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Research paper

Water transport and drug release study from cross-linked polyacrylamide grafted guar gum hydrogel microspheres for the controlled release application ☆

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Abstract

The graft copolymer (pAAm-*g*-GG) of guar gum with acrylamide was prepared and cross-linked with glutaraldehyde to form the hydrogel microspheres by the water-in-oil (w/o) emulsification method. The microspheres were loaded with two antihypertensive drugs, verapamil hydrochloride (water-soluble) and nifedipine (water-insoluble) to investigate their controlled release characteristics. The drugs were incorporated either during cross-linking by dissolving it in the reaction medium or after cross-linking by the soaking technique. The microspheres were characterized by Fourier transform infrared spectroscopy, thermogravimetry, differential scanning calorimetry, equilibrium water uptake and dynamic swelling. The microspheres are spherical with smooth surfaces. Dynamic swelling experiments indicated that with an increase in cross-linking, water transport deviates from Fickian to non-Fickian mechanism. The in vitro drug release showed a dependence on the extent of cross-linking, amount of drug loading, nature of drug molecule and method of drug loading. Even though the release of drugs is swelling controlled in the initial stages, in the later stage diffusion of the solute is dominating. Various transport parameters have been calculated and the results are discussed in terms of the nature of the drug and the polymer.

Keywords: Guar gum; Graft copolymer; Microsphere; Water transport; Drug release

1. Introduction

Hydrogels are the three-dimensional network polymers that are known to swell in aqueous solutions. In the swollen state, these become soft and rubbery, resembling a living tissue and some possess excellent biocompatibility [1]. Thus, polymeric hydrogels are of considerable interest as biomaterials in drug delivery research [2–6]. Natural polymers are often preferred to synthetic materials due to their non-toxic, low cost, free availability, biodegradability. It should be noted that many 'old' materials compete successfully today after almost a century of efforts to replace them. They can be modified to over come certain draw backs, like uncontrolled rate of hydration, microbial contamination, drop in viscosity on storing, etc. The hydrogel of modified natural polymers finds application in drug delivery and other fields [7–9]. Graft copolymerization is an easier method to modify the structure of natural polymers and thus makes them attractive biomaterials in controlled release (CR) applications.

Guar gum (GG), a plant polysaccharide, has advantages such as low cost, easy availability and is biodegradable. However, uncontrolled rate of hydration, decreased viscosity on storage and microbial contamination limit its application. The GG-based formulations developed earlier for the CR of several antihypertensive drugs have yielded successful clinical trials [10,11]. The structure of GG can be modified to tailor its properties to be a useful CR device. Earlier, it was shown [12] that when GG is cross-linked with borax, a decrease in viscosity is observed in the presence of enzymes, suggesting that GG retains its degradation properties even after cross-linking. However, the borax cross-linked GG was not very successful due to its high swelling in the presence of gastric and intestinal fluids. Rubinstein et al. [13,14] have reported some CR devices of GG cross-linked with glutaraldehyde (GA) and phosphate for applications in colon targeting. Recently, Soppimath et al. [15] have reported the cross-linked interpenetrating network microspheres of poly(vinyl alcohol)-GG for the CR of nifedipine. In this paper, we report the synthesis of graft copo-

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lymer of polyacrylamide (pAAm) with natural guar gum, i.e. pAAm-*g*-GG, to improve physicochemical properties of GG and its application in drug delivery.

In continuation of our ongoing program of research to develop the polymeric microspheres for the release of antihypertensive drugs [15–18], we now report the synthesis of GA cross-linked hydrogel microspheres of pAAm-*g*-GG, which are used for the CR of two antihypertensive drugs: verapamil hydrochloride, VRP (water-soluble) and nifedipine, NFD (water-insoluble). Both NFD and VRP are calcium blockers. However, the bioavailability of NFD is low due to first-pass hepatic metabolism and it has a short biological half-life of 3–4 h with its antihypertensive effect lasting only up to few hours [19]. The conventional formulations of NFD have not been successful due to their rapid elimination with significant fluctuations in plasma drug concentrations [20]. On the other hand, VRP has a very low bioavailability of about 10–20% when administered by oral/i.v. routes. The low bioavailability is due to the rapid biotransformation in the liver with a biological half-life of 4.2 h [21].

The hydrogel microspheres were characterized by Fourier transform infrared (FTIR) spectroscopy to confirm grafting and cross-linking reactions. Their shapes and surface morphologies were studied by scanning electron microscopy (SEM). The influence of water transport and swelling properties of the microspheres were studied to understand their in vitro release characteristics. The diffusion as well as other related parameters like penetration front velocity (u) and swelling interface number (Sw) have been calculated and used to investigate the mechanism of drug release. The procedures developed by Peppas et al. [22–25] were used in the analysis of data for the present systems.

2. Experimental

2.1. Materials

Acrylamide, ceric ammonium nitrate, Tween 80, sodium laurel sulfate, glutaraldehyde (25% w/v), light liquid paraffin, hexane and guar gum are used in this study. Guar gum was purified by refluxing with solvent ether before use. All chemicals were purchased from s.d. Fine Chemicals (Mumbai, India). The verapamil hydrochloride and nifedipine (both USP grade) were received as gift samples from Lincoln Pharmaceuticals (Ahmedabad, India).

2.2. Synthesis of graft copolymer of guar gum–acrylamide

The grafted copolymer of guar gum and acrylamide was prepared by free radical polymerization. Briefly, 2 g of GG was dispersed in 150 ml of water and allowed to hydrate and dissolve overnight under stirring in a 250-ml round-bottom flask; 0.12 mol pAAm was dissolved in 20 ml water, added to the GG solution and mixed uniformly for 1 h. To this solution, 30 ml of 0.005 mol ceric ammonium nitrate was added. Polymerization was carried out at 60°C with a continuous purging of nitrogen gas for 6 h in a water bath with constant stirring. After complete polymerization, a sufficient amount of acetone was added to precipitate the graft copolymer. The polymer was dried under vacuum (60 mmHg) at 40°C overnight. Mass of the polymer was taken and the percent grafting was calculated as:

$$\text{Percent AAm grafting} = \left(\frac{\text{Mass of the AAm in grafted polymer}}{\text{Mass of the grafted polymer}}\right) \times 100 \quad (1)$$

2.3. Preparation of pAAm-g-GG cross-linked hydrogel microspheres and drug loading

Different batches of microspheres of pAAm-*g*-GG were prepared by a water-in-oil (w/o) emulsification method. Briefly, 20 ml of 5.0% (w/v) polymer solution was prepared and acidified with 5 ml dilute sulfuric acid. In order to cross-link the polymer, 2.5, 5 and 7.5 ml of 25% (w/v) glutaraldehyde (GA) solutions were added to the polymer solution separately. These solutions were then emulsified into 100 ml of light liquid paraffin with 2% (w/v) Tween 80 using a Eurostar high-speed stirrer (IKA Labortechnik, Germany) at 600 rpm for 5 h at room temperature. The total time of cross-linking was kept constant. Hardened microspheres were filtered and washed repeatedly with hexane and water to remove liquid paraffin, unreacted GA and any adhered Tween 80. The hydrogel microspheres were then dried under vacuum at 40°C overnight and kept in a desiccator until further use.

Microspheres were loaded with the drug by soaking in 0.1 N HCl saturated with VRP for 3 days at 37°C. In the case of NFD, the swollen microspheres were soaked in a saturated solution of NFD in acetone. NFD was also loaded during cross-linking at three different loadings of 5, 10 and 20% (w/w) (based on the dry mass of the polymer) and cross-linked with 5 ml of GA. The drug-loaded microspheres were then dried and stored under desiccant until further use.

3. Characterization of microspheres

3.1. Fourier transform infrared spectroscopy

Guar gum-grafted copolymer and the cross-linked empty microspheres were analyzed by FTIR spectroscopy to confirm grafting and cross-linking reactions. The polymer samples were crushed with potassium bromide to make pellets. Spectra were taken on a Nicolet, Model Impact 410, USA and scanned between 400 and 3000 cm^{-1}.

3.2. Thermal analyses

Both thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed on GG,

pAAm-*g*-GG and the cross-linked microspheres by using a DuPont-2000 microcalorimeter. TGA experiments were performed with 2–3 mg of the sample under a dynamic nitrogen atmosphere flowing at a rate of 50 ml/min and at a heating rate of 10°C/min. For DSC, the samples were heated from sub-zero temperature to 300°C at a heating rate of 10°C/min under argon atmosphere.

3.3. Microscopic studies

Dried microspheres were studied using an optical microscope to measure the particle size and surface characteristics. The particle size was measured by taking 100–200 particles on a glass slide under regular polarized light. SEM analysis was performed to know the topographical characterization of the microspheres. The sample was deposited on a brass holder and sputtered with gold. The SEM photographs were taken with JSM 6400 Scanning Microscope (Japan) at the required magnification.

3.4. Swelling studies

Equilibrium water uptake of the cross-linked empty microspheres and the drug-loaded microspheres were determined by measuring the extent of swelling of the matrices in water. To ensure complete equilibration, the samples were allowed to swell for 24 h. The excess surface-adhered liquid drops were removed by blotting and the swollen microspheres were weighed to an accuracy of 0.01 mg using an electronic microbalance (Mettler, AT 120, Switzerland). The hydrogel microspheres were then dried in an oven at 60°C for 5 h until there was no change in the dried mass of the samples. The percent equilibrium water uptake was calculated as:

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

Drug release from the cross-linked hydrogels depends upon the extent of water ingression into the cross-linked hydrogel matrices. In order to understand the molecular transport of water into the cross-linked microspheres, the microscopic technique reported by Robert et al. [22] was used. In this method, the change in diameter of the microspheres in the presence of distilled water was monitored. Experiments were carried out in triplicate and the average values were considered for data treatment and calculations. In all systems, the standard deviation was less than 5% of the measured value.

3.5. Estimation of drug loading

As hydrogels cannot be dissolved completely in organic solvents or acids, the drug loading was estimated by mechanically crushing the microspheres in the presence of a suitable media such as 0.1 N HCl for VRP and methanol + aqueous solution of 0.05% (v/v) Tween 80 for NFD-loaded microspheres. The solution was then gently heated for 2 h to extract the drugs completely and filtered to analyze both VRP and NFD by using a UV-visible spectrophotometer (Model Anthelie, Secomam, France) at 238 and 278 nm, respectively.

3.6. In vitro drug release

The in vitro drug release from the pAAm-*g*-GG hydrogel microspheres was carried out in a USP-II rotating paddle dissolution apparatus (Disotest Labindia, India) at a rotation speed of 100 rpm and at 37°C. The dissolution media used for the release of NFD was 900 ml of 0.1 N HCl with 1.0 w/v of sodium laurel sulfate, but for VRP, the dissolution medium was 900 ml of 0.1 N HCl. At regular intervals of time, the aliquots were withdrawn and analyzed for drugs using the UV-visible spectrophotometer.

4. Results and discussion

Graft copolymerization of GG with acrylamide was attempted by Ce(IV) catalyzed free radical reaction. The chelate complex formed between –OH group of GG decomposes to generate the free radical site, facilitating grafting to occur at the active site of GG with acrylamide monomer. This reaction is shown in Scheme 1. Owen and Shen [26] observed if the monomer concentration is more than 2.0 M, then homopolymer is formed. In order to minimize the formation of acrylamide homopolymer, we used 0.5 M monomer and even with this the grafting efficiency was 81.12%.

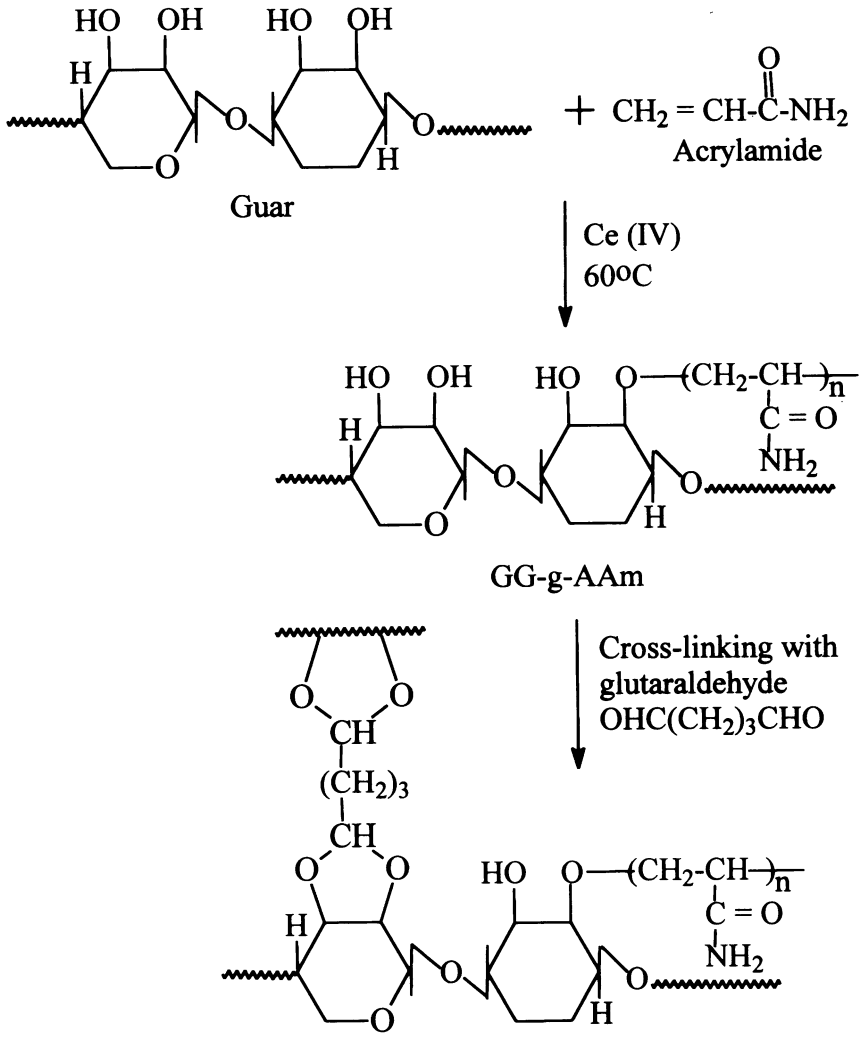
4.1. FTIR characterization of graft copolymer and cross-linked microspheres

The grafting reaction between acrylamide and guar gum was confirmed by FTIR spectroscopy (see Fig. 1). A sharp peak observed at ~1659 cm^{-1}, which represents the carbonyl group of amide moiety of the grafted acrylamide chain is not observed in the spectrum of GG. The –NH stretching. which appeared as a shoulder band around ~3200 cm^{-1} of the graft copolymer, has overlapped with a broad peak between 3650 and 3200 cm^{-1} of the hydroxyl group. These data are supportive of the grafting reaction between acrylamide and GG.

FTIR spectra of the cross-linked microspheres are presented in Fig. 1C. During cross-linking, GA might have reacted with the –OH groups of the graft copolymer through the formation of ether linkages. Hence, the appearance of a sharp peak at ~1245 cm^{-1} in the spectra of the cross-linked microspheres confirms the formation of more ether linkages. This could be further supported by the presence of a sharp high intensity peak due to $–CH_2$ group of the alkyl chain formed by cross-linking.

4.2. Thermal analyses

TGA thermograms of GG, pAAm-*g*-GG and of the cross-



Scheme 1. Formation of cross-linked pAAm-g-GG.

linked microspheres are presented in Fig. 2. The observed initial mass loss up to 120°C may be due to the presence of moisture, solvents, the unreacted cross-linking agents or the monomers. However, no mass loss occurred at the later stage, i.e. up to ~240°C. In the case of GG, a sharp mass loss of about 50–55% is observed between 230 and 350°C and this may be attributed to the loss of hydroxyl group of GG as water molecules. For the pAAm-*g*-GG grafted copolymer, mass loss was small initially, but at a later stage, it became constant. However, the grafted copolymer had a higher mass percentage of about 50% until 400°C. The percent residual mass of pAAm-*g*-GG was higher than observed for GG at 550°C. This supports that modification of GG by grafting with acrylamide renders GG thermally more stable. With the cross-linked microspheres, a similar thermal behavior as that of pAAm-g-GG was observed around ~320–490°C. No sharp mass loss was observed at 490°C and the matrix maintained a mass of ~26% even at 600°C. This may be due to the formation of a rigid polymer network, making it thermally more stable.

DSC thermogram of pAAm-*g*-GG empty microspheres given in Fig. 3 exhibited endothermic peaks around ~75–100°C and ~270–300°C. A shift in endothermic peaks to higher temperatures was observed -for microspheres prepared with a higher concentration of the cross-linking agent along with increased values of specific heat of fusion. These results indicate that the energy needed for the fusion of polymer increased with an increase in cross-linking, and support the fact that an increase in cross-linking increases the polymer chain rigidity and hence, higher energy is required to break the highly cross-linked polymer than the loose network.

4.3. Microscopic study

The pAAm-*g*-GG microspheres are almost spherical in shape as indicated by the SEM photograph shown in Fig. 4A. The surfaces are smooth without any porous structure (see Fig. 4B). The particles have a mean diameter ranging from 391 to 594 μm (see data in Table 1) and are free

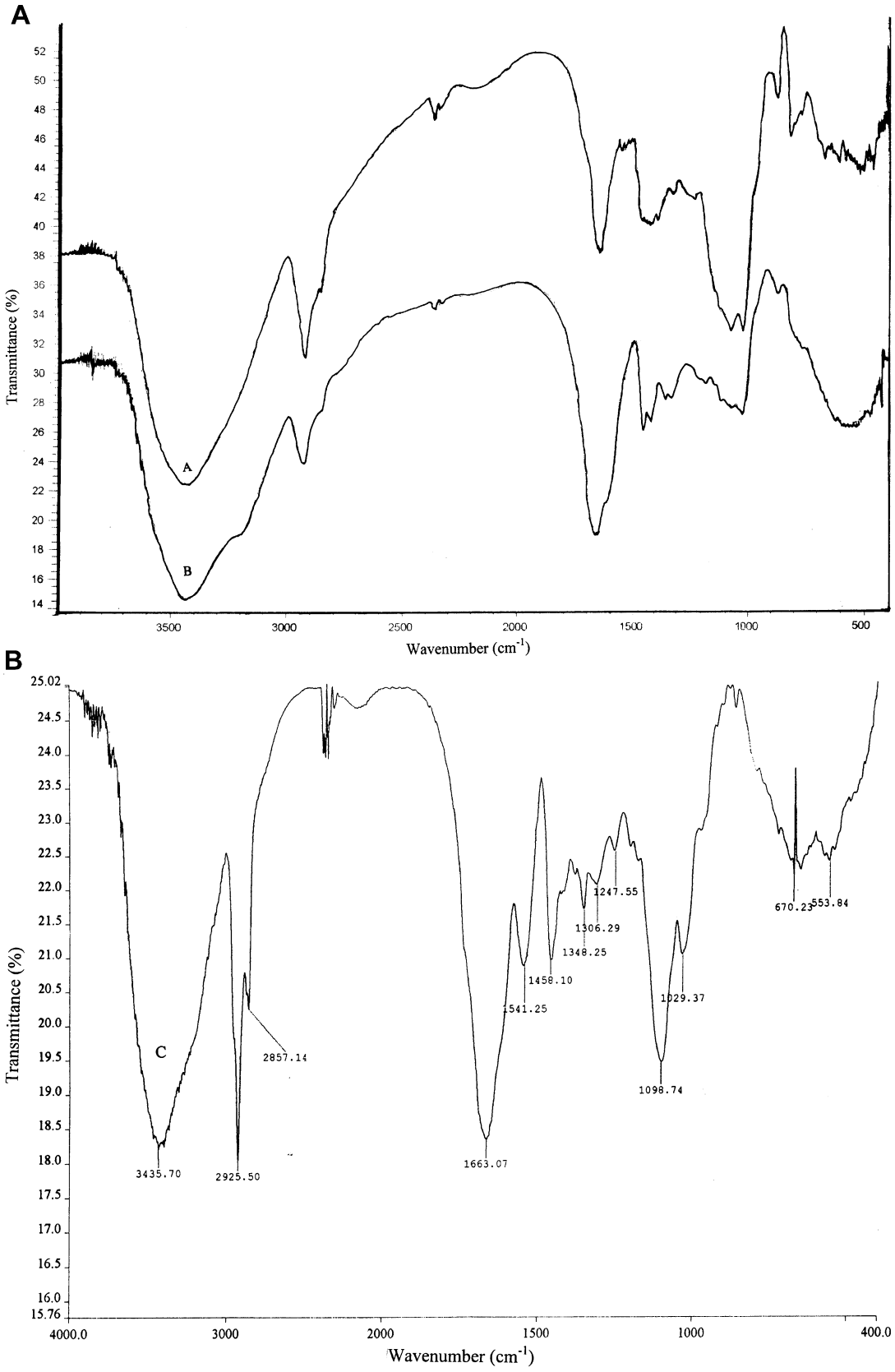


Fig. 1. FTIR spectrum of GG (A), pAAm-*g*-GG (B) and cross-linked microspheres (C).

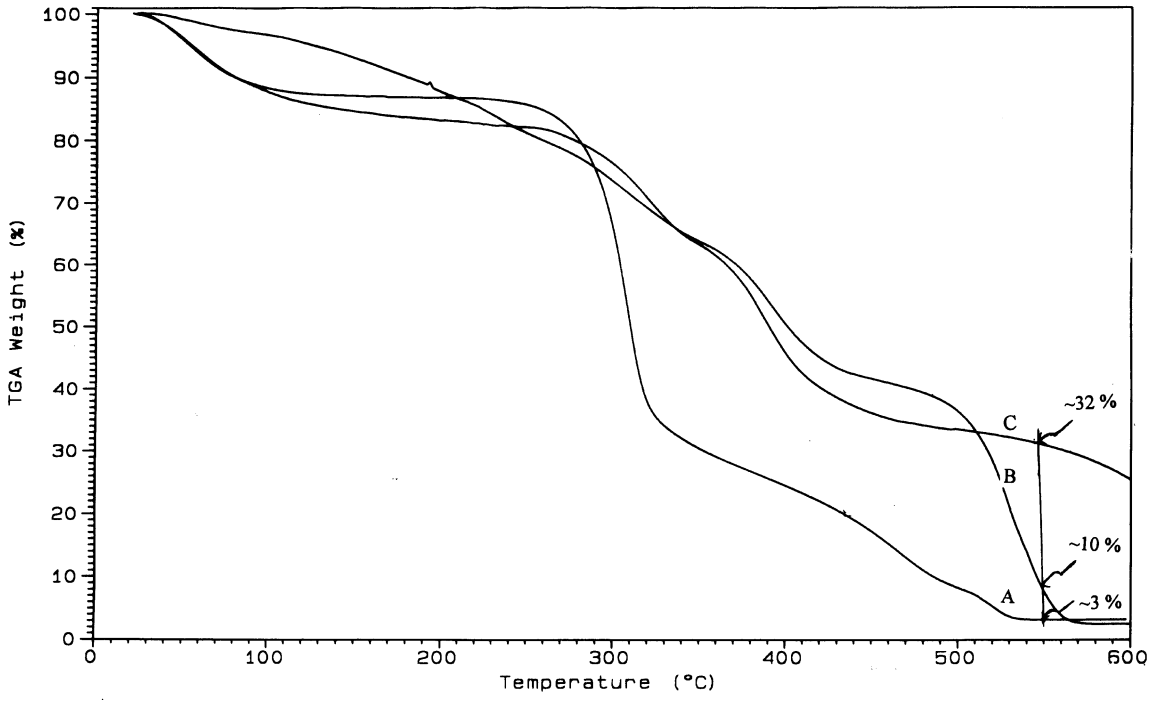


Fig. 2. TGA thermograms of GG (A), pAAm-*g*-GG (B) and cross-linked microspheres with 7.5 ml of GA (C).

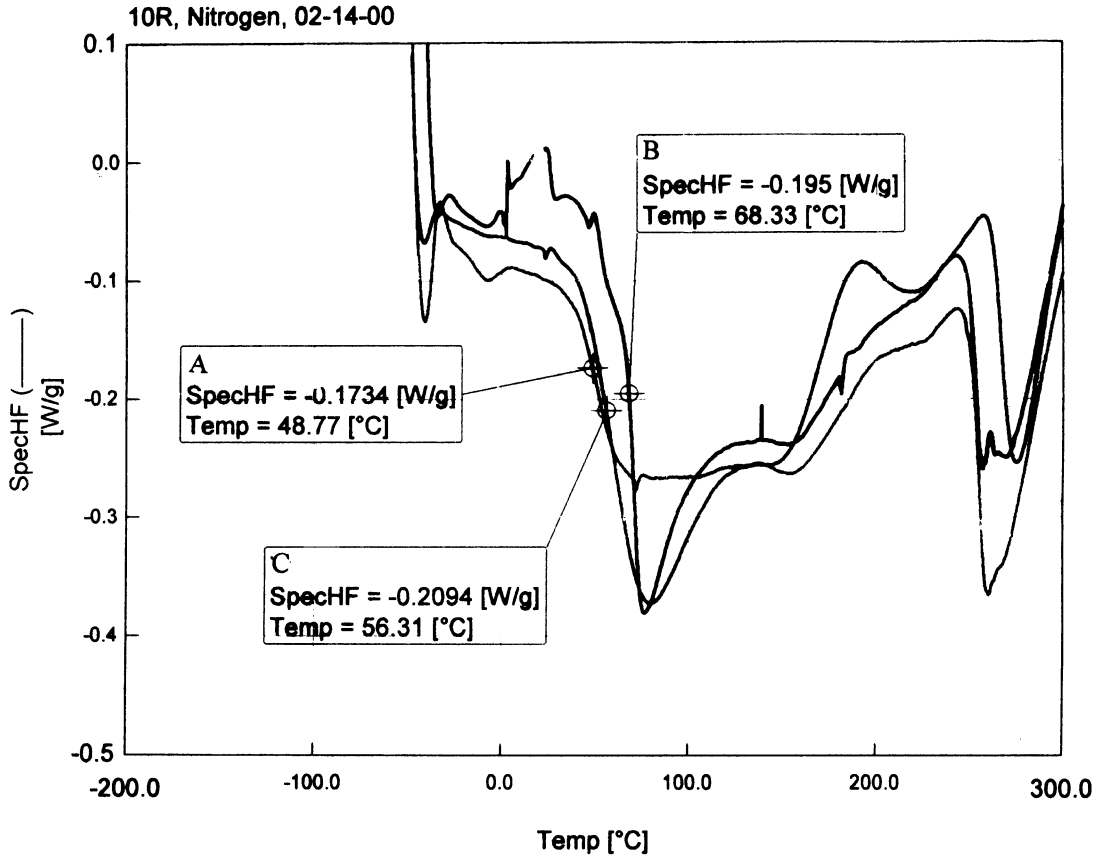


Fig. 3. DSC thermograms of pAAm-*g*-GG empty microspheres cross-linked with (A) 2.5 ml, (B) 5 ml and (C) 7.5 ml of GA.

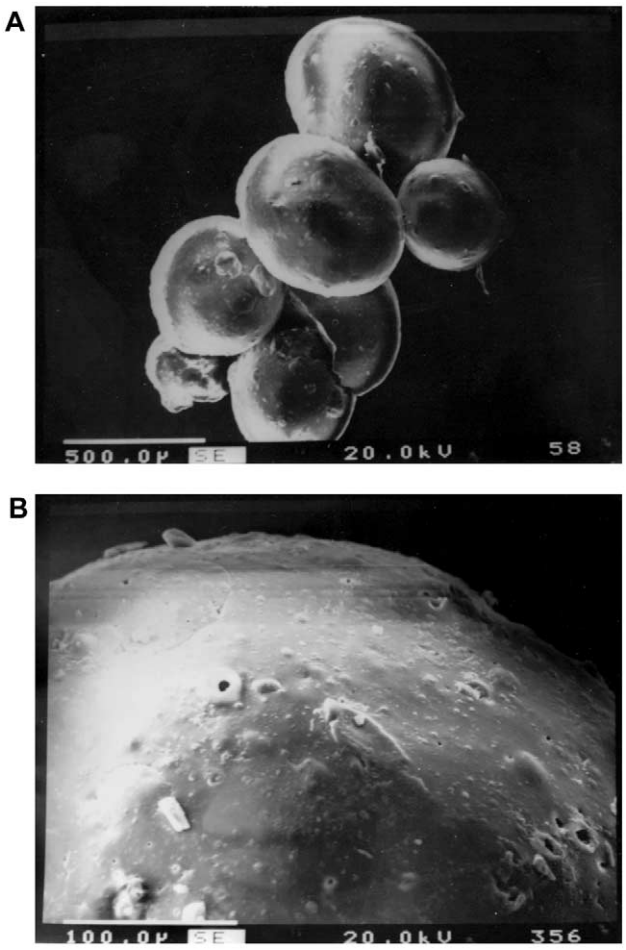


Fig. 4. Scanning electron microscopic photographs of pAAm-*g*-GG empty microspheres cross-linked with 5 ml GA (A) and surface photographs of the same (B).

flowing without any aggregation. The extent of cross-linking showed an effect on particle size. For instance, with an increasing amount of GA from 2.5 to 7.5 ml, a considerable decrease in particle size from 594 to 391 μm is observed. This suggests that during cross-linking, the hydrogels might have undergone shrinkage (syneresis) leading to the formation of smaller particles at higher cross-link densities. Similar shrinkages have been observed by Korsmeyer et al. [27] at higher cross-link densities in the case of poly(vinyl alcohol) hydrogels.

Size of the NFD-loaded microspheres presented in Table 2 depends upon the method of drug loading as well as the extent of drug loading (entrapment efficiency). For the NFD-loaded microspheres, during the cross-linking process, the particle size increased from 474 to 744 μm when NFD loading was increased from 5 to 10%. This effect can be explained on the hydrodynamic viscosity concept, i.e. as the concentration of NFD in the microspheres increases, the interfacial viscosity of the polymer droplet in the emulsion also increases, which will then hinder breaking of the dispersed phase into smaller size particles during emulsification. The other explanation is that NFD particles might have occupied the free volume spaces within the gel polymer matrix, which will hinder the inward shrinkage of the polymer chain during cross-linking.

4.4. Encapsulation efficiency

The cross-linked pAAm-*g*-GG microspheres were loaded with NFD before cross-linking and by the soaking method, i.e. by soaking the microspheres in the saturated solutions of the drugs. The VRP was loaded with soaking method only as it was difficult to wash the microspheres without concomitant loss of drug. If unwashed the unreacted GA cannot be removed, the cross-linking reaction may continue during storage. For these reasons, VRP was loaded by the soaking method and NFD was loaded both during and after cross-linking. When NFD was loaded during cross-linking, the encapsulation efficiency was as high as 90% (see Table 2). For the soaking method, lower drug loading is observed in case of the highly cross-linked microspheres when compared to the lightly cross-linked matrices. Thus, the drug loading was influenced by the extent of equilibrium water uptake of the matrices (see Table 1).

4.5. Water uptake studies

Equilibrium uptake of the cross-linked microspheres exerts a profound influence on their release rates [15]. The percent equilibrium water uptake data of the cross-linked empty microspheres presented in Table 1 indicate that, as the amount of GA in the matrices increases from 2.5 to 7.5 ml, a significant decrease in equilibrium water uptake from 307 to 128% is observed.

When the drug is loaded by the soaking method, water uptake remains the same as these of the unloaded microspheres indicating that the amount of drug loaded is small to exhibit any effect on the water uptake. However, the NFD-loaded microspheres during cross-linking exhibited significantly lower equilibrium percent water uptake values than the unloaded microspheres (see Table 2) probably because

Table 1
Formulation results of the drug-loaded pAAm-*g*-GG microspheres

Cross-linking agent used (ml)	% Water uptake	% VRP loading[a]	% NFD loading	Mean size (μm)
2.5	307 ± 17.1	15.2 ± 2.1	4.4 ± 0.4	594
5.0	259 ± 9.4	12.6 ± 1.2	3.7 ± 0.9	424
7.5	128 ± 10.1	7.9 ± 0.5	2.0 ± 0.2	391

[a] Expressed as % of polymer dry mass.

Table 2
Formulation results of microspheres loaded with NFD before cross-linking with 5.0 ml of GA

% Loading[a]	% Water uptake	% Entrapment efficiency	Mean size (μm)
5	210 ± 19.2	81.1 ± 4.5	474
10	172 ± 4.1	88.7 ± 2.5	699
20	162 ± 2.5	90.0 ± 2.1	744

[a] Expressed as % of polymer dry mass.

of the hydrophobic nature of NFD, which acts as an inert filler, thereby restricting water transport. The uniformly distributed NFD fillers will occupy the free volume of the swollen hydrogel and create a tortuous path for the water to permeate. However, the degree of tortuosity depends upon the volume fraction of the filler in addition to its shape, size and orientation [28,29].

In the present study, the dynamic swelling was determined by monitoring the change in microsphere diameter, D_t, as a function of time using an optical microscope. Fig. 5 displays the normalized diameter, D_t/D_0 (where D_0 is initial diameter of the microsphere) as a function of time for different amounts of GA added. With an increase in cross-linking, a decrease in water uptake is observed. As the amount of GA increases, swelling capacity of the microspheres decreased considerably. The results of equilibrium swelling diameter, D_∞ normalized to original diameter, D_0 are presented in Table 3.

The dimensional changes of the microspheres due to swelling (i.e. volume change ΔV_t with time with respect to initial volume time, V_0) have been analyzed to compute diffusion coefficient, D_v of water molecules using a modified theory proposed earlier by Harogoppad and Aminabhavi [30,31]:

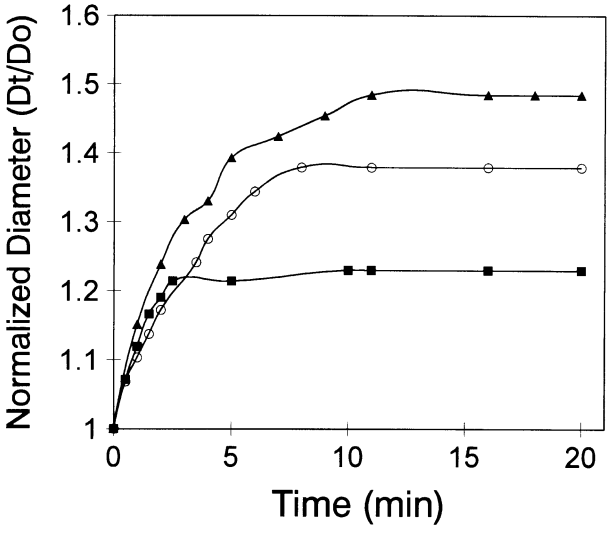


Fig. 5. Plot of D_t/D_0 vs. swelling time, t for pAAm-g-GG microspheres cross-linked with: 2.5 ml (▲), 5.0 ml (○) and 7.5 ml (■) of GA.

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

The symbol ΔV_∞ represents the change in volume at equilibrium condition. Eq. (3) allows us to compute D_v from the slope of the $\Delta V_t/V_0$ vs. $t^{1/2}$ plots. The results are included in Table 3.

The solvent front velocity, u, of the advancing boundary for the spherical microspheres was calculated using the relation [23,24]:

$$u = \left(\frac{dv}{dt}\right)\frac{1}{A} \quad (4)$$

Here, dv/dt is the change in volume of the microsphere per unit time and A is the area of the microsphere. The results of u are also included in Table 3.

The values of diffusion coefficients and front velocity decrease with an increase in cross-linking. For instance, with an increasing amount of GA from 2.5 to 7.5 ml, a considerable decrease in diffusion coefficient from 5.44×10^{-5} to 0.88×10^{-5} cm^2/s and similarly, a decrease in solvent front velocity from 6.66 to 4.56 cm/s is observed. Molecular transport in the microspheres is dependent upon the extent of cross-linking.

These results support that the more tightly cross-linked matrix does not expand in water as much as the loosely cross-linked matrix. At lower amounts of GA (i.e. lower cross-link density), the network is loose and has a high hydrodynamic free volume to accommodate more of the solvent molecules, thereby inducing matrix swelling. The water uptake in hydrogels depends upon the extent of hydrodynamic free volume and the availability of hydrophilic functional groups for the water to establish hydrogen bonds. Higher water uptake values observed at lower levels of cross-linking and vice versa observed in the present systems confirm the formation of rigid polymeric networks due to cross-linking.

The swelling data presented in Fig. 5 have been hued to the empirical equation of the type [16,22]

$$\frac{D_t}{D_\infty} = kt^n \quad (5)$$

Here, k is a rate constant and n is a parameter that represents the type of transport mechanism. The least squares estimations of the values of n from the dynamic swelling data fitted to Eq. (5) are presented in Table 3. The results of n increase from 0.46 to 0.78 with increasing amount of GA from 2.5 to 7.5 ml. These data are in agreement with our earlier results on poly(vinyl alcohol)–GG based hydrogel microspheres [15–17] and suggest that an increase in cross-linking reverts the transport mechanism from Fickian to non-Fickian.

4.6. Drug release vs. molecular transport

Drug release is closely related to molecular transport of

Table 3
Transport data of water in pAAm-*g*-GG microspheres

GA used (ml)	Equilibrium normalized diameter (D_∞/D_0)	n[a]	$D_v \times 10^5$ (cm^2/s)	$u \times 10^3$ (cm/s)
2.5	1.56	0.46	5.443	6.66
5.0	1.37	0.66	1.194	4.84
7.5	1.23	0.78	0.883	4.56

[a] Computed from Eq. (3): the values of n and D_v are estimated by the least squares method at 95% confidence limit.

drug containing solution within the domain of microspheres. The in vitro release of the VRP-loaded cross-linked GG-*g*-pAAm was carried out with the dried (glassy) as well as the fully swollen (rubbery) microspheres in 0.1 N HCl. Average values of the triplicate measurements are used for graphical presentation as well as for the mathematical treatment. In all cases, standard deviations were less than 5%. The release profiles of VRP from the dried and swollen microspheres cross-linked with different amounts of GA are displayed, in Figs. 6 and 7, respectively, and the release from the dried polymer is relatively slower than swollen microspheres. As the transport of water into glassy microsphere is under the control of the polymer chain relaxation, on the other hand it is not the case in swollen gels.

These results were also supported by the diffusion coefficient D, values calculated by using the following equation assuming initial time approximation [32]:

$$\frac{M_t}{M_\infty} = \left(\frac{36Dt}{\pi r^2}\right)^{1/2} - \left(\frac{3Dt}{r^2}\right) \quad (6)$$

where r is radius of the microsphere. The fractional release, M_t/M_∞ from 0 to 0.4 was taken into account. The results of D presented in Table 4 decrease considerably with an increase in cross-linking of the network polymer. Thus, the drug diffusion is dependent upon the rate of water permeation, i.e. extent of cross-linking. Drug diffusion in the swollen microspheres will be higher when compared to that observed in the dried (glassy) microspheres as diffusion of water in the rubbery microspheres is not controlled by the polymer chain relaxation, which is the case in glassy polymer.

The release profiles of NFD-loaded microspheres prepared by the soaking method are presented in Fig. 8. Here, the release is somewhat biphasic with an initial burst effect, followed by a subsequent slower release. In the case of microspheres when the drug was loaded during cross-linking, the release showed a dependency on the amount of drug loaded (see Fig. 9). In formulations with lower drug loading (5% w/w), NFD was released more quickly when compared with the higher drug-loadings. At lower drug loading, large pore fractions may be formed rapidly, which may increase the water uptake (see Table 2) and consequently, release the drug more quickly. The other explanation might be that the lower drug-loaded microspheres are smaller in size (see Table 2) and hence, the water uptake and the drug release process are faster due to their larger surface area. A slight increase in drug release was observed when the drug loading was increased from 10 to 20% (w/w). Similar findings were reported [33] for theophylline-loaded formulations when the drug loading was $<10\%$. The apparent diffusion coefficients of NFD as calculated from Eq. (6) are included in Table 5. The values

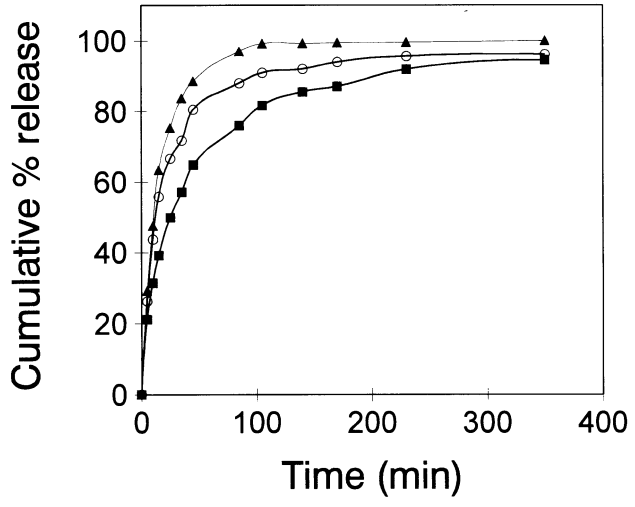


Fig. 6. Release profiles of VRP from the dried pAAm-*g*-GG microspheres cross-linked with: 2.5 ml (▲), 5 ml (○) and 7.5 ml (■) of GA.

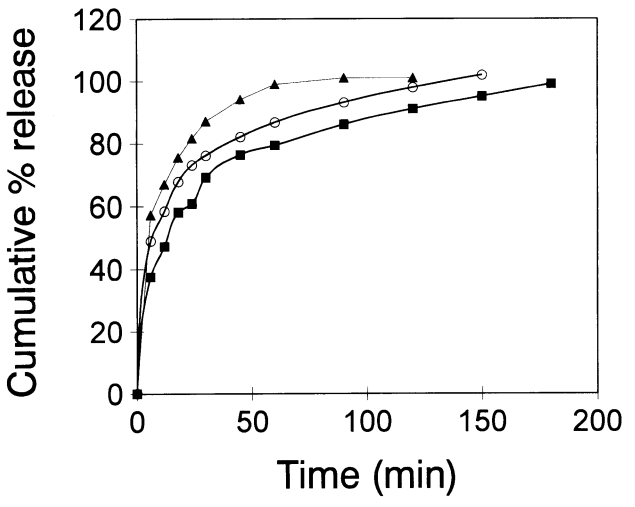


Fig. 7. Release profiles of VRP from the swollen pAAm-*g*-GG cross-linked with: 2.5 ml (▲), 5 ml (○) and 7.5 ml (■) of GA.

Table 4
Release kinetic parameters of VRP from pAAm-*g*-GG dried and swollen microspheres

GA (ml)	n[a]	D 10^6 (cm^2/s)[b]	*Sw*
Dried microspheres			
2.5	0.54	11.16	
5.0	0.52	4.51	
7.5	0.51	3.23	
Swollen microspheres			
2.5	0.27	19.69	30.67
5.0	0.28	8.33	35.03
7.5	0.39	5.61	42.26

[a] Computed from Eq. (7), estimated by the least squares method at 95% confidence limit
[b] Computed from Eq. (6), estimated by the least squares method at 95% confidence limit.

of diffusion coefficients exhibit a relationship with the extent of cross-linking and percent drug loading.

In order to establish a link between drug release and molecular transport parameters, we have analyzed the transport results by fitting the fraction of the drug released, M_t/M_∞ vs. time t, data using the empirical equation [34]

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

Here, k and n have the same meanings as before; The values of n for VRP are presented in Table 4 for both the dried and rubbery microspheres. For the dried microspheres, the values of n are > 0.5, indicating that the release in these systems is non-Fickian. For the rubbery microspheres, the values of n are < 0.5, indicating the drug release in the swollen microspheres follows the Fickian diffusion. These results agree with those published by Korsmeyer and Peppas [27] and confirms that the release from the rubbery polymer

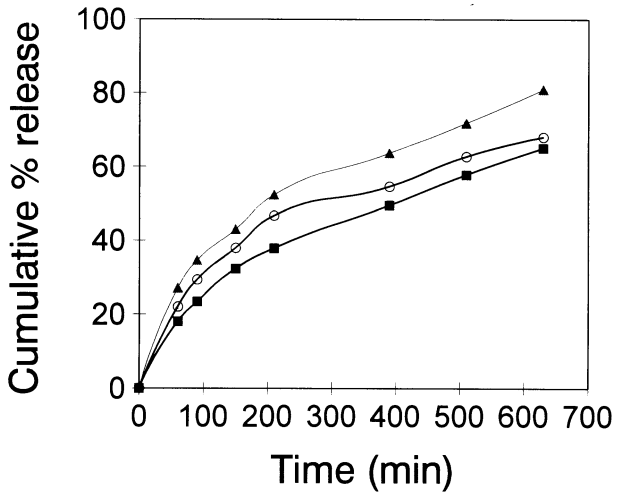


Fig. 8. Release profiles of NFD-loaded pAAm-*g*-GG microspheres by soaking method and cross-linked with: 2.5 ml (▲), 5 ml (○) and 7.5 ml (■) of GA.

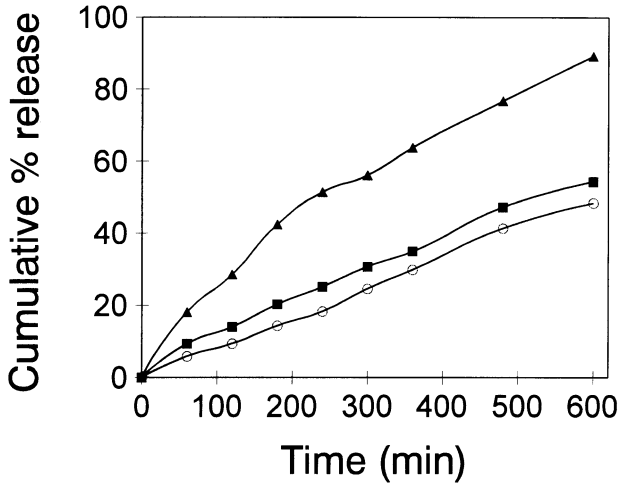


Fig. 9. Release profiles of NFD-loaded pAAm-*g*-GG microspheres before cross-linking. Symbols for NFD loadings are: 5% (▲), 10% (○) and 20% (■).

is Fickian, while it follows anomalous/or non-Fickian behavior for the glassy polymer.

The release of NFD is mainly non-Fickian. since the values of n are > 0.5 (see Table 5). The values of n increase with an increase in drug loading as well as cross-linking for those formulations that are loaded with NFD before and after cross-linking. The release occurs by non-Fickian mechanism for the NFD-loaded formulations before cross-linking, and the value of $n = 0.88$ for 20% loading is suggestive of Case-II transport at higher drug loading.

4.7. Dimensionless analysis

In order to explain the observed non-Fickian behavior and to understand the mechanism of solute release from the dynamically swelling polymer, we have attempted to use the dimensionless analysis approach as suggested earlier by Peppas and Franson [35]. A dimensionless parameter

Table 5
Release kinetic parameters of microspheres loaded with NFD before and after cross-linking

GA (ml)	% NFD loading	n[a]	D 10^6 (cm^2/s)[b]	*Sw*
Drug loading by soaking				
2.5	4.4	0.50	2.79	216
5.0	3.7	0.59	1.41	206
7.5	2.0	0.60	0.98	241
Drug loading during cross-linking				
5.0	16.0	0.88	3.19	
5.0	9.0	0.78	1.85	
5.0	3.9	0.72	2.01	

[a] Computed from Eq. (7), estimated by the least squares method at 95% confidence limit.
[b] Computed from Eq. (6), estimated by the least squares method at 95% confidence limit.

called swelling interface number and designated by *Sw* was calculated by using Eq. (8):

$$Sw = \frac{u\delta_{max}}{D_s} \quad (8)$$

where u is average penetration velocity of the aqueous release medium calculated from Eq. (4), δ_{max} is the maximum thickness of the swollen microspheres through which solute diffusion occurs and D_s is diffusion coefficient of the drug in the swollen region. When the rate of drug transport through the swollen gel, D_s/δ_{max}, is faster than the rate at which the dissolution media penetrates, then Sw is < 1 and the drug release is controlled by swelling rather than drug diffusion. On the other hand, if the values of Sw are $\cong 1$, then both swelling and diffusion control the drug release and the mechanism is non-Fickian. If Sw is > 1, then drug diffusion becomes dominating with a Fickian transport.

In the present systems, the values of Sw are >1 for the VRP-loaded microspheres, indicating that the rate of solvent penetration is higher than drug diffusion from the swollen microspheres (see Table 4). However, the release may not be completely diffusion-controlled because 40–60% of the drug is released in the transition period of 10–20 min from rubbery-to-glassy state. This suggests that the release is also controlled by swelling, but the subsequent release is controlled by both polymer swelling and drug diffusion. The *Sw* values for the NFD-loaded microspheres by the soaking method (see Table 5) are also > 1, indicating that the release is dominated by the diffusion process rather than swelling, i.e. by polymer relaxation process. The *Sw* values of the NFD-loaded microspheres are much higher than those observed for the VRP-loaded microspheres. This is further supportive of the fact that the release of water-insoluble drug from hydrogels occurs mainly by solute diffusion. Davidson and Peppas [23,24] obtained $n = 0.749$ and $Sw = 11.4$ for the cross-linked (pHEMA-co-MMA) loaded with theophylline. Similarly, Gander et al. [36] obtained $Sw > 1$ for $n > 0.5$ for the cross-linked poly(vinyl alcohol) matrices loaded with proxyphylin. Thus, it appears that *Sw* will add towards an understanding of the mechanism of drug release, but it is not possible to predict the release mechanism solely on the values of *Sw*. Thus, another dimensionless parameter called Debhorah number was used to study the release kinetics [24], but this approach is not attempted in this paper.

5. Conclusions

Guar gum has been successfully modified by grafting with acrylamide to form graft copolymer pAAm-*g*-GG, which showed a better thermal stability than guar gum. The graft copolymer was cross-linked with GA to form the hydrogel microspheres by using the water-in-oil emulsification method. The study indicated that it is possible to load the water-insoluble drug like NFD with higher encapsulation efficiencies when loaded before cross-linking. A soaking method can be used for loading both the VRP and NFD. The swelling of polymer matrices depends upon the extent of cross-linking and drug loaded. The molecular transport depends on the extent of cross-linking of the matrix.

The release of VRP is fast in the initial stages with a burst effect, but the subsequent release is slow and continues for up to 3–4 h. The fast release during initial stages is attributed to swelling or polymer relaxation in the case of water-soluble drug. An understanding of water transport mechanism is important while investigating the release characteristics of the microspheres. For the NFD-loaded formulations, the release was longer (>12 h). However, a zero order release could be achieved by loading the higher concentration of drug (>20% w/w). The release in this case is mainly diffusion-controlled rather than swelling-controlled. The dimensionless number, *Sw*, was useful in explaining the release mechanism. On the whole, the microspheres developed here may serve to be good biomaterials for the controlled release of both water-soluble and water-insoluble bioactive materials.

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