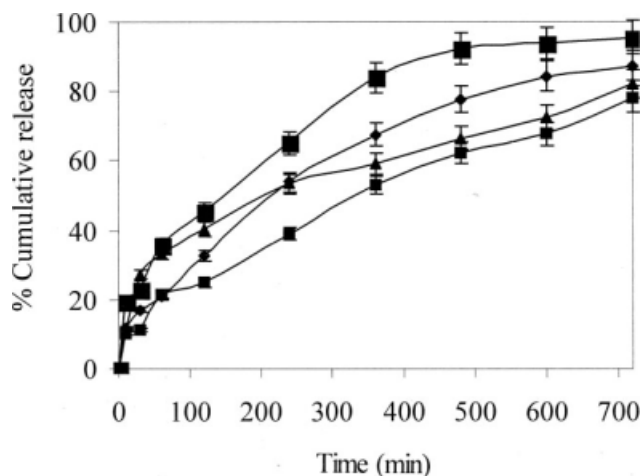


Figure 4 % Cumulative release of NFD through AAm-g-MC/Ge microspheres containing different amount of MC. Symbols: (■) Pure MC, (◆) 10 wt % Ge, (●) 20 wt % Ge, and (▲) 30 wt % of Ge.

Effect of gelatin content

Figure 4 shows the effect of gelatin content with a constant loading of 5 wt % NFD. It is found that pure MC produced almost 100% cumulative drug release in about 10 h, whereas MC-g-AAm/Ge microspheres produced up to 90% cumulative release in 12 h. This could be due to the fact that during the dissolution process, microspheres are systematically swollen at decreasing amounts of gelatin because of the formation of loosely crosslinked network of MC. When the amount of gelatin increases, the cumulative release has decreased due to lesser swelling of gelatin than MC. This could be due to increased hydrophobicity of the matrix with increasing Ge content of the blend matrix; hydrophobicity effect of the blend is due to the presence of NH_2 groups in gelatin with the lesser number of residual —OH groups. Thus, polymer matrix responds in response to stresses induced by the surrounding solvent media during the process of dissolution, resulting in a decrease of dimension (radius of gyration) of the polymer; this would further result in a decrease of molecular volume of the hydrated polymer due to decreased swelling of gelatin component of the matrix, thereby reducing the free volume space in the overall matrix. Notice that the nature of release profiles remains almost identical in all the formulations containing different amounts of GA; however, swelling of gelatin segments in the matrix vary in a linear fashion with the drug release profiles.

Effect of acrylamide content

Figure 5 shows the *in vitro* release data of NFD from different microspheres performed with different

ratios of AAm. These data show that higher amount of AAm containing particles have higher encapsulation efficiencies and also release data indicated that microspheres containing higher amount of AAm exhibited prolonged release rates than those containing lower amount of AAm. Generally, the drug release profiles depend on factors such as particle size, crystallinity, surface morphology, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity, and rate of hydration of the polymeric materials.³² One can also consider the binding affinity of drug and swelling property of AAm segments of the matrix. A rapid release of more than 98% of drug was observed within 12 h from the microspheres containing lower amount of AAm, indicating some of type physical interaction between the two polymers.

Effect of crosslinking agent

The % cumulative release data vs. time plots with varying amounts of GA, i.e., 2.5, 5.0, and 7.5 mL at a fixed amount of NFD (5 %) are displayed in Figure 6. The % cumulative release is quite fast and large at lower amount of GA (i.e., 2.5 mL), whereas release is quite slower at higher amount of GA (i.e., 7.5 mL). The cumulative release is somewhat smaller when lower amount of GA was used, probably because at higher concentration of GA, polymeric chains would become rigid due to the contraction of microvoids, thereby reducing the % cumulative release of NFD through the polymeric matrices. As expected, drug release becomes slower at higher amount of GA but becomes faster at lower amount of GA.

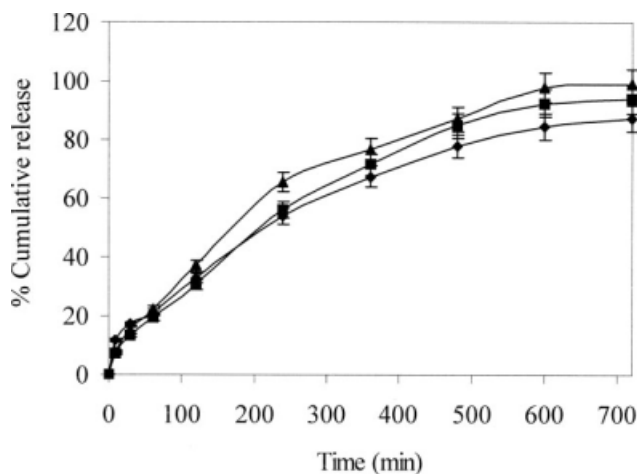


Figure 5 % Cumulative release of NFD through MC-g-AAm/Ge microspheres containing different amount of acrylamide. Symbols: (◆) 10 wt % AAm, (■) 20 wt % AAm, and (▲) 30 wt % of AAm.

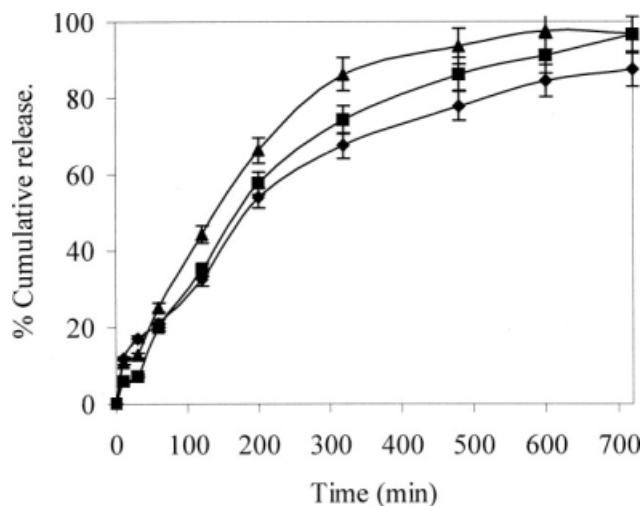


Figure 6 % Cumulative release of NFD through MC-g-AAm/Ge microspheres containing different amounts of crosslinking agent. Symbols: (■) 2.5 mL, (◆) 5 mL, and (▲) 7.5 mL.

Effect of % drug loading

Figure 7 shows the release profiles of NFD-loaded MC-g-AAm/Ge microspheres at different amounts of drug loading. Release data showed that formulations containing the highest amount of drug (15 wt %) displayed fast and higher release rates than those formulations containing small amount of NFD. The prolonged release was observed for formulation containing a lower amount of NFD. In other words, with a decreasing amount of drug in the matrix, a shift from anomalous type release to Case II is observed. Notice that release rate becomes quite slower at lower amount of drug in the matrix, probably due to the availability of more free void spaces through which lesser number of drug molecules

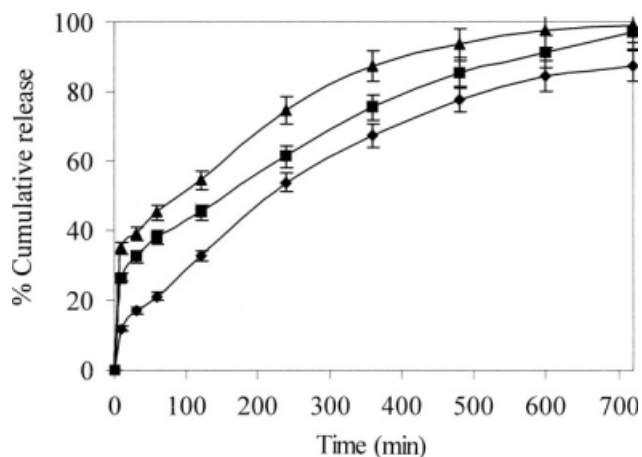


Figure 7 % Cumulative release of NFD through MC-g-AAm/Ge microspheres containing different amount of NFD. Symbols: (◆) 5 wt %, (■) 10 wt %, and (▲) 15 wt %.

will transport. For all the NFD-loaded formulations, complete release of NFD was not observed even after 600 min, but the release rates were around 700 min.

CONCLUSIONS

Microspheres prepared from a combination of carbohydrate polymers using MC and Ge with acrylamide yielded useful information on the controlled release of NFD. The particles prepared were characterized by DSC, SEM, and particle size distribution. DSC thermograms confirmed molecular distribution of drug particles in the polymer matrix, whereas SEM suggested the spherical nature with smooth surface of the microspheres. Laser particle size analyzer estimated the size of particles in the range of 112–205 μm . NFD was released in a controlled manner through the developed formulations. Swelling of the microspheres exerted an influence on drug release characteristics at higher amount of gelatin. The reduction in water uptake was correlated with drug release characteristics of the microspheres that contained different amounts of gelatin. The microspheres of this study have lower densities and, hence, could be retained in the gastric environment for more than 12 h, which might help to improve the bioavailability of NFD.

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