CHAPTER-V

Development of Chitosan Based Microspheres for the Controlled Release of Non-Steroidal Anti-Inflammatory Drugs

This chapter deals with the synthesis and characterization of chitosan microspheres either by the use of different crosslinking agents or by grafting of chitosan with poly(acrylamide) followed by crosslinking. Applications of these microspheres for the controlled release of non-steroidal anti-inflammatory drugs viz. diclofenac sodium and indomethacin are discussed. The investigations are presented in two sections.
V.A. Crosslinked Chitosan Microspheres for Encapsulation of Diclofenac Sodium: Effect of Crosslinking Agent

Abstract: Microspheres of chitosan crosslinked with three different crosslinking agents viz., glutaraldehyde, sulfuric acid (SA) and heat treatment have been prepared for the encapsulation of diclofenac sodium. Chitosan microspheres are produced in water in oil (w/o) emulsion followed by crosslinking in the water phase by one of the crosslinking agents. Encapsulation of diclofenac sodium (DS) was carried out by soaking the already swollen crosslinked microspheres in the saturated solution of DS. Microspheres are further characterized by FTIR, x-RD and SEM. The in vitro release studies are performed in 7.4 pH buffer solution. The release data are treated with the empirical equation to investigate the type of release mechanism. Microspheres produced are spherical and have smooth surfaces with the sizes ranging between 40 and 230 μm as evidenced by SEM. The crosslinking of chitosan takes place at the free amino group in all the cases as evidenced by FTIR. This leads to the formation of imine group or ionic bond. Polymer crystallinity increases after crosslinking as determined by x-RD. The method adopted for drug loading into the microspheres is satisfactory and up to 28 to 30 % w/w loading is observed for the sulfuric acid crosslinked microspheres, whereas 23 to 29 and 15 to 23 % of loadings are obtained for the glutaraldehyde (GA)- and heat-crosslinked microspheres, respectively. Among all the systems studied, the 32 % GA crosslinked microspheres have shown the least release i.e., 41 % at 420 min and a maximum release of 81 % at 500 min is shown by heat crosslinking for 3 h. Drug release from the matrices deviates slightly from the Fickian trend.

Results of this section are presented at:
(1) Pharmaceutical Association of the Americas, 24-29 March, 2001 at Orlando, FL, USA and published in
V.A.1. INTRODUCTION

Chitin is a naturally occurring and the second most abundant organic material next to cellulose. Due to its inertness, less attention has been given to chitin than cellulose. Several biomedical applications of chitosan have already been reported\(^1^\)\(^-^\)\(^4\). Deacetylation of chitin yields chitosan, which is relatively reactive and can be produced in numerous forms, such as powder, paste, film, fiber, etc.\(^5^,^6\). Chitosan has some advantages over other polysaccharides due to its nontoxicity and biodegradability without damaging the environment as it is broken down in the human system to harmless products (amino sugars) that can be easily absorbed.

In many studies, chitosan has been crosslinked with glutaraldehyde to make it a rigid polymer to be used as a core material in controlled release (CR) research\(^7\). However, the biological acceptance of such crosslinked products depends upon the amount of hazardous material (crosslinking agent) present in the final products. In order to avoid the use of such toxic crosslinking agents, we have developed a new method to crosslink chitosan by heat treatment, to produce microspheres for the CR of diclofenac sodium (DS).

The advantages of CR formulations containing non-steroidal anti-inflammatory drugs (NSAID) over the conventional dosage forms have been reported; such formulations minimize the serious gastric irritant side effects of the conventional NSAID preparations. In this pursuit, we have undertaken a detailed study to prepare the CR formulations containing DS by using the newly developed polymeric systems to improve upon the therapeutic effects. The microsphere crosslinking mechanism and the topological properties have been characterized by FTIR, x-RD and SEM spectral methods.
V.A.2. RESULTS AND DISCUSSION

V.A.2.1. Matrix Crosslinking and its Characterization

Chitin-/chitosan-based derivatives have been widely used in biomedical applications, since they are biodegradable, nontoxic and biocompatible\textsuperscript{2,3}. In the formulation procedures many toxic substances have been used to cross-link the polymer or to modify its structure chemically. In these steps, there is a good possibility of contamination of the agents in the end polymer product, which may not be biocompatible\textsuperscript{8,9}. In order to prepare the microspheres, it is possible to cross-link the matrices in the presence of sulphuric acid\textsuperscript{10} or glutaraldehyde\textsuperscript{7} or by heat treatment. During such reactions, chemical interactions may take place between the -NH\textsubscript{2} group of chitosan and the -CHO group of GA leading to the formation of imine group i.e., (C=\textit{N})\textsuperscript{11}. The reaction scheme is shown in Figure V.A.1.

When sulfuric acid is used as a crosslinking agent, it forms an ionic bond with the -NH\textsubscript{2} group of chitosan\textsuperscript{12}. Polysaccharides can give rise to a large variety of oxidation products when heated alone or in the presence of dilute acid\textsuperscript{13}. Chitosan, being a polysaccharide, upon heating it forms aldehydic groups and the cross-linking mechanism is quite similar to that of GA-crosslinked microspheres leading to the formation of Schiff base i.e., imine group (see Figure V.A.1). After heat treatment of the matrix, the polymeric chains expand to a large extent so that an immediate deformation of the polymer takes place and after the polymer chain relaxes, the three-dimensional network structure is formed. At higher temperatures, chitosan loses water molecules and forms an imine group (see Figure V.A.1).
Figure V.A.1. Reaction scheme of crosslinking of chitosan by different methods.
These reactions are confirmed by FTIR spectra of the uncrosslinked chitosan and the GA-crosslinked chitosan microspheres (i.e., GA-8, GA-16 and GA-32) are displayed in Figure V.A.2. The peak at 1647 cm\(^{-1}\) in the FTIR spectra of the uncrosslinked chitosan is assigned to the amino absorption peak. In contrast to the spectra of the uncrosslinked chitosan, a new peak appearing at 1567 cm\(^{-1}\) in the spectra of GA-8, GA-16 and GA-32 is due to the formation of -C=\(\text{N}\) (imine) group; however, the intensity of the peak increases with increasing crosslinking, which is evident from the Figure V.A.2. FTIR spectra of the SA-crosslinked microspheres (i.e., SA-20, SA-40 and SA-60) are presented in Figure V.A.3. A strong peak appearing around 1120 - 1250 cm\(^{-1}\) is assigned to the formation of ionic bond between two chitosan chain molecules. Because of this, a broad peak at 2091 cm\(^{-1}\) is attributed to the -NH\(_3^+\) group\(^{12}\). A sharp peak at 622 cm\(^{-1}\) is due to the trace amount of H\(_2\)SO\(_4\) remaining in the polymer matrix even after repeated washings. FTIR spectra of the heat-crosslinked microspheres are quite similar to those of the GA-crosslinked matrices as shown in Figure V.A.4. A characteristic peak at 1579 cm\(^{-1}\) represents the formation of a -C=\(\text{N}\) moiety. This is due to the reaction taking place between -NH\(_2\) and -CHO, which is formed by the oxidation of -CH\(_2\)OH groups of chitosan thereby resulting in the removal of water and thus forming the -C=\(\text{N}\) group.

Crosslinked chitosan microspheres have also been analyzed by x-ray diffraction method. The x-RD tracings of the uncrosslinked chitosan presented in Figure V.A.5 indicate that only one peak appearing at \(\theta\) of 20° is related to a crystal in the chitosan polymer and this result is similar to Samuels\(^{14}\) and Ge, et al.\(^{10}\). In case of the crosslinked chitosan, in addition to the peak observed at 20°, two new peaks appeared at 15° and 23° for the heat-crosslinked, at 11° and 27° for the SA-crosslinked and at 22° and 46° for the GA-crosslinked matrices. This indicates the increased crystallinity of the matrices with an increase in crosslinking. The x-ray diffraction patterns of DS (curve a), microspheres
Figure V.A.2. FTIR spectra of pure chitosan (a), chitosan-crosslinked with GA-8 (b), GA-16 (c) and GA-32 (d).
Figure V.A.3. FTIR spectra of pure chitosan (a), chitosan-crosslinked with SA-20 (b), SA 40 (c) and SA-60 (d).
Figure V.A.4. FTIR spectra of pure chitosan (a), chitosan-crosslinked with H-6 (b), H-4 (c) and H-3 (d).

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Figure V.A.5. x-RD spectra of pure chitosan (a), chitosan-crosslinked with GA (b), SA (c) and Heat (d).
containing DS (curve c) and chitosan (un-crosslinked, curve b) are shown in Figure V.A.6. The characteristic sharp peaks appeared in the x-ray diffraction (curve a) for the pure DS did not appear in the x-ray diffraction (curve c) pattern of DS-loaded crosslinked chitosan microspheres and a similar pattern was observed for the chitosan matrix (curve b). This further confirms the molecular level dispersity of the drug in the microspheres.

Topology and size distribution studies on the microspheres have been carried out by SEM. Typical SEM photographs of SA-, GA- and heat-crosslinked microspheres are presented in Figure V.A.7. An example of the heat-crosslinked microsphere is shown in Figure V.A.8. The microspheres are almost spherical in nature and have smooth surfaces. In order to calculate the mean particle size, SEM photographs were taken at the lower magnification such that the photograph covers about 10 particles in polydisperse region. These results included in Table V.A.1, indicate that the particle size decreases with increasing extent of crosslinking. The GA-crosslinked microspheres have smaller particle size (i.e., between 83.76 and 73.51 μm) than the other microspheres. The SA- and heat-crosslinked microspheres have the particle sizes ranging from 180 to 230 and 40 to 124 μm, respectively.

V.A.2.2. Erosion Studies of the Matrices

In order to understand the nature of crosslinking process, we have studied the erosion of the uncrosslinked polymers in the presence of 2 % acetic acid solution. The microspheres formed by crosslinking with different crosslinking agents are stirred in 1 % acetic acid solution for 6 h. The calculated mass loss of the microspheres represents the % erosion of chitosan. These results given in Table V.A.1 and presented in Figure V.A.9 indicate that erosion decreases with an increase in crosslinking of the matrices.
Figure V.A.6. x-RD spectra of pure DS (a), pure chitosan (b) and GA-crosslinked DS loaded microspheres (c).
Figure V.A.7. Scanning electron micrograph of chitosan-crosslinked with GA (a), SA (b) and heat (c) microspheres.
Figure V.A.8. Scanning electron micrograph of a heat-crosslinked chitosan microsphere.
<table>
<thead>
<tr>
<th>Matrix Type</th>
<th>Crosslinking Agent</th>
<th>Cross Linking Extent</th>
<th>% Erosion</th>
<th>% Equilibrium Swelling</th>
<th>% Encapsulation of DS</th>
<th>Mean Particle Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-8</td>
<td>GA (40 %)</td>
<td>8 %</td>
<td>4.3 ± 0.2</td>
<td>38.0</td>
<td>29.4 ± 0.25</td>
<td>83.8 ± 13.2</td>
</tr>
<tr>
<td>GA-16</td>
<td>16 %</td>
<td>3.01 ± 1.1</td>
<td>31.6</td>
<td>26.6 ± 2.26</td>
<td>78.6 ± 12.8</td>
<td></td>
</tr>
<tr>
<td>GA-32</td>
<td>32 %</td>
<td>2.1 ± 0.89</td>
<td>24.5</td>
<td>23.6 ± 0.49</td>
<td>73.5 ± 11</td>
<td></td>
</tr>
<tr>
<td>SA-20</td>
<td>SA (3.6 N)</td>
<td>20 %</td>
<td>0.91 ± 0.5</td>
<td>39.8</td>
<td>30.1 ± 0.61</td>
<td>230.6 ± 92</td>
</tr>
<tr>
<td>SA-40</td>
<td>40 %</td>
<td>0.73 ± 1.1</td>
<td>37.6</td>
<td>29.2 ± 0.71</td>
<td>198.4 ± 70</td>
<td></td>
</tr>
<tr>
<td>SA-60</td>
<td>60 %</td>
<td>0.53 ± 1.3</td>
<td>35.6</td>
<td>28.4 ± 0.68</td>
<td>180.3 ± 90</td>
<td></td>
</tr>
<tr>
<td>H-3</td>
<td>Heat (90°C)</td>
<td>3 h</td>
<td>4.92 ± 2.1</td>
<td>25.1</td>
<td>23.8 ± 0.77</td>
<td>124.4 ± 27</td>
</tr>
<tr>
<td>H-4</td>
<td>4 h</td>
<td>4.62 ± 1.0</td>
<td>22.3</td>
<td>22.6 ± 1.55</td>
<td>94.8 ± 30</td>
<td></td>
</tr>
<tr>
<td>H-6</td>
<td>6 h</td>
<td>4.32 ± 1.0</td>
<td>8.0</td>
<td>15.1 ± 0.28</td>
<td>40.8 ± 58</td>
<td></td>
</tr>
</tbody>
</table>

**Figure V.A.9.** % Erosion of uncrosslinked chitosan from chitosan microspheres crosslinked with various crosslinking agents.

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Among the three-crosslinking agents used, the sulphuric acid-crosslinked microspheres show the least erosion i.e., 0.53 to 0.91 %, but the GA- and heat-crosslinked microspheres show the erosion of 2.1 to 4.3 % and 4.32 to 4.92 %, respectively. Since the crosslinking of chitosan with SA was carried out at 50°C, this might have resulted in a more rigid polymer structure thereby giving the less erosion when compared to other matrices. The % erosion of heat-crosslinked microspheres is higher than the GA-crosslinked matrices because the latter involves the actual chemical reactions between GA and chitosan and therefore, it is a stronger and rigid matrix than the heat-crosslinked matrices.

V.A.2.3. Drug Loading

The chitosan microspheres are formed by emulsification in the light liquid paraffin followed by crosslinking. The routine method of drug loading in the above mentioned method is dispersing the drug with the polymer solution and followed by emulsification. Here, the polymer solution is prepared in 2 % acetic acid solution in which DS is insoluble. In the present research, drug loading is carried out by soaking the microspheres in a saturated solution of DS followed by washing with distilled water to remove the surface-adhered DS.

Among all the systems studied, the heat-crosslinked microspheres have shown the least swelling i.e., 8-22 % w/w. The GA-crosslinked microspheres exhibited 24-38 % swelling in water, whereas the SA-crosslinked microspheres have shown 35-39 % swelling. These results indicate that in all the cases, the % swelling decreases with an increase in crosslinking. The % loading by this soaking method mainly depends upon the swelling of microspheres in water. In doing so, the drug increases with increase in swelling.

Results of % DS loading in the crosslinked microspheres are also included in Table V.A.1. In all the matrices, the % loading of the drug decreases with increasing crosslinking because the polymer matrices formed at higher crosslinking show lesser swelling. Highest % loading of 28 to 30 % w/w was
observed for the SA-crosslinked microspheres whereas 23 to 29 % and 15 to 23 % of loadings were obtained for the GA- and heat-crosslinked microspheres, respectively.

V.A.2.4. In Vitro Release Study

In vitro release studies are performed in 7.4 pH phosphate buffer. Plots of % release vs time are presented in Figure V.A.10, V.A.11 and V.A.12 for the GA-, SA- and heat-crosslinked chitosan microspheres, respectively. In all the cases, the % release decreases with increasing crosslinking. The release of DS from the microspheres depends upon the type of matrix and their rigidity. The release of the active agent from the matrix involves first swelling and then erosion of the drug. Among all the systems studied, GA-32 crosslinked microspheres have shown the least release (i.e., 41 % up to 420 min), but maximum release, 81% was observed for H-3 at 500 min. However, the % release of SA-crosslinked microspheres shows an intermediary release of 72 % at 435 min.

The in vitro release data have been analyzed using the empirical equation of the type IV.A.1 as discussed earlier to estimate the values of $n$ and $k$. The fractional release ($M_t/M_{\infty}$) up to 60 % of DS at time $t$ are fitted to the above equation and the values of $k$ and $n$ have been calculated by the least squares method. These values are presented in Table V.A.2. In the present systems, the $n$-values are in between 0.47 and 0.61 with the correlation coefficient of 0.99, indicating the release mechanism to be slightly deviating from the Fickian transport\textsuperscript{15,16}. The $n$ value increases with an increase in % loading of DS in different crosslinked formulations. The calculated values of $k$, being independent on either the release or loading of DS, vary from 0.011 to 0.068; this indicates a mild interaction between the drug and the polymer.
Figure V.A.10. % Cumulative release of DS from GA-32 (Δ), GA-16 (□) and GA-8 (O).

Figure V.A.11. % Cumulative release of DS from SA-60 (Δ), SA-40 (□) and SA-20 (O).
Figure V.A.12. % Cumulative release of DS from H-6 (Δ), H-4 (□) and H-3 (○).

**Table V.A.2**

Calculated Values of $k$, $n$ and $r$ from Eq. (IV.A.1) of *In Vitro* Release Kinetics of DS from the Microspheres

<table>
<thead>
<tr>
<th>Matrix Type</th>
<th>$k$ (min$^{-n}$) $10^2$</th>
<th>$n$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-8</td>
<td>2.7</td>
<td>0.61</td>
<td>0.999</td>
</tr>
<tr>
<td>GA-16</td>
<td>1.3</td>
<td>0.56</td>
<td>0.987</td>
</tr>
<tr>
<td>GA-32</td>
<td>4.9</td>
<td>0.49</td>
<td>0.988</td>
</tr>
<tr>
<td>SA-20</td>
<td>1.1</td>
<td>0.66</td>
<td>0.992</td>
</tr>
<tr>
<td>SA-40</td>
<td>5.8</td>
<td>0.57</td>
<td>0.989</td>
</tr>
<tr>
<td>SA-60</td>
<td>3.4</td>
<td>0.56</td>
<td>0.999</td>
</tr>
<tr>
<td>H-3</td>
<td>1.3</td>
<td>0.55</td>
<td>0.989</td>
</tr>
<tr>
<td>H-4</td>
<td>6.8</td>
<td>0.49</td>
<td>0.984</td>
</tr>
<tr>
<td>H-6</td>
<td>6.5</td>
<td>0.47</td>
<td>0.987</td>
</tr>
</tbody>
</table>

$r$- Correlation Coefficient
V.A.3. SUMMARY

In conclusion, the chitosan-based drug delivery systems are limited to in vivo tests as injectables because of the hazardous nature of the crosslinking agents used in the formulated products. However, it is extremely important to understand the crosslinking mechanism of various agents and their biocompatibility. The heat-crosslinked chitosan microspheres developed in this research have shown promising results on the extent of drug loading and crosslinking. Moreover, such heat-crosslinked formulations have lesser toxicity when compared to microspheres formed by crosslinking with glutaraldehyde or sulfuric acid. In future, it is realized that the nontoxic and crosslinked chitosan-based oligomers will be of value in biomedical applications.
V.A.4. LITERATURE CITED

V.B. Synthesis and Characterization of Poly(acrylamide) Grafted Chitosan Hydrogel Microspheres for the Controlled Release of Indomethacin

Abstract: The microspheres of poly(acrylamide)-grafted-chitosan crosslinked with glutaraldehyde have been prepared and used to encapsulate indomethacin, a non-steroidal anti-inflammatory drug. Microspheres were produced by the water in oil (w/o) emulsion technique and encapsulation of indomethacin is carried out before crosslinking of the matrix. Extent of crosslinking was analyzed by Fourier transform infrared spectroscopy and differential scanning calorimetry. Microspheres were characterized for drug encapsulation efficiency, particle size, and water transport into the polymeric matrix as well as for drug release kinetics. Scanning electron microscopy confirmed the spherical nature and surface morphology of the particles with mean particle size of 525 μm. Dynamic swelling experiments, suggested that with an increase in cross-linking, the transport mechanism changes from Fickian to non-Fickian. Release of indomethacin depends upon crosslinking of the network and also on the amount of drug loading. This is further supported by the calculation of drug diffusion coefficients using the initial time approximation. The drug release in all the formulations followed a non-Fickian trend and the diffusion was relaxation-controlled.

Results of this section are presented at:
(1) 29th International Symposium on Controlled Release of Bioactive Materials, July 22-25, 2002, Seoul, Korea
V. B. 1. INTRODUCTION

Polymeric microsphere hydrogels have been extensively used in the delivery of a several of drugs\(^1\)\(^-\)\(^6\). The preparation of such microspheres is generally based on the water in oil (w/o) emulsion technique\(^7\). It was found in our previous papers\(^8\)\(^-\)\(^13\) that a number of modified forms of natural polymers can be used for the delivery of drugs. The main advantages of using such natural polymers are that they can be biocompatible, biodegradable and produce no systemic toxicity upon administration\(^14\),\(^15\). Several biopolymers belonging to the class of polysaccharides have some inherent disadvantages such as poor mechanical strength, uncontrolled water uptake, and microbial contamination. In order to overcome these problems, efforts have been made to develop the chemically modified matrices by combining with the synthetic monomers\(^16\)\(^-\)\(^18\).

In continuation of research efforts\(^4\),\(^11\), the development of hydrogels of polyacrylamide (PAAm) grafted with the natural polymers is reported here. Procedures are developed to modify chitosan by grafting with PAAm.

The microspheres were prepared by the water in oil (w/o) emulsion method by using glutaraldehyde (GA) as the crosslinking agent. Indomethacin (IM), a non-steroidal anti-inflammatory drug (NSAID), was used as a model drug and loaded before crosslinking of the matrix. The advantages of such CR formulations containing NSAID over the conventional dosage forms have been reported earlier\(^12\),\(^13\),\(^19\); such formulations help to minimize the serious gastric irritation side effects of the conventional dosage NSAID formulations. The microspheres prepared were characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and laser beam particle size analyzer. Microspheres were evaluated for their thermal behavior, water-transport properties as well as in vitro drug release kinetics.
V.B.2. RESULTS AND DISCUSSION

Graft copolymerization of chitosan with acrylamide was achieved in the presence of potassium persulphate catalyzed free radical polymerization. The chelate formed by the reaction between $-\text{NH}_2$ and $-\text{OH}$ groups of chitosan decomposes to generate the free radical sites at 50°C facilitating the reaction site for acrylamide (AAm) monomer on chitosan backbone, as shown in Scheme V.B.1. The grafting reaction carried out at the temperature higher than 50°C resulted in decreased grafting possibility due to the possible formation of homopolymer\textsuperscript{17}. At the monomer concentration of 0.16 M used in the present study, no homopolymer was formed and the grafting efficiency was 100 % with the % grafting of 92. A considerable decrease in viscosity of the PAAm-g-chitosan polymer is observed when compared to the neat chitosan solution. PAAm-g-chitosan at the concentration of 0.002 g/dL has a viscosity of 1.342 mPa.s.

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

FTIR spectral analyses were carried out to confirm grafting as well as cross-linking of PAAm-g-chitosan microspheres. The spectra of pure chitosan and PAAm-g-chitosan are shown in Fig. V.B.1. A broad band appearing at $\sim$3400 cm$^{-1}$ corresponds to the associated $-\text{OH}$ stretching vibrations of the hydroxy groups, and a peak at 1647 cm$^{-1}$ corresponds to the N-H deformation were observed for chitosan. In the spectra of the copolymer, a new peak at 3200 cm$^{-1}$ is observed which corresponds to the bonded $-\text{NH}$ stretching vibrations and antisymmetric $-\text{N-H}$ bending at 1659 cm$^{-1}$ of the primary amides. A relatively high intense band at 2919 cm$^{-1}$ corresponds to the aliphatic $-\text{CH}$ stretching in the graft copolymer further confirms the grafting of acrylamide onto chitosan backbone.
Scheme V.B.1. Grafting and crosslinking reactions of AAm onto chitosan

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Figure V.B.1. FTIR spectra of (A) neat chitosan and (B) PAAm-g-chitosan
Crosslinking of PAAm-g-chitosan leads to the formation of an imine moiety, which is due to the reaction between free -NH$_2$ groups of the copolymer and the -CHO groups of GA and this appeared at 1665 cm$^{-1}$ as shown in the FTIR spectra given in Fig. V.B.2. With an increase in GA concentration, the intensity of this peak has increased probably due to the formation of more imine groups.

DSC performed on plain chitosan and PAAm-g-chitosan shows the $T_g$ at ~ 46°C and ~ 52°C, respectively. This supports that modification of chitosan by grafting with AAm makes chitosan polymer thermally more stable (see Fig. V.B.3). DSC performed on the IM-loaded microspheres as well as neat IM are also presented in Fig. V.B.3. For IM, a sharp endothermic peak is observed at 161°C; this peak was also observed in the IM-loaded microspheres; its intensity is smaller when compared to the neat IM suggesting the less crystalline nature of IM in the microspheres.

PAAm-g-chitosan empty microspheres presented in Fig. V.B.4 exhibit a (endothermic peaks) step transition at $T_g$ around 46 to 58°C. A shift in $T_g$ at higher temperature is attributed to an increase in the cross-linking density of the matrix. Increase in $T_g$ is due to the high energy required to break the highly crosslinked polymer network. This confirms the formation of highly crystalline microspheres with an increase in cross-linking.

The particle size of PAAm-g-chitosan microspheres was measured using an optical microscopy and these results are given in Table V.B.1. Three samples i.e., empty microspheres crosslinked with 7.5 and 10 mL GA as well as drug-loaded microspheres IM-9 were analyzed by HELOS particle size analyzer. The results indicated the size range of 464-682 μm with a narrow size distribution (see Fig. V.B.5) for the empty microspheres. With an increase in cross-linking particle size decreases and this may be due to the formation of more rigid polymer network. Also, an increase in % drug loading increases the size of the microspheres, which may be due to the filling of the free volume of the microspheres. For example, 7.5
Figure V.B.2. FTIR spectra of (A) GA crosslinked PAAm-g-chitosan with 5 mL, (B) 7.5 mL of GA and (C) 10 mL of GA.
Figure V.B.3. DSC thermograms of (A) chitosan, (B) PAAm-g-chitosan and (C) indomethacin (D) indomethacin loaded PAAm-g-chitosan microspheres with 10 mL of GA crosslinked.
Figure V.B.4. DSC thermograms of PAAm-g-chitosan empty microspheres crosslinked with (A) 5 mL, (B) 7.5 mL and (C) 10 mL of GA.
<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Amount of Crosslinking Agent (mL)</th>
<th>Initial % Drug Loading (w/w)</th>
<th>Encapsulation Efficiency (%)</th>
<th>Mean Particle Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM-1</td>
<td>5</td>
<td>10</td>
<td>53.05 ± 2.21</td>
<td>651</td>
</tr>
<tr>
<td>IM-2</td>
<td>7.5</td>
<td>10</td>
<td>53.57 ± 2.92</td>
<td>536</td>
</tr>
<tr>
<td>IM-3</td>
<td>10</td>
<td>10</td>
<td>64.65 ± 3.12</td>
<td>464</td>
</tr>
<tr>
<td>IM-4</td>
<td>5</td>
<td>20</td>
<td>47.21 ± 3.41</td>
<td>672</td>
</tr>
<tr>
<td>IM-5</td>
<td>7.5</td>
<td>20</td>
<td>54.15 ± 3.25</td>
<td>558</td>
</tr>
<tr>
<td>IM-6</td>
<td>10</td>
<td>20</td>
<td>53.44 ± 3.62</td>
<td>480</td>
</tr>
<tr>
<td>IM-7</td>
<td>5</td>
<td>30</td>
<td>48.89 ± 3.92</td>
<td>682</td>
</tr>
<tr>
<td>IM-8</td>
<td>7.5</td>
<td>30</td>
<td>41.91 ± 3.78</td>
<td>641</td>
</tr>
<tr>
<td>IM-9</td>
<td>10</td>
<td>30</td>
<td>50.65 ± 2.98</td>
<td>520</td>
</tr>
</tbody>
</table>

*Formulation Codes:
IM-1: 10 % IM loaded 5 mL GA crosslinked microspheres
IM-2: 10 % IM loaded 7.5 mL GA crosslinked microspheres
IM-3: 10 % IM loaded 10 mL GA crosslinked microspheres
IM-4: 20 % IM loaded 5 mL GA crosslinked microspheres
IM-5: 20 % IM loaded 7.5 mL GA crosslinked microspheres
IM-6: 20 % IM loaded 10 mL GA crosslinked microspheres
IM-7: 30 % IM loaded 5 mL GA crosslinked microspheres
IM-8: 30 % IM loaded 7.5 mL GA crosslinked microspheres
IM-9: 30 % IM loaded 10 mL GA crosslinked microspheres
Figure V.B.5. Particle size distribution for (●) 7.5 mL GA crosslinked empty microspheres, (■) 10 mL GA crosslinked empty microsphere and (σ) 40 % IM loaded microspheres crosslinked with 10 mL GA.
mL crosslinked empty microspheres has a (volume) mean diameter of 617 \( \mu \text{m} \) whereas the 10 mL crosslinked empty microspheres have the diameter of 459 \( \mu \text{m} \).

In all the cases, particles are spherical and aggregated with rough surfaces as evidenced by the SEM photographs shown in Figs. V.B.6 and V.B.7 and photographs did not show any porous structure and no crystals of the drug were present on the surface.

Drug loading was done at the time of cross-linking of the microspheres. The results of encapsulation efficiency are given in Table V.B.1 and these values range between 41 to 64 \%\). The lower values are probably due to the loss of the drug in liquid paraffin during the process of crosslinking in the presence of polysorbate-80. However, the extent of drug loading and crosslinking did not show any systematic effect on the encapsulation efficiency.

Dynamic swelling of the PAAm-g-chitosan microspheres was studied by measuring the change in particle diameter, \( D \), as a function of time using an optical microscope. Figure V.B.8 represents the dynamic swelling data wherein an increase in cross-linking showed a decrease in their water uptake characteristics. These results indicated that a dense three-dimensional network structure might have been formed by increasing the amount of GA (cross-linking agent); these results are also supported by FTIR and DSC data. The molecular transport of liquids within the polymeric matrices are influenced by polymer swelling. The loose polymer network generally absorbs higher quantity of liquid thereby giving higher swelling. Equilibrium swelling studies were performed in triplicate, but the average values are presented in Table V.B.2. The 5 mL GA crosslinked microspheres shows 368 \%, equilibrium water uptake whereas 7.5 and 10 mL GA crosslinked microspheres showed 314 and 233 \% respectively; this supports the above explanations.

Since the drug release from swollen polymers is mainly controlled both by the swelling or relaxation of the chain, the dynamic swelling data have been analyzed using an empirical equation\(^ {20} \):
Figure V.B.6. Scanning electron microscopic photographs of (A) a single IM loaded PAAm-g-chitosan microsphere and (B) surface photographs of the same
Figure V.B.6. Scanning electron microscopic photographs of (A) a single IM loaded PAAm-g-chitosan microsphere and (B) surface photographs of the same.
Figure V.B.8. Normalized diameter of PAAm-g-chitosan crosslinked microspheres as a function of swelling time for GA: (O) 5 mL, (□) 7.5 mL, and (Δ) 10 mL.

**Table V.B.2**

Transport Data on Empty Microspheres at 37°C

<table>
<thead>
<tr>
<th>GA (mL)</th>
<th>$D_i/D_o$</th>
<th>$n$</th>
<th>Mean</th>
<th>Upper limit</th>
<th>Lower limit</th>
<th>$r$</th>
<th>$Q^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.74</td>
<td>0.50</td>
<td>0.56</td>
<td>0.46</td>
<td>0.997</td>
<td>367.77±81.95</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>1.51</td>
<td>0.66</td>
<td>0.69</td>
<td>0.62</td>
<td>0.989</td>
<td>314.10±58.42</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.39</td>
<td>0.90</td>
<td>0.94</td>
<td>0.85</td>
<td>0.998</td>
<td>232.85±54.51</td>
<td></td>
</tr>
</tbody>
</table>

$^a$% Equilibrium water uptake

$r$- Correlation Coefficient
\[
\frac{D_{t}}{D_{\infty}} = k t^n
\]  

(V.B.1)

Where \(D_t\) is the change in microsphere diameter at time \(t\) and \(D_{\infty}\) is the equilibrium diameter of the swollen microsphere. The exponent value of \(n\) indicates the type of transport mechanism. The least-squares method was used to estimate the values of \(n\) and \(k\), at the 95% confidence limit, but only the \(n\) values are presented in Table V.B.2. It is observed that \(n\) increases with increasing extent of crosslinking agent suggesting the deviation of Fickian to non-Fickian transport. The equilibrium swelling particle diameter, \(D_{\infty}\) normalized with respect to the original diameter, \(D_0\) decreased significantly (Table V.B.2) indicating that the more rigid the crosslinked matrix network becomes, it does not expand in water as much as the loosely crosslinked matrix.

In order to understand the release of IM from the crosslinked chitosan-g-pAAm microspheres, the in vitro release study was carried out in pH 7.4 phosphate buffer at 37°C. These experiments are performed in triplicate, but the average values were considered for graphical presentation as well as data treatment. The standard deviations in all the cases were less than 5%. Figures V.B.9-V.B.11 represents the release profiles of PAAm-g-chitosan microspheres loaded with 10, 20 and 30% of IM as well as those matrices prepared with three levels of cross-linking. These results showed a systematic effect of the extent of cross-linking on the drug release profiles in all the formulations. This may be because of the fact that diffusion of the drug from the hydrogel depends upon the mesh size of the polymer network, which will be decreased with increased cross-linking. The crosslinking also increases the crystallinity of the polymer and hence the rigidity of the polymeric chain, which was evident from the DSC analyses. For instance, 10% drug loaded microspheres when crosslinked with 5 mL of GA showed a release of 89% whereas, when 10 mL of GA was used, the crosslinked microspheres exhibit drug release up to 73%.
Figure V.B.9. *In vitro* % cumulative release vs time for IM-loaded PAAm-g-chitosan microspheres of (O) IM-1, (□) IM-2, and (Δ) IM-3.

Figure V.B.10. *In vitro* % Cumulative release vs time for IM-loaded PAAm-g-chitosan microspheres of (O) IM-4, (□) IM-5, and (Δ) IM-6.
**Figure V.B.11.** *In vitro* % cumulative release vs time for IM-loaded PAAm-g-chitosan microspheres: of (O) IM-7, (□) IM-8, and (Δ) IM-9.
The drug release also showed a dependence on the extent of drug loading. In case of formulations containing 10 % IM, the release was fast when compared to the formulations containing 20 % of the drug. This indicates that the drug release at lower loadings (< 10 %) is somewhat quicker. This may be due to the fact that at lower drug loadings there is a possibility of the formation of large pore volume, which in turn, might enhance the drug release. Any further increase in drug loading increased the drug release.

The \textit{in vitro} release data have been analyzed by using an empirical equation of the type IV.A.1 to estimate the values of $n$ and $k$ as explained earlier. The least-squares estimations of the fractional release data along with the estimated correlation coefficient values, $r$ are presented in Table V.B.3. From these data, we notice that $n$ value ranged between 0.38 and 0.66, with the correlation coefficient values of 0.99, indicating the drug release deviating slightly from the Fickian transport\textsuperscript{7,17,19}. Even though water uptake by the microspheres reaches equilibrium very fast (i.e. 10-20 min) converting the glassy polymer into the rubbery polymer the release was continued for several hours indicating that the polymer chain relaxation has a little influence on the drug release, but governed by the molecular diffusion. In an earlier study on water transport characteristics PVA-GG crosslinked matrix loaded with nifedipine, it was found that the release is not only governed by drug diffusion through the polymeric network, but also by the polymer chain relaxation processes.

In order to calculate the values of apparent diffusion coefficients, $D$ of IM from the hydrogel microspheres, the initial portion of the release profiles as shown in Figs. V.B.9 – V.B.11 i.e. $0 < M_t/M_\infty < 0.4$ were analyzed by making use of the Eqs.IV.A.2 and IV.B.1 as explained earlier. These data reported in Table V.B.3 show a relationship between extent of cross-linking and drug loading. An increase in amount of GA from 5 to 10 mL has shown a decrease in diffusion coefficients from $6.90 \times 10^{-7}$ cm$^2$/s to $2.02 \times 10^{-7}$ cm$^2$/s for the 10 % IM-loaded microspheres. As explained before diffusion coefficient values decreased with higher drug loadings.
### Table V.B.3

*Analysis of Release Kinetics from Eq. (IV.A.1) of the IM-Loaded from PAAm-g-Chitosan Microspheres*

<table>
<thead>
<tr>
<th>Formulations</th>
<th>$k$ (min$^{-1}$) $10^2$</th>
<th>$n$</th>
<th>$r$</th>
<th>$D\times10^9$ (cm$^2$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM-1</td>
<td>1.22</td>
<td>0.41</td>
<td>0.992</td>
<td>6.90</td>
</tr>
<tr>
<td>IM-2</td>
<td>0.95</td>
<td>0.38</td>
<td>0.994</td>
<td>2.58</td>
</tr>
<tr>
<td>IM-3</td>
<td>0.37</td>
<td>0.58</td>
<td>0.988</td>
<td>2.02</td>
</tr>
<tr>
<td>IM-4</td>
<td>0.69</td>
<td>0.49</td>
<td>0.997</td>
<td>4.13</td>
</tr>
<tr>
<td>IM-5</td>
<td>0.43</td>
<td>0.54</td>
<td>0.991</td>
<td>2.25</td>
</tr>
<tr>
<td>IM-6</td>
<td>0.21</td>
<td>0.66</td>
<td>0.993</td>
<td>1.97</td>
</tr>
<tr>
<td>IM-7</td>
<td>0.99</td>
<td>0.47</td>
<td>0.999</td>
<td>3.35</td>
</tr>
<tr>
<td>IM-8</td>
<td>0.43</td>
<td>0.62</td>
<td>0.993</td>
<td>2.02</td>
</tr>
<tr>
<td>IM-9</td>
<td>0.58</td>
<td>0.52</td>
<td>0.997</td>
<td>1.73</td>
</tr>
</tbody>
</table>
V.B.3. SUMMARY

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


