CHAPTER III

Development of Novel Guar Gum Modified Hydrogel Microspheres with Poly(vinyl alcohol) and Acrylamide for the Controlled Release of Antihypertensive Drugs

This chapter presents experimental results on the development of guar gum (GG) modified interpenetrating network structures with poly(vinyl alcohol) (PVAL) as well as GG grafted acrylamide (AAm) cross-linked hydrogel microspheres. These matrices are used for the controlled release of two antihypertensive (calcium channel blockers) drugs: nifedipine (NFD) and verapamil hydrochloride (VRP).

III.A. Nifedipine-Loaded Full-Interpenetrating Network Poly(vinyl alcohol)-Guar Gum Hydrogel Microspheres

Abstract: Poly(vinyl alcohol)-guar gum interpenetrating network microspheres have been prepared by cross-linking with glutaraldehyde and used to study the release kinetics of nifedipine. Drug was loaded into microspheres before and after cross-linking. Cross-linking was confirmed by FTIR and DSC techniques. Drug entrapment efficiency, particle size, water uptake properties and release kinetics of the microspheres have been investigated. SEM was used to understand their spherical nature and surface morphology. Mean particle size of the microspheres was ~ 300 μm. The dynamic swelling results showed that an increase in cross-linking modified the mechanism of water transport from Fickian to non-Fickian. The in-vitro data indicated that the release depends upon the extent of cross-linking, the amount and method of drug loading.

III.A.1. Introduction

Polymeric hydrogels are being increasingly studied in controlled release (CR) applications because of their excellent biocompatibility and ease of fabrication. The CR of entrapped drugs from such hydrogels can be monitored by regulating their water uptake properties or by cross-linking the polymer [1]. Hydroxy propyl methylcellulose, Na-carboxymethylcellulose and other derivatives of cellulose have been extensively studied in the literature for the CR of cardiovascular drugs [2], but the cross-linked hydrogels have still greater opportunities in drug delivery research. In such systems, the extent of cross-linking can be monitored to control the drug release. One of the advantages of these polymers is that semi-interpenetrating and full interpenetrating polymer networks can be engineered as external stimuli response systems [3,4]. These polymers have also been used to prepare the cross-linked microspheres to deliver the bioactive agents [5,6]. Among various polymers, cross-linked poly(vinyl alcohol) (PVAL) was studied earlier in CR applications [7-11]. However, hitherto no reports are available in the literature on the use of PVAL microspheres for the CR of nifedipine (NFD), a calcium channel blocker, which is used in the treatment of hypertension, angina and myocardial infarction [12].

This section presents results on the development of interpenetrating microspheres of PVAL with the naturally occurring guar gum (GG). Glutaraldehyde (GA) was used earlier as a cross-linking agent for both PVAL [8] and guar gum [13]; when these polymers are cross-linked in the presence of each other, they will form interpenetrating networks (IPNs) [14]. The microspheres prepared were used for the CR of NFD. However, the short acting formulations of NFD should be used with great caution because of their associated problems of increased dose dependent death from myocardial infarction.
Thus, there is a great need to develop the CR devices of NFD that are important in the effective management of hypertension. In this study, NFD was loaded in the microspheres before cross-linking and also by soaking the cross-linked microspheres in a saturated drug solution. Microspheres were characterized by FTIR, SEM and DSC techniques.

III.A.2. Results and Discussion

III.A.2.1. FTIR

FTIR confirmed the cross-linking of PVAL-GG IPN matrix. Acetal ring formation due to cross-linking of hydroxyl groups of the polymers with aldehydes of GA shows a peak at 1251 cm⁻¹ (see Figure III.A.1). However, cross-linking at different GA concentrations has increased the intensity of this peak due to the formation of more acetal rings, confirming the cross-linking reaction. Even though the particles are repeatedly washed to remove the unreacted GA, a peak observed at 1733 cm⁻¹ is due to the presence of the unreacted aldehyde in the polymer [15]; however, in toluene concentrated GA, this peak is shifts to 1722 cm⁻¹. Thus, FTIR suggests the involvement of a single aldehyde group of GA while the free aldehyde groups may still be present in the pendant form.

III.A.2.2. Differential Scanning Calorimetry

The PVAL-GG empty microspheres presented in Figure III.A.2 exhibit endothermic peaks at around ~ 86-100°C and ~306-312°C. A shift in endothermic peaks observed at higher temperatures is attributed to an increased cross-linking. The heat of enthalpy i.e., ΔH (given in J/g), shows an increase with increasing cross-linking due to the high energy required to break the highly cross-linked polymer network. This also confirms the formation of highly crystalline microspheres with an increase in cross-linking. Crystalline nature of polymer further influences water uptake and other physical properties.
DSC was performed on the NFD-loaded microspheres before cross-linking and after cross-linking (i.e., by soaking). These are presented in Figure III. A. 3. For NFD, a sharp endothermic peak is observed at \( \sim 173^\circ C \); this peak was also observed in the NFD-loaded microspheres, but the peak magnitude was smaller in the PVAL-GG microspheres when loading was done by soaking in the NFD saturated methanolic solution than when compared to the drug-loaded microspheres before cross-linking; this indicates a less crystalline nature of NFD in the microspheres. This explains the effect of drug loading method on the nature of the drug in the microspheres.

**III.A.2.3. Microscopic Study**

Particle size of PVAL-GG microspheres was measured by using an optical microscopy; these data are presented in Table III.A.1. The particle sizes range from 260 to 300 \( \mu m \) (narrow size distribution) for all the formulations. Particles are spherical with rough surfaces as seen by SEM photographs (Figure III A.4). However, the SEM pictures do not indicate any porous structure and no drug crystals were appeared on the surface.

**III.A.2.4. Drug Loading**

Drug loading in PVAL-GG matrix was done by two methods: (i) during cross-linking and (ii) soaking in the presence of drug solution (after cross-linking). Entrapment efficiency for the first type of microspheres presented in Table III.A.1 range from 20 to 62 %. For formulations where drug is loaded before cross-linking, the drug might be leached out in liquid paraffin during cross-linking. Increase in initial loading of NFD also increased the entrapment efficiency. The % loadings of NFD were 11.01, 8.69 and 7.12 respectively, for the PVAL-GG microspheres cross-linked with 5, 10, and 15 mL of GA when soaked in drug.
saturated methanol solution for three days. The extent of drug loading decreased
with an increase in cross-linking and this may be due to a decrease in water uptake
by the microspheres.

III.A.2.5. Transport Studies

Dynamic swelling of PVAL-GG matrices was followed by measuring the
change in microsphere diameter with time, \( D_t \) using an optical microscope. Figure
III.A.5 presents the dynamic swelling data vs time wherein, an increase in cross-
linking leads to a decrease in water uptake. Due to the formation of a dense
macromolecular network; FTIR and DSC also support this observation. Molecular
transport of liquids depends on polymer swelling. For instance, a loose network
absorbs larger amount of liquid and produce higher swelling. Swelling results are
important in understanding drug release characteristics.

In order to substantiate the effect of swelling on release kinetics, dynamic
swelling results have been fitted to an empirical equation proposed by Robert et
al., [5].

\[
\frac{D_t}{D_\infty} = k t^n 
\]  

(III.A.1)

Here, \( D_t \) is change in microsphere diameter at time, \( t \) and \( D_\infty \) is the equilibrium
diameter i.e., fully swollen microsphere. The value of \( n \) indicates the type of
transport mechanism. Using the least squares method, the values of \( n \) and \( k \) have
been estimated, but only the values of \( n \) are included in Table III.A.2. The values
of \( n \) increase with increasing cross-linking, indicating the change of transport from
Fickian to non-Fickian. Normalized values of equilibrium diameter, \( D_\infty/D_0 \) (where
\( D_0 \) is the original diameter) decrease from 2.21 to 1.53 with increasing cross-
linking indicating that more tightly cross-linked matrix does not expand in the
presence of water as much as the loosely cross-linked matrix.
III.A.2.6. In-Vitro Release

To understand the release kinetics of NFD-loaded PVAL-GG IPN microspheres, the *in-vitro* release study was carried out in 6.8 pH phosphate buffer containing 0.05 % polysorbate-80 (to maintain perfect sink conditions). These experiments were performed in triplicate and the average values were used for graphical presentation and data treatment. The standard deviations were less than 5 % in all the cases. Figures III.A.6, III.A.7 and III.A.8 display the release profiles of the cross-linked PVAL-GG microspheres loaded with 10, 20 and 30 % of NFD. These results exhibit a pronounced effect of cross-linking on drug release kinetics. As the solvent uptake by the microspheres decreases with an increase in cross-linking and additional support also comes from DSC wherein it is shown that an increase in cross-linking shows an increase in crystallinity and hence, rigidity of the IPN there by hindering the release. Even though water uptake by the microspheres reaches equilibrium very fast (i.e., within 10 to 15 min) the release continues for several hours indicating that the release is governed mainly by the diffusion mechanism.

Effect of drug loading exhibits a significant effect on the release profiles. Upon comparing the release profiles of NFD-7, NFD-8 and NFD-9 formulations with NFD-4, NFD-5 and NFD-6 (Figures III.A.7 and III.A.8), we found a considerable decrease in drug release with increasing drug loadings. This may be due to the formation of larger NFD crystalline domains in PVAL-GG microspheres at higher loadings. Benita et al., [16] reported similar findings for the NFD-loaded polyacrylate microspheres. Gander et al., [11] in a study of cross-linked PVAL matrices loaded with proxyphyllin reported that the release is governed by both drug diffusion and polymer relaxation due to solvent penetration. For NFD-1, NFD-2 and NFD-3 (i.e., with 10 % initial drug loading), release is fast and about 70% of the drug is released within 30 min. The final drug contents in these microspheres were quite small (i.e., 2.0 to 2.6 %) producing large pores in the
microspheres, which enhances the water uptake. Thus, a rapid release of NFD occurs at lower drug loadings. Similar findings have been reported by Steendam and Lerk [17] wherein, with < 10% of theophylline loading, a rapid release was observed.

The release profiles of formulations loaded with NFD by soaking method are presented in Figure III.A.9. The % release data of PVAL-GG cross-linked with 5 mL of GA are higher than those observed for the PVAL-GG cross-linked matrices with 10 mL or 15 mL of GA. The lowest values of % release for the PVAL-GG matrices cross-linked with 15 mL of GA are due to the rigidity of the IPN polymer. In these formulations biphasic release was observed with an initial burst release and with subsequent delayed release for more than 8 h (see Figure III.A.9). In Figure III.A.10, a comparison is made between the directly loaded formulations and those prepared by the soaking method. Release from the soaked formulations is fast when compared to those formulations where NFD is directly loaded at the time of cross-linking (Figure III.A.10.). A fast release of NFD from the soaked matrices may be due to the less crystalline nature of the drug as evidenced from the DSC analysis (Figure III.A.3.). The other possible explanation is that when the drug was loaded before cross-linking, it might have been entrapped within the IPN matrix and thus hindering the drug release. On the other hand, when NFD is loaded by the soaking method, the drug gets concentrated in low cross-linked regions of the matrix [12]. Similar results have been reported by Peppas and Mongia [18] for theophylline loaded in PVAL cross-linked matrices before and after freezing/thawing.

Analogous to (Eq. III.A.1), the release kinetics data have also been analyzed by an empirical equation proposed by Ritger and Peppas [19] and Peppas [20]:

$$\left( \frac{M_t}{M_\infty} \right) = k_1 t^n$$ (III.A.2)
Here, \((M/M_\infty)\) represents fractional drug release at time \(t\), \(k_1\) is a constant characteristic of the drug-polymer system and \(n\) is an empirical parameter that characterizes the release mechanism. For instance, a value of \(n = 0.5\) indicates the presence of Fickian transport, while \(n = 1.0\) indicates Case II transport. The intermediary values ranging between 0.5 and 1.0 are attributed to anomalous transport \([21,22]\). Least squares analyses of the fractional release data along with the values of correlation coefficient, \(r\) are presented in Table III.A.3.

Quite lower values of \(n\) are found for NFD-1, NFD-2 and NFD-3 formulations and hence, these are not included in the Table III.A.3, whereas for NFD-4 to NFD-7 formulations the \(n\) values range between 0.267 and 0.424. For polydisperse spherical systems, the values of \(n\) will be lower than the expected Ritger and Peppas \([19]\) reported smaller \(n\) values of 0.3 and 0.45 for Fickian and Case II transport, respectively. Still smaller values of \(n\) have been reported by Shukla et al., \([23]\) for the cross-linked starch-urea formaldehyde matrices loaded with carbofuran. Based on these observations, the release in the present systems follows the Fickian mode of transport. For the NFD-8 and NFD-9 formulations, \(n\) values are respectively, 0.650 and 0.640, indicating non-Fickian or Case II transport. The values of \(n\) are dependent upon the extent of drug loading. For instance, \(n\) values increase with increasing the % drug loading. On the other hand, with NFD-I, NFD-II and NFD-III, in which drug is loaded by the soaking method, the \(n\) values range between 0.260 and 0.348. The values of \(n\) calculated from Eq. (III.A.1) presented in Table III.A.2 and those computed form Eq. (III.A.2) presented in Table III.A.3, are not comparable. In former case the exponent values are computed from the dimensional response in the radial direction, which is fully governed by the cross-linking i.e., polymer chain relaxation. Where as in the later case the exponent is computed for the drug release, which is not only influenced by the water uptake, but also depends upon the drug diffusion.
IIIA.2.7. Diffusion Coefficients

Diffusion data of the drug-loaded microspheres are very important in the quantitative understanding of the release kinetics. In the literature of membrane science, diffusion coefficients have been calculated for variety of geometries [24,25]:

In the present research, apparent diffusion coefficients, $D$ of NFD from the PVAL-GG IPN hydrogel microspheres, were calculated from the release data in the range $0.6 < \frac{M}{M_{\infty}} < 1.0$, by considering the transport through the fully swollen hydrogel (wherein drug release is mainly by diffusion) using the following relation [26].

$$\frac{M_t}{M_{\infty}} = 1 - \frac{6}{\pi^2} \exp\left(\frac{\pi^2 D t}{r^2}\right)$$ (III.A.3)

Here, $r$ is the average diffusional distance, which corresponds to the radius of the fully swollen microsphere. The values of diffusion coefficients are also included in Table III.A.3. These data show a wide variation for the microspheres depending upon the extent of cross-linking. For instance, $D$ decreases with increasing cross-linking as well as drug loading. The same dependency is also observed for the soaked hydrogel microspheres.

III.A.3. Summary

PVAL-GG full-interpenetrating network microspheres were successfully prepared by cross-linking with glutaraldehyde for the controlled release of NFD. The drug was loaded before cross-linking as well as by the soaking method (after cross-linking). The drug release from PVAL-GG IPN microspheres can be manipulated by varying the extent of cross-linking. The release of the drug from
these microspheres is initially by the polymer relaxation process and the later part of the release from the swollen polymer is mainly controlled by the diffusion mechanism. By loading a higher amount of NFD before cross-linking, it is possible to achieve prolonged release for more than 8 h. On the contrary, the lower drug-loaded microspheres exhibit a quick release of NFD. The better drug loading can also be achieved by soaking method and shows acceptable controlled release profiles for NFD. In conclusion, the PVAL-GG IPN microspheres are useful in the CR of nifedipine.
III.A.4. Literature Cited


Figure III.A.1. FTIR spectra of PVAL-GG empty microspheres cross-linked with GA (A) 5 mL, (B) 10 mL and (C) 15 mL.
Figure III.A.2. DSC thermograms of the PVAL-GG empty microspheres cross-linked with: GA (A) 5 mL, (B) 10 mL and (C) 15 mL.
Figure III.A.3. DSC thermograms of the cross-linked PVAL-GG microspheres (A) NFD-5, (B) NFD-II and (C) pure NFD.
Figure III.A.4. Scanning electron microscopic photographs of the cross-linked PVAL-GG empty microspheres: (A) NFD-8 and (B) same as (A) at higher magnification.
Figure III.A.5. Normalized diameter of PVAL-GG cross-linked microspheres as a function of swelling time for GA (●) 5 mL, (▲) 10 mL and (■) 15 mL.

Figure III.A.6. *In-vitro* percent cumulative release vs time for the NFD-loaded PVAL-GG microspheres: (O) NFD-1, (▲) NFD-2 and (■) NFD-3.
Figure III.A.7. In-vitro percent cumulative release vs time for the NFD-loaded PVAL-GG microspheres: (O) NFD-4, (▲) NFD-5 and (■) NFD-6.

Figure III.A.8. In-vitro percent cumulative release vs time for the NFD-loaded PVAL-GG microspheres: (O) NFD-7, (▲) NFD-8 and (■) NFD-9.
Figure III.A.9. *In-vitro* percent cumulative release vs time for the PVAL-GG microspheres loaded with NFD by the soaking method: (O) NFD-I, (▲) NFD-II and (■) NFD-III.

Figure III.A.10. Comparison of release profiles of PVAL-GG microspheres loaded with NFD before cross-linking (O) NFD-7, (▲) NFD-8 and (■) NFD-9 and by the soaking method (+) NFD-I, (♦) NFD-II and (□) NFD-III.
### Table III.A.1
Initial Drug Loading, % Entrapment Efficiency and Particle Size of the Cross-linked PVAL-GG Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Amount of Cross-linking Agent (mL)</th>
<th>Initial % Drug Loading (w/w)</th>
<th>% Entrapment Efficiency</th>
<th>Particle Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFD-1</td>
<td>5</td>
<td>10</td>
<td>27.0</td>
<td>273</td>
</tr>
<tr>
<td>NFD-2</td>
<td>10</td>
<td>10</td>
<td>22.3</td>
<td>269</td>
</tr>
<tr>
<td>NFD-3</td>
<td>15</td>
<td>10</td>
<td>20.6</td>
<td>293</td>
</tr>
<tr>
<td>NFD-4</td>
<td>5</td>
<td>20</td>
<td>49.4</td>
<td>282</td>
</tr>
<tr>
<td>NFD-5</td>
<td>10</td>
<td>20</td>
<td>38.6</td>
<td>290</td>
</tr>
<tr>
<td>NFD-6</td>
<td>15</td>
<td>20</td>
<td>37.8</td>
<td>291</td>
</tr>
<tr>
<td>NFD-7</td>
<td>5</td>
<td>30</td>
<td>54.2</td>
<td>294</td>
</tr>
<tr>
<td>NFD-8</td>
<td>10</td>
<td>30</td>
<td>61.0</td>
<td>300</td>
</tr>
<tr>
<td>NFD-9</td>
<td>15</td>
<td>30</td>
<td>62.2</td>
<td>310</td>
</tr>
</tbody>
</table>

*a Toluene concentrated with GA.

*b Expressed as % of dry mass of the polymer.

### Table III.A.2.
Results of Transport Studies Performed on Empty Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Cross-linking Agent Added (mL)</th>
<th>Equilibrium Normalized Diameter ( (D_e/D_o) )</th>
<th>Exponent ( n ) of Eq.(III.A.1)</th>
<th>Correlation Coeff. ( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFD-I</td>
<td>5</td>
<td>2.21</td>
<td>0.48</td>
<td>0.997</td>
</tr>
<tr>
<td>NFD-II</td>
<td>10</td>
<td>1.93</td>
<td>0.54</td>
<td>0.988</td>
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<tr>
<td>NFD-II</td>
<td>15</td>
<td>1.53</td>
<td>0.60</td>
<td>0.999</td>
</tr>
</tbody>
</table>

CHAPTER III
Table III.A.3
Release Kinetics Data of NFD-Loaded PVAL-GG Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>n</th>
<th>r</th>
<th>$D \cdot 10^6$ (cm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFD-1</td>
<td>—</td>
<td>—</td>
<td>110.6</td>
</tr>
<tr>
<td>NFD-2</td>
<td>—</td>
<td>—</td>
<td>47.87</td>
</tr>
<tr>
<td>NFD-3</td>
<td>—</td>
<td>—</td>
<td>37.53</td>
</tr>
<tr>
<td>NFD-4</td>
<td>0.318</td>
<td>0.976</td>
<td>9.422</td>
</tr>
<tr>
<td>NFD-5</td>
<td>0.267</td>
<td>0.989</td>
<td>6.079</td>
</tr>
<tr>
<td>NFD-6</td>
<td>0.303</td>
<td>0.984</td>
<td>4.610</td>
</tr>
<tr>
<td>NFD-7</td>
<td>0.424</td>
<td>0.996</td>
<td>8.754</td>
</tr>
<tr>
<td>NFD-8</td>
<td>0.650</td>
<td>0.998</td>
<td>6.839</td>
</tr>
<tr>
<td>NFD-9</td>
<td>0.640</td>
<td>0.980</td>
<td>3.955</td>
</tr>
<tr>
<td>NFD-I*</td>
<td>0.260</td>
<td>0.992</td>
<td>11.31</td>
</tr>
<tr>
<td>NFD-II*</td>
<td>0.348</td>
<td>0.974</td>
<td>7.599</td>
</tr>
<tr>
<td>NFD-III*</td>
<td>0.278</td>
<td>0.999</td>
<td>4.255</td>
</tr>
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</table>

*a Drug loaded by soaking method
III.B. Cross-Linked Guar Gum Grafted Acrylamide Hydrogel Microspheres for the Controlled Release of Nifedipine and Verapamil Hydrochloride

Abstract: This section presents results on the syntheses of guar gum (GG) grafted acrylamide copolymer (GG-g-AAm), which is cross-linked with glutaraldehyde to develop the hydrogel microspheres by water-in-oil (W/O) emulsification method. Two antihypertensive drugs: verapamil hydrochloride (VRP) (water-soluble) and nifedipine (NFD) (water-insoluble) were loaded into the hydrogel microspheres either during cross-linking by dissolving it in the reaction medium or after cross-linking by the soaking technique. The microspheres were characterized by FTIR, DSC, TGA, equilibrium water uptake and dynamic swelling studies. The microspheres are spherical in shape with smooth surfaces. Dynamic swelling experiments indicated that with an increase in cross-linking water transport deviates from Fickian to non-Fickian. The in-vitro drug release depends upon the extent of cross-linking, the amount of drug loading, nature of the drug molecule and the method of drug loading. Even though initially the drug release follows swelling-controlled process at later stages, diffusion dominates. Transport parameters have been calculated and the results are discussed in terms of the nature of the drug and the polymer.


CHAPTER III

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III.B.1. Introduction

Hydrogels are the three-dimensional network polymers that are known to swell in water. In the swollen state, they become soft and rubbery, resembling a living tissue, and some of them possess excellent biocompatibility [1]. Thus, polymeric hydrogels are of considerable interest as biomaterials in drug delivery research [2-6]. Water uptake properties of the hydrogels are important because these can be monitored to control the release of drugs by modifying their structures. However, the hydrogel forming ability of cross-linked natural polymers like gelatin, starch, gums and chitosan has been documented in the earlier literature [7-9]. Through graft copolymerization, it is possible to modify the structure of the natural polymers to make them more attractive as biomaterials in CR applications.

This section reports, the synthesis of graft copolymer (GG-g-AAm) of natural GG with acrylamide (AAm). GG, a plant polysaccharide, has many advantages such as low cost, easy availability and higher propensity to form gels at lower concentration. The GG-based formulations developed earlier for the CR of antihypertensive drugs have yielded successful clinical trials [10,11]. The structure of GG can be modified to tailor its properties for useful CR application. Earlier, Tayal et al., [12] shown 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, borax cross-linked GG was not very successful due to its high swelling in the presence of gastric and intestinal fluids. Rubinstein and co-workers [13,14] have reported the CR systems of GG cross-linked with glutaraldehyde (GA) and phosphate for applications in colon targeting. Recently, Soppimath et al., [15] reported the cross-linked IPN microspheres of poly(vinyl alcohol)-GG for the CR of nifedipine.

In an ongoing program of research to develop the polymeric microspheres for the CR of antihypertensive drugs [15-18], the GA cross-linked hydrogel
microspheres of GG-g-AAm, have been prepared for the CR of VRP and NFD; both of these are antihypertensive drugs belonging to the class of calcium channel blockers. Bioavailability of NFD is low due to the first-pass hepatic metabolism with a short biological half-life of 3-4 h; its antihypertensive effect lasts only up to few hours [19]. In the literature, 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. route due to its low biological half-life of 4.2 h. Several studies [21-23] have been reported on the iontophoretic transdermal permeation of VRP to avoid its bioavailability problems. This section presents the results on the development of cross-linked GG-g-AAm hydrogel microspheres for the CR of NFD and VRP.

The hydrogel microspheres developed have been characterized by FTIR to confirm grafting and cross-linking reactions. SEM was used to study the particle shape and surface morphology. The effect of water transport and swelling of the microspheres have been studied to understand their in-vitro release characteristics. Diffusion as well as other related parameters like penetration front velocity \( (u) \) and swelling interface number \( (Sw) \) have been calculated to discuss the mechanism of drug release [24-27].

### III.B.2. Results and Discussion

Graft copolymerization of GG with acrylamide was attempted by Ce (IV) catalyzed free radical reaction. The chelate complex formed between the -OH group of GG decomposes to generate free radical site, facilitating the grafting reaction at the active site of GG with acrylamide monomer. The reaction scheme is shown in Figure III.B.1. Owen and Shen [28] observed that when the monomer concentration is more than 2.0 M, then the homopolymer is likely to be formed. In
order to minimize the formation of acrylamide homopolymer, a monomer concentration of 0.12 M was used giving a grafting efficiency of 81.12%.

![Chemical structure of guar gum and acrylamide](image)

**Figure III.B.1. Synthesis and Cross-linking Reaction of GG-g-AAm**

**III.B.2.1. FTIR Analyses**

Grafting reaction between acrylamide and guar gum was confirmed by FTIR (see Figure III.B.2). A sharp peak observed at ~1659 cm\(^{-1}\) due to the carbonyl group of amide moiety of the grafted acrylamide chain for GG-g-AAm is not observed in the spectrum of GG (Figure III.B.2.A and III.B.2.B). The -NH\(_2\) stretching, appearing as a shoulder band at ~3200 cm\(^{-1}\) in case of 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 AAm and GG.

FTIR spectra of the cross-linked microspheres are presented in Figure III.B.2.C. During cross-linking, GA might have reacted with -OH groups of the graft copolymer through the formation of ether linkages. Hence, the appearance of a sharp peak at \(\sim1245\) cm\(^{-1}\) in the spectra of the cross-linked microspheres confirms the formation of more ether linkages. This is further supported by the presence of a sharp high intensity peak due to -CH\(_2\) group of alkyl chain due to cross-linking.

III.B.2.2. Thermal Analyses

TGA thermograms of GG, GG-g-AAm and of the cross-linked microspheres are presented in Figure III.B.3. The observed initial mass loss up to 120\(^\circ\)C may be due to the presence of moisture, solvents and unreacted cross-linking agents or monomers. However, no mass loss occurred at the later stage i.e., up to \(\sim240\)\(^\circ\)C. In the case of GG, a sharp mass loss of about 50-55 % is observed between 230\(^\circ\) and 350\(^\circ\)C, and this may be attributed to a loss of hydroxyl group of GG as water molecules. For the GG-g-AAm grafted copolymer, mass loss was small initially, but at a later stage, it was constant. However, the grafted copolymer had retained a 50 % of initial mass until 400\(^\circ\)C. The % residual mass of GG-g-AAm was higher than observed for GG at 550\(^\circ\)C. This supports that modification of GG by grafting with AAm renders GG thermally more stable. With the cross-linked microspheres, a similar thermal behavior as that of GG-g-AAm was observed around \(\sim320\) to 490\(^\circ\)C. However, no sharp mass loss is observed at 490\(^\circ\)C and the matrix maintained a mass of \(\sim26\) % at 600\(^\circ\)C. This may be due to the formation of a rigid network, making it thermally more stable.
DSC was used to characterize the cross-linked hydrogels. DSC thermograms of the cross-linked microspheres in the temperature range of 50 to 300°C are presented in Figure III.B.4. The GG-g-AAm empty microspheres exhibit endothermic peaks in the range between ~75 - 100°C and ~270 - 300°C. As shown in Figure III.B.4, a shift in endothermic peaks at higher temperatures is observed for microspheres prepared with higher concentration of GA. The values of specific heat of fusion ($\Delta H$) indicate that the energy required for the fusion of polymer increased with an increase in cross-linking. These observations support 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 structure.

**III.B.2.3. Microscopic Study**

The GG-g-AAm microspheres are almost spherical in shape as indicated by SEM photographs (Figure III.B.5.A). The surfaces are smooth without porous structure (see Figure III.B.5.B). Particles have the mean diameter ranging from 391 to 594 µm (see data in Table III.B.1) and are free flowing without 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, 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., [29] at higher cross-link densities in the case of poly(vinyl alcohol) hydrogels. At the end of the cross-linking process, the rejected "sol fraction" during polymer shrinkage was found to emulsified into the oil phase.

Size of the NFD-loaded microspheres presented in Table III.B.2 depends upon the method of drug loading as well as the extent of drug loading (entrapment efficiency). For the NFD-loaded microspheres, during cross-linking, the particles
size increased from 474 to 744 μm with an increasing amount of NFD loading from 10 to 20%. Katti Krishnamurti [30] observed similar trends for the cross-linked albumin microspheres. 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 hinder breaking of the dispersed phase into smaller size particles during emulsification. The other explanation is that the NFD particles might have occupied the free volume spaces within the gel matrix, thereby hindering the inward shrinkage of the polymer chain during cross-linking.

**III.B.2.4. Encapsulation Efficiency**

The cross-linked GG-g-AAm microspheres have been loaded with VRP and NFD before cross-linking and by the soaking method i.e., by soaking the microspheres in the saturated solutions of drugs. When 10 % (w/w) of VRP was loaded before cross-linking, the observed loading efficiency is about 10 %. This may be due to the shrinkage in polymer chain during cross-linking. Along with the sol fraction, VRP might have oozed out giving lower encapsulation efficiency. Since 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 III.B.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, drug loading is clearly influenced by the extent of equilibrium water uptake of the matrices (see Table III.B.1).
**III.B.2.5. Equilibrium Water Uptake**

Equilibrium uptake of the cross-linked microspheres exerts a profound influence on their release rates [15]. The % equilibrium water uptake data of the cross-linked empty microspheres presented in Table III.B.1 indicate that as the amount of GA increased from 2.5 mL to 7.5 mL, a significant decrease in equilibrium water uptake from 307 to 128 % is observed. At lower amount of GA (i.e., lower cross-link density), the network is loose and has a high hydrodynamic free volume to accommodate more number of solvent molecules, thereby inducing the matrix swelling. The water uptake in hydrogels depends upon the extent of hydrodynamic free volume and the availability of hydrophilic functional groups for 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 network due to cross-linking.

When drug is loaded by the soaking method, water uptake remains similar to the unloaded microspheres, indicating that the amount of drug loaded is small to exhibit any effect on water uptake by the microspheres. The NFD-loaded microspheres during cross-linking exhibited significantly lower equilibrium % water uptake when compared to the unloaded microspheres (see Table III.B.2) probably because of the hydrophobic nature of NFD, which acts as an inert filler, thereby restricting the 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 [31,32]. In the present study, an increase in loading of NFD resulted in a decrease of equilibrium water uptake, further supporting that NFD acts as a filler with the hydrogel matrix used.
III.B.2.6. Swelling-Induced Transport

Molecular transport is closely related to swelling as well as drug release characteristics. When a glassy hydrogel is exposed to water, the latter will diffuse into it by creating a solvent front, which moves at a front velocity, \( u \). During this process, the migrating water molecules move across the glassy/rubbery interface, thereby inducing a change in the wall thickness of the material with a lapse of time. The drug containing water front moves quite rapidly in the rubbery region than in the glassy region. Thus, the rate of drug release through the rubbery region depends upon the amount of liquid uptake as well as the rate of drug diffusion through the rubbery layer of the gel matrix [24-27]. Therefore, it is important to investigate the transport of drug containing water molecules into the GG-g-AAm cross-linked hydrogel microspheres using the dynamic swelling data.

Swelling was monitored by measuring the change in the microsphere diameter, \( D_n \), as a function of time using an optical microscope. Figure III.B.6 displays the normalized diameter, \( D/D_0 \) (where \( D_0 \) is initial diameter of the microsphere) as a function of time for different added amounts of GA. With increasing cross-linking, a decrease in water uptake is observed. However, the shapes of the swelling curves depend upon the amount of GA added. For instance, when 2.5 mL of GA was added, swelling increases linearly up to 5 min and then levels off. As the amount of GA increases, swelling capacity of the microspheres decreases considerably. The results of equilibrium swelling diameter, \( D_e \), normalized to the original diameter, \( D_0 \), are presented in Table III.B.3. These results support that the more tightly cross-linked matrix does not expand in water as much as the loosely cross-linked matrix.

Swelling results presented in Figure III.B.6 have been fitted to the empirical relation i.e., (III.A.1) [24-27]. The least squares estimations of the values of \( n \) from the dynamic swelling data fitted to Eq. (III.A.1) are presented in Table III.B.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 from Fickian to non-Fickian mode.

### III.B.2.7. Dimensional Response and Evaluation of Transport Parameters

Dimensional changes of the microspheres due to swelling with time have been analyzed to compute the diffusion coefficient, \( D_\nu \) of the water molecules using the theory proposed earlier by Harogoppad and Aminabhavi [33]:

\[
M_t = \left( \frac{W_t}{W_0} \right) = \left( \frac{4W_\infty}{d_0 W_0} \right) \left( \frac{D_\nu}{\pi} \right)^{1/2} \left( \frac{t}{t_a} \right)^{1/2} \tag{III.B.1}
\]

Here, \( M_t \) is mass % uptake, \( W_0, W_t \) and \( W_\infty \) are respectively, the mass of the microsphere at zero time \( t_0 \), at time, \( t \) and at equilibrium time \( t_\infty \); \( d_0 \) is the initial diameter of the microspheres and \( \pi \) is a geometric parameter. Assuming that an increase in volume at any time is proportional to the mass of the liquid transported until that time, so that: \( W_t \propto (\text{swollen volume of the microsphere at time } t - \text{ initial volume of the microsphere}) \). The mass gain, \( W_t \) at time, \( t \) and at equilibrium time, \( W_\infty \) are calculated using:

\[
W_t = b\pi (r_t^3 - r_0^3) \tag{III.B.2}
\]
\[
W_\infty = b\pi (r_\infty^3 - r_0^3) \tag{III.B.3}
\]

where \( b \) is a proportionality constant, \( r_0, r_t \) and \( r_\infty \) are, respectively the radii of spherical microsphere, initially at zero time, after lapse of time, \( t \) and at equilibrium time, \( t_\infty \). By substituting the values of \( W_t \) and \( W_\infty \) into Eq. (III.B.1), one obtains
where \( (r_t^3 - r_0^3) \) and \( (r_{\infty}^3 - r_0^3) \) represent, respectively the volume changes \( (\Delta V_t) \) at time, \( t \) and at equilibrium time \( (\Delta V_{\infty}) \). Thus, Eq. (III.B.4) can be rewritten as

\[
\Delta V_t = \left( \frac{4(\Delta V_{\infty})}{d_0} \right) \left( \frac{D_v}{\pi} \right)^{1/2} t^{1/2}
\]  

(III.B.5)

For changes in volume per unit volume, Eq. (III.B.5) becomes

\[
\left( \frac{\Delta V_t}{V_0} \right) = \left( \frac{4(\Delta V_{\infty})}{d_0} \right) \left( \frac{D_v}{\pi} \right)^{1/2} t^{1/2}
\]  

(III.B.6)

Equation (III.B.6) allows us to compute \( D_v \) from the slope of the \( \Delta V/V_0 \) vs \( t^{1/2} \) plots. These results are included in Table III.B.3.

The solvent front velocity, \( u \) of the advancing boundary in the spherical microspheres was calculated using the relation [25,26]:

\[
u = \left( \frac{dV}{dt} \right) \frac{1}{A}
\]  

(III.B.7)

Here, \( dV/dt \) is a 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 III.B.3.

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

III.B.2.8. Drug Release vs Molecular Transport

The release of the drug depends upon its transport across the polymeric matrix. The \textit{in-vitro} release studies of the VRP loaded cross-linked GG-g-AAm were carried out with the dried (glassy) as well as fully swollen (rubbery) microspheres in 0.1N HCl. Average values of the triplicate measurements are used for the graphical presentations as well as for the mathematical treatment. In all cases, the 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, respectively in Figures III.B.7 and III.B.8. As shown in Figure III.B.7, the release of VRP occurs with an initial burst, which is probably due to a sudden release of VRP from the surface of the microspheres. At later time, the release becomes slow and continues up to 4-5 h. A considerable decrease in drug release with a decrease in burst release was observed at higher cross-linking. Release of VRP from the initially swollen microspheres shown in Figure III.B.8 is relatively quick when compared to the initially dried glassy microspheres. In the case of dried microspheres, initially the polymer was glassy and later, it becomes rubbery. Hence, the polymer chain relaxation governs the transport process and water diffusion into the glassy region of the polymer becomes slower than that of the rubbery region. Therefore, the release from the dried polymer is relatively slower.

The release profiles of NFD-loaded microspheres prepared by the soaking method are presented in Figure III.B.9. Here, the release is somewhat biphasic with an initial burst effect, probably due to a release of the surface adhered drug molecules from the microspheres. However, the subsequent release is slower, which continued for > 10 h. Cross-linking has an effect on drug release. In case of
microspheres cross-linked with 5 mL of GA and when the drug was loaded during cross-linking, the release showed a dependency on the amount of drug loaded (see Figure III.B.10.). In formulations with lower drug loading (5 % w/w), NFD was released quicker when compared to higher drug-loadings. At lower drug loading, large pores will be formed rapidly in the microspheres, which may increase the water uptake and consequently, release the drug. A slight increase in drug release is observed when drug loading was increased from 10 to 20 % (w/w). Similar findings have been reported [34] for theophylline-loaded formulations when drug loading was < 10 %.

In order to establish a link between drug release and molecular transport parameters, fractional drug release data, $M/M_{\infty}$ have been analyzed using the empirical equation (III.A.2) [35]. The values of $n$ for VRP are presented in Table III.B.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 swollen microspheres follows Fickian diffusive transport. These results agree with those published by Korsmeyer and Peppas [29], and confirm that the release from the rubbery polymer is Fickian while it follows the anomalous/or non-Fickian trend for the glassy polymer. The kinetic rate constants, $k$ for the release, which incorporates the overall solute diffusion coefficient and geometric parameters of the system, decrease with a decrease in cross-linking. The $k$ values for the dried microspheres are smaller than the swollen microspheres because the release from the glassy polymer is slower than for the rubbery polymer.

The release of NFD is non-Fickian because the values of $n$ are > 0.5 (see Table III.B.5). The values of $n$ increase with an increase in drug loading as well as increase in cross-linking for those formulations that are loaded with NFD before and after cross-linking. The increase in $n$ from 0.5 to 0.6 is mainly attributed to an increase in cross-linking, which is responsible in changing the water transport mechanism from Fickian to non-Fickian. The release occurs by a non-Fickian
transport for the NFD-loaded formulations before cross-linking and the value of \( n = 0.88 \) for the 20% loading is suggestive of the Case-II transport at higher drug loading.

Water transport into microspheres and glassy-to-rubbery transition of the matrices are relatively fast processes (of the order of minutes) when compared to the slow drug release, which normally takes more than 8-12 h. Thus, one may assume that drug release from the cross-linked matrices is mainly diffusion-controlled and hence, it becomes essential to estimate the apparent diffusion coefficient, \( D \) by using the simplified relation to study drug release from spherical microspheres. The values of \( D \) calculated for the VRP loaded formulations in dried and swollen microspheres were determined by using the following equation assuming initial time approximation [36]

\[
\frac{M_t}{M_\infty} = \left( \frac{36D_t}{\pi r^2} \right)^{\frac{1}{2}} - \left( \frac{3D_t}{r^2} \right)
\]

(III.B.8)

where \( r \) is radius of the microsphere. In the calculation, the fractional release, \( M_t/M_\infty \) from 0 to 0.4 was taken into account. The results of \( D \) presented in Table III.B.4 decrease considerably with an increase in cross-linking of the network polymer. Thus, drug diffusion is dependent upon the rate of water permeation i.e., the extent of cross-linking. However, drug diffusion in the swollen microspheres is higher than observed in the dried (glassy) microspheres because diffusion of water in rubbery microspheres is fast when compared to the polymer chain relaxation processes.

The apparent diffusion coefficients of NFD as calculated from (Eq. III.B.8) are included in Table III.B.5. The values of diffusion coefficients exhibit a relationship with the extent of cross-linking and % drug loading. An increase in GA from 5.0 to 7.5 mL has shown a decrease in diffusion coefficients from 2.79 x
$10^{-6}$ to $0.98 \times 10^{-6}$ cm$^2$/s. The decrease in diffusion values with an increase in drug loading from 5 to 10% for the microspheres loaded with drug before cross-linking supports the fact that at lower levels of drug loading, a faster release occurs.

**III.B.2.9. Dimensionless Analysis**

In order to explain the observed non-Fickian behavior and to understand the mechanism of solute release from a dynamically swelling polymer, an effort was made to use the dimensionless analysis approach as suggested by Peppas and Franson [37]. Thus, a dimensionless parameter, called swelling interface number, $S_w$ was calculated by using Eq. (III.B.9):

$$S_w = \frac{u \delta_{\text{max}}}{D_s}$$  \hspace{1cm} (III.B.9)

where $u$ is average penetration velocity of the aqueous release medium calculated from Eq. III.B.7, $\delta_{\text{max}}$ is maximum thickness of the swollen microsphere 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, $D_s/\delta_{\text{max}}$, is faster than the rate at which the dissolution media penetrates, then $S_w$ is $< 1$ and the drug release is controlled by swelling phenomenon rather than drug diffusion. On the other hand, if the values of $S_w$ are $\approx 1$, then both swelling and diffusion control the drug release and the transport mechanism is non-Fickian. If $S_w$ is $> 1$, then drug diffusion is dominated by a Fickian transport.

In the present systems, values of $S_w$ 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 III.B.4). However, the release may not be completely diffusion-controlled because about 40 to 60% of the drug is released in the transition period of 10-20 min from glassy-to-rubbery state. This suggests that swelling also controls the release, but the subsequent release is
controlled by both polymer relaxation and drug diffusion. Values of $Sw$ for the NFD-loaded microspheres prepared by the soaking method (see Table III.B.5.) are also $> 1$ indicating that the release is dominated by diffusion 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 [25,26] obtained $n = 0.749$ and $Sw = 11.4$ for the cross-linked (pHEMA-co-MMA)-loaded with theophylline. Similarly, Gander et al., [38] obtained $Sw > 1$ for $n > 0.5$ for the cross-linked poly(vinyl alcohol) matrices loaded with proxphylin. Thus, $Sw$ is an important parameter in understanding the mechanism of drug release. However, it is not possible to predict the release mechanism solely on the values of $Sw$. Thus, another dimensionless parameter called Debhorah number could be used to study the release kinetics [26], but this approach is not attempted here.

**III.B.3. Summary**

Guar gum was successfully grafted with acrylamide to give GG-g-AAm which exhibited better thermal stability than guar gum. Cross-linking was done by GA and the hydrogel microspheres were formed by water-in-oil emulsification method. Microspheres with higher encapsulation efficiencies can be obtained for the water-insoluble drug such as NFD. Efficient loading can also be achieved for both VRP and NFD by the soaking method. Molecular transport of solvent and drug 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 becomes slow and continues up to 3-4 h. The fast release during initial stages is attributed to swelling or polymer relaxation in 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, release was slower (>12 h). A zero order release is achieved by loading > 20% (w/w) of the drug. The release in this case is mainly diffusion-controlled rather than swelling-controlled.

The microspheres appear to be good biomaterials for the CR of both water-soluble and water-insoluble drugs. Since VRP has pH-dependant solubility with low and variable bioavailability, the microspheres developed may exhibit uniform drug distribution to avoid the vagaries of gastric emptying and different transit rates [39]. However, the NFD-based formulations can be useful as the CR devices for once a day oral formulation in the effective management of hypertension.
III.B.4. Literature Cited


Figure III.B.2. FTIR spectra of: GG (A) and GG-g-AAm (B)
Figure III.B.2. (contd.) FTIR spectra of: cross-linked GG-g- AAm microspheres (C).

CHAPTER III
Figure III.B.3. TGA thermograms of: GG (A) GG-g-AAm (B) and cross-linked microspheres with 7.5 mL of GA (C).
Figure III.B.4. DSC thermograms of: GG-g-AAm empty microspheres cross-linked with (A) 2.5 mL, (B) 5 mL and (C) 7.5 mL of GA.
Figure III.B.5. Scanning electron microscopic photographs of: GG-g-AAm empty microspheres cross-linked with 5 mL GA (A) and surface photographs of the same (B).
Figure III.B.6. Plot of \( D_t/D_0 \) vs swelling time, \( t \) for GG-g-AAm microspheres cross-linked with: 2.5 mL (▲), 5.0 mL (O) and 7.5 mL (■) of GA.

Figure III.B.7. Release profiles of VRP from the dried GG-g-AAm microspheres cross-linked with: 2.5 mL (▲), 5 mL (O) and 7.5 mL (■) of GA.
Figure III.B.8. Release profiles of VRP from the swollen GG-g-AAm cross-linked with: 2.5 mL, (▲) 5 mL (O) and 7.5 mL (■) of GA.

Figure III.B.9. Release profiles of NFD-loaded GG-g-AAm microspheres by soaking method and cross-linked with: 2.5 mL, (▲) 5 mL (O) and 7.5 mL (■) of GA.
Figure III.B.10. Release profiles of NFD-loaded GG-g-AAm microspheres before cross-linking. Symbols for NFD loadings are: 5 % (▲), 10 % (○) and 20 % (■).
<table>
<thead>
<tr>
<th>Cross-linking Agent Used (mL)</th>
<th>% Water Uptake</th>
<th>% VRP&lt;sup&gt;a&lt;/sup&gt; Loading</th>
<th>% NFD Loading</th>
<th>Mean Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>307 ± 17.1</td>
<td>15.2 ± 2.1</td>
<td>4.4 ± 0.4</td>
<td>594</td>
</tr>
<tr>
<td>5.0</td>
<td>259 ± 9.4</td>
<td>12.6 ± 1.2</td>
<td>3.7 ± 0.9</td>
<td>424</td>
</tr>
<tr>
<td>7.5</td>
<td>128 ± 10.1</td>
<td>7.9 ± 0.5</td>
<td>2.0 ± 0.2</td>
<td>391</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as % of polymer dry mass

<table>
<thead>
<tr>
<th>% Loading&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Water Uptake</th>
<th>% Entrapment Efficiency</th>
<th>Mean Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>210 ± 19.2</td>
<td>81.1 ± 4.5</td>
<td>474</td>
</tr>
<tr>
<td>10</td>
<td>172 ± 4.1</td>
<td>88.7 ± 2.5</td>
<td>699</td>
</tr>
<tr>
<td>20</td>
<td>162 ± 2.5</td>
<td>90.0 ± 2.1</td>
<td>744</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as % of polymer dry mass
Table III.B.3
Transport Data of Water in GG-g-AAm Microspheres

<table>
<thead>
<tr>
<th>GA Used (mL)</th>
<th>Equilibrium Normalized Diameter ($D_e/D_o$)</th>
<th>$n^a$</th>
<th>$D_e \times 10^5$ (cm$^2$/sec)</th>
<th>$u \times 10^3$ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>1.56</td>
<td>0.46</td>
<td>5.443</td>
<td>6.66</td>
</tr>
<tr>
<td>5.0</td>
<td>1.37</td>
<td>0.66</td>
<td>1.194</td>
<td>4.84</td>
</tr>
<tr>
<td>7.5</td>
<td>1.23</td>
<td>0.78</td>
<td>0.883</td>
<td>4.56</td>
</tr>
</tbody>
</table>

$^a$ Computed from Eq. (III.A.1); the values of $n$ and $D_e$ are estimated by the least squares method at 95% confidence limit.

Table III.B.4
Release Kinetics Parameters of VRP from GG-g-AAm Dried and Swollen Microspheres

<table>
<thead>
<tr>
<th>Amount of GA (mL)</th>
<th>$k^a$ (min$^{-n}$)</th>
<th>$n^a$</th>
<th>$D \times 10^6$ (cm$^2$/s)</th>
<th>$S_w$ Swelling No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried Microspheres</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.132</td>
<td>0.54</td>
<td>11.16</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0.122</td>
<td>0.52</td>
<td>4.51</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>0.096</td>
<td>0.51</td>
<td>3.23</td>
<td></td>
</tr>
<tr>
<td>Swollen Microspheres</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.352</td>
<td>0.27</td>
<td>19.69</td>
<td>30.67</td>
</tr>
<tr>
<td>5.0</td>
<td>0.297</td>
<td>0.28</td>
<td>8.33</td>
<td>35.03</td>
</tr>
<tr>
<td>7.5</td>
<td>0.180</td>
<td>0.39</td>
<td>5.61</td>
<td>42.26</td>
</tr>
</tbody>
</table>

$^a$ Computed from Eq. (III.A.2); the values of $n$ and $D$ are estimated by the least squares method at 95% confidence limit.
<table>
<thead>
<tr>
<th>Amount of GA Loading (mL)</th>
<th>% NFD Loading</th>
<th>$k^a$ (min^{-n})</th>
<th>$n^a$</th>
<th>$D \times 10^6$ (cm^2/s)</th>
<th>Swelling No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Loading by Soaking</td>
<td>2.5</td>
<td>4.4</td>
<td>0.034</td>
<td>0.50</td>
<td>2.79</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>3.7</td>
<td>0.020</td>
<td>0.59</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>2.0</td>
<td>0.016</td>
<td>0.60</td>
<td>0.98</td>
</tr>
<tr>
<td>Drug Loading During Cross-linking</td>
<td>5.0</td>
<td>16.0</td>
<td>0.002</td>
<td>0.88</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>9.0</td>
<td>0.003</td>
<td>0.78</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>3.9</td>
<td>0.010</td>
<td>0.72</td>
<td>2.01</td>
</tr>
</tbody>
</table>

*a Computed from Eq. (III.B.2); the values of $n$ and $D$ are estimated by the least squares method at 95% confidence limit.