CHAPTER VII

Novel Interpenetrating Polymer Network Microspheres of Chitosan and Methylcellulose for Controlled Release of Theophylline

Abstract

This chapter presents preparation of novel interpenetrating polymer network (IPN) microspheres of chitosan (CS) and methylcellulose (MC) by emulsion-crosslinking in the presence of glutaraldehyde (GA) as a crosslinker. Theophylline (THP), an antiasthmatic drug was encapsulated into IPN microspheres under varying ratios of MC and CS, % drug loading and amount of GA added. IPNs have shown better mechanical properties than pure CS. Cross-link density of the matrices was significantly affected by the amount of GA and MC. Microspheres were characterized by Fourier transform infrared (FTIR) spectroscopy to assess the formation of IPN structure and to confirm the absence of chemical interactions between drug, polymer and crosslinking agent. Particle size was measured by laser light scattering technique. Scanning electron microscopy (SEM) was performed to study the surface morphology of the microspheres. Differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of drug after encapsulation into IPN microspheres. Equilibrium swelling was performed in distilled water. In vitro release studies were performed in both 0.1 N HCl and pH 7.4 buffer solutions.

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VII.1. Introduction

The importance of biocompatible and biodegradable carbohydrate polymers is continuously increasing in pharmaceutical applications [1] because of their propensity to form crosslinked three-dimensional network hydrogels that tend to swell in water or biological fluids. Such systems have been the potential candidates to deliver bioactive molecules, particularly in controlled release applications [2-4]. Hydrogels based on carbohydrate polymers swell in water and retain a significant fraction of water within their network structures. They are insoluble in water at physiological temperature, pH and ionic strengths [5]. Soft tissue biocompatibility and an open porous structure allows for easy transport of the incorporated drugs in a controlled manner. Self-regulated release from such hydrogels would enhance the drug release with a sustained action. However, biocompatibility is the major determinant for their successful functioning, since noncompatible materials can elicit inflammatory responses \textit{in vivo} and thus limit their usage in living systems.

An interpenetrating polymer network (IPN) is a combination of two polymers exhibiting varied characteristics. Whenever an IPN hydrogel is formed from two polymers at a given temperature, the physical phase separation between the component polymers would be almost impossible because of the infinite zero-viscosity of the gel. IPN is also attractive in producing synergistic properties from the component polymers. For example, when a hydrophilic gelling polymer is interpenetrated with a relatively hydrophobic gelling polymer, the resultant IPN hydrogel is expected to have an improved capability of immobilizing a drug. This would open up new avenues to use IPN in designing the novel drug release systems [6,7].

Even though several polymers have been used in pharmaceutical industry, chitosan (CS) has been one of the most widely used polymers due to its reduced toxicity and better patient compliance [8]. Drug release from such
matrices can be controlled and enhanced by the addition of another water-soluble or water swellable carbohydrate polymer such as methylcellulose (MC). Methylcellulose, a polyhydroxy water-soluble carbohydrate polymer can be chemically crosslinked with dialdehyde in the presence of a strong acid to generate a hydrogel [9-11]. On the other hand, chitosan is a unique cationic polymer with excellent gel and film-forming properties. This polymer has been investigated extensively for many years in the pharmaceutical area [12-16].

In continuation of our earlier research on the development of controlled release (CR) devices utilizing carbohydrate polymers, we herein present the work on hydrogel microspheres of chitosan and methylcellulose. The effect of composition of these polymers on the CR of theophylline (THP) was investigated. THP is an effective drug used in the treatment of asthma and pulmonary disease [17] and has been widely used as a model drug in various controlled release studies [18-21]. The release data have been measured in 0.1 N HCl and pH 7.4 buffer solutions. Diffusion anomalies of the drug through the developed matrices have been investigated.

VII.2. Results and Discussion

VII.2.1. Preparation and Characterization of Microspheres

Theophylline-loaded IPN microspheres based on two well-known carbohydrate polymers viz., chitosan and methylcellulose were prepared by crosslinking with GA. By this method, % encapsulation efficiency was found to be in the range between 58.29 and 82.06. The % encapsulation efficiency showed a dependence on MC content, extent of crosslinking and % drug loading. By increasing the amount of MC, a slight decrease in % encapsulation efficiency was observed, which is due to the formation of a loose network that allows for leaching out of more of drug particles during microsphere preparation. The % encapsulation efficiency also shows a dependence on %
drug loading. The formulations loaded with higher amount of drug exhibited higher encapsulation efficiencies (see Table VII.1). This is due to the accumulation of more amounts of drug particles at higher % drug loading. The effect of crosslinking on % encapsulation efficiency showed a significant effect. As the concentration of crosslinking agent increased, a reduction in % encapsulation efficiency was observed. This could be due to higher extent of crosslinking, resulting in the formation of a more rigid network. This caused retention of more drug particles during the microsphere preparation.

Particle size (see Table VII.1) revealed an increase with increasing amount of MC. It was found that particle size of F3 (20 %, w/w, MC) was higher than that of F2 (15 %, w/w, MC) and particle size of F2 was greater than F1. Similar findings were observed for other formulations. This could be due to the higher amount of MC present, leading to viscosity increase in polymer solution, thereby producing bigger droplets during emulsification that were later hardened in the presence of GA. Particle size also showed a dependence on drug loading. Formulations containing 50 % drug loading exhibited higher particle sizes as compared to formulations containing 25 % drug loading. This could be due to the accumulation of more drug particles during microsphere preparation. Another interesting observation is that particle size decreased with an increase in crosslinking extent. It is observed that particle size of F1 (5 mL GA added) is higher than that of F7 (10 mL GA added). Similar findings were observed for F2, F3, F4, F5 and F6 formulations as compared to F7, F8, F9, F10, F11 and F12. This could be due to the formation of more rigid network structures at higher crosslinking. Microspheres of this study were spherical with smooth surfaces and also have shown surface adhered drug particles as revealed by SEM micrographs as shown in Figure VII.1.
Table VII.1

Results of % Entrapment Efficiency, Volume Mean Particle Size, % Water Uptake and $n$ Values, $r$ Values and Diffusion Coefficient

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% Entrapment efficiency</th>
<th>Volume mean particle size ($\mu$m)</th>
<th>% Water uptake</th>
<th>$n$</th>
<th>$r$</th>
<th>$D \times 10^5$ (cm$^2$/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>68.54</td>
<td>209</td>
<td>217</td>
<td>0.522</td>
<td>0.994</td>
<td>2.64</td>
</tr>
<tr>
<td>F2</td>
<td>61.17</td>
<td>238</td>
<td>281</td>
<td>0.549</td>
<td>0.994</td>
<td>3.24</td>
</tr>
<tr>
<td>F3</td>
<td>58.29</td>
<td>269</td>
<td>304</td>
<td>0.563</td>
<td>1.0</td>
<td>5.57</td>
</tr>
<tr>
<td>F4</td>
<td>72.44</td>
<td>282</td>
<td>231</td>
<td>0.508</td>
<td>1.0</td>
<td>2.83</td>
</tr>
<tr>
<td>F5</td>
<td>66.10</td>
<td>301</td>
<td>254</td>
<td>0.459</td>
<td>1.0</td>
<td>3.74</td>
</tr>
<tr>
<td>F6</td>
<td>62.33</td>
<td>318</td>
<td>289</td>
<td>0.363</td>
<td>0.999</td>
<td>6.21</td>
</tr>
<tr>
<td>F7</td>
<td>76.60</td>
<td>119</td>
<td>148</td>
<td>0.494</td>
<td>0.993</td>
<td>1.04</td>
</tr>
<tr>
<td>F8</td>
<td>72.23</td>
<td>156</td>
<td>178</td>
<td>0.504</td>
<td>0.995</td>
<td>2.67</td>
</tr>
<tr>
<td>F9</td>
<td>70.16</td>
<td>175</td>
<td>192</td>
<td>0.502</td>
<td>0.995</td>
<td>3.33</td>
</tr>
<tr>
<td>F10</td>
<td>82.06</td>
<td>219</td>
<td>140</td>
<td>0.532</td>
<td>0.995</td>
<td>1.95</td>
</tr>
<tr>
<td>F11</td>
<td>77.72</td>
<td>233</td>
<td>182</td>
<td>0.414</td>
<td>1.0</td>
<td>2.92</td>
</tr>
<tr>
<td>F12</td>
<td>72.19</td>
<td>251</td>
<td>197</td>
<td>0.398</td>
<td>0.994</td>
<td>4.16</td>
</tr>
</tbody>
</table>
Figure VII.1. SEM micrographs of (a) group of microspheres and (b) a single microsphere.
VII.2.2. *Fourier Transform Infrared (FTIR) Spectral Studies*

FTIR spectra of plain CS, plain MC, placebo microspheres, drug-loaded microspheres and plain THP were studied to investigate the effect of crosslinking and chemical stability of the drug after encapsulation into the matrix. Figure VII.2 depicts the FTIR spectra of (a) plain chitosan, (b) plain methylcellulose and (c) placebo microspheres. In case of CS, a broad band at 3422 cm\(^{-1}\) is attributed to N-H stretching vibrations. Bands at 2922 and 2810 cm\(^{-1}\) represent the aliphatic C-H stretching vibrations. Three bands observed at 1649, 1594 and 1379 cm\(^{-1}\) indicate amide-I, amide-II and amide-III, respectively. The MC showed a broad band at 3457 cm\(^{-1}\) due to O-H stretching vibrations. Two bands at 2930 and 2834 cm\(^{-1}\) show the presence of C-H aliphatic stretching vibrations. In case of placebo microspheres, all the bands of both CS and MC were observed in addition to a new band observed at 1647 cm\(^{-1}\), which confirmed the C=N stretching vibration of the imine group of Schiff base. The band at 1014 cm\(^{-1}\) is due to the presence of an acetal group, which is formed due to the reaction of GA with hydroxyl groups of MC. Thus, FTIR confirms the cross-linking reaction of GA with CS and MC.

FTIR spectral data were also used to confirm the chemical stability of THP in IPN microspheres. For instance, FTIR spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pure THP are presented in Figure VII.3. In case of THP, a broad band at 3432 cm\(^{-1}\) is due to N-H stretching vibrations. Bands at 3061, 2986, 2918 and 2827 cm\(^{-1}\) are attributed to both aromatic and aliphatic C-H stretching vibrations. A band at 1715 cm\(^{-1}\) represents the imide group stretching of the heterocyclic ring. A sharp band at 1670 cm\(^{-1}\) is due to tertiary amide group stretching vibrations. N-H bending vibration is represented by a band at 1566 cm\(^{-1}\). A band at 1243 cm\(^{-1}\) shows C-N stretching vibrations. In case of drug-loaded microspheres, all the bands that
were observed in THP have also appeared, indicating the chemical stability of THP after encapsulation into the polymer matrix.

Figure VII.2. FTIR spectra of (a) plain chitosan, (b) plain methylcellulose and (c) placebo microspheres.
Figure VII.3. FTIR spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.
VII.2.3. Differential Scanning Calorimetric (DSC) Study

DSC thermograms of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pure THP are displayed in Figure VII.4. In case of placebo microspheres, a small peak and two broad peaks were observed at 35°, 57° and 75°C, respectively due to endothermic transition of the polymer matrix. Thermogram of THP showed a sharp peak at 277°C, indicating the melting of the drug. In case of drug-loaded microspheres, three peaks were observed at 41°, 74° and 181°C due to endothermic transitions. However, there was no peak corresponding to THP, indicating the amorphous dispersion of THP into IPN matrix.

![DSC spectra](image)

**Figure VII.4.** DSC spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.
VII.2.4. X-Ray Diffraction (X-RD) Studies

X-ray diffractograms of (a) placebo microspheres, (b) drug loaded microspheres and (c) pure THP are presented in Figure VII.5. The diffraction pattern of THP has the characteristic intense peaks at $2\theta$ of 12.4°, identical to stable anhydrous theophylline crystal. This peak has disappeared in the THP-loaded microspheres, but only peaks observed in placebo polymer matrix were seen. X-RD peak depends on the crystal size; but in the present study, for all the drug-loaded matrices, the characteristic peak of THP could overlap with the noise of the coated polymer itself. Further, the loaded drug is amorphous, which is very difficult to measure at the detection limit of the crystal size in the present case. This indicates that the drug is dispersed molecularly in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

VII.2.5. Water Uptake Studies

The % equilibrium water uptake of the crosslinked microspheres presented in Table VII.1 indicate that, as the amount of GA in the matrices increased from 5 to 10 mL, the equilibrium water uptake decreased significantly from 304 to 140 %. Such a reduction in water uptake capacity is due to the formation of a rigid network structure at higher concentration of crosslinking. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake as well as the release rates. Notice that formulations containing higher amounts of MC showed higher swelling rates than formulations containing lesser amount of MC. Thus, formulation F3 (20 %, w/w, MC) exhibited a higher swelling than formulation F2 (15 %, w/w, MC); similarly, formulation F2 exhibited a greater swelling than formulation F1, due to hydrophilic nature of MC, thereby leading to higher water uptake capacity.
Figure VII.5. X-RD spectra of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine THP.
VII.2.6. Tensile Strength Measurements

Young’s modulus of the crosslinked CS and MC membranes was used to estimate the average molecular weight between crosslinks \( M_c \) as well as the effective crosslink density \( V_e \). The \( M_c \) was determined using Equation (VII.1) derived from the rubber elasticity theory [22,23]:

\[
M_c = \frac{3\rho RT}{E}
\]  

(VII.1)

where \( \rho \) is specific density (g/cm\(^3\)), \( R \) is gas constant, \( T \) is absolute temperature and \( E \) is Young’s modulus. The effective crosslink density was calculated using the following equation [22,24]:

\[
V_e = \frac{\rho}{M_c}
\]  

(VII.2)

The results of Young’s modulus, \( M_c \) and \( V_e \) estimated for CS and MC formulations are shown in Table VII.2. Notice that the \( M_c \) values decreased with an increase in GA content of the formulation, since the network becomes denser. Also, the \( M_c \) values decreased with an increase in MC content of the formulation, indicating a dense structure. Similarly, GA and MC contents of the formulations significantly affected the crosslink density of IPNs. Thus, from the results of tensile strengths, the formation of IPN between CS and MC was confirmed.
Table VII.2

Results of Tensile Properties of Various Formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>CS (% w/w)</th>
<th>MC (% w/w)</th>
<th>GA (mL)</th>
<th>ρ (g/cm³)</th>
<th>E (MPa)</th>
<th>M₆ (kg/mol)</th>
<th>Vₑ × 10⁻⁵ (mol/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslinked</td>
<td>0</td>
<td>100</td>
<td>5</td>
<td>0.67</td>
<td>3.4±0.1</td>
<td>14.94±1.45</td>
<td>4.45±0.32</td>
</tr>
<tr>
<td>Crosslinked CS</td>
<td>100</td>
<td>0</td>
<td>5</td>
<td>1.30</td>
<td>7.1±0.21</td>
<td>10.67±0.44</td>
<td>12.2±0.43</td>
</tr>
<tr>
<td>MC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>90</td>
<td>10</td>
<td>5</td>
<td>0.733</td>
<td>3.65±0.13</td>
<td>15.22±1.12</td>
<td>4.8±0.91</td>
</tr>
<tr>
<td>F2</td>
<td>85</td>
<td>15</td>
<td>5</td>
<td>0.765</td>
<td>8.06±0.31</td>
<td>8.67±1.06</td>
<td>7.2±0.58</td>
</tr>
<tr>
<td>F3</td>
<td>80</td>
<td>20</td>
<td>5</td>
<td>0.80</td>
<td>10.0±0.50</td>
<td>6.06±0.89</td>
<td>13.2±0.88</td>
</tr>
<tr>
<td>F7</td>
<td>90</td>
<td>10</td>
<td>10</td>
<td>0.733</td>
<td>7.98±0.26</td>
<td>6.96±0.72</td>
<td>10.5±0.62</td>
</tr>
<tr>
<td>F8</td>
<td>85</td>
<td>15</td>
<td>10</td>
<td>0.765</td>
<td>11.97±0.29</td>
<td>4.84±0.34</td>
<td>15.8±0.74</td>
</tr>
<tr>
<td>F9</td>
<td>80</td>
<td>20</td>
<td>10</td>
<td>0.80</td>
<td>21.25±1.87</td>
<td>2.85±0.17</td>
<td>28.0±1.07</td>
</tr>
</tbody>
</table>

VII.2.7. In Vitro Release Studies

Drug release behavior of the formulations based on CS and MC polymers were evaluated by performing the in vitro release experiments in simulated gastric and intestinal pH conditions. Results of % cumulative release vs time for drug-loaded microspheres for formulations F1, F7, F2, F8, F3 and F9 are compared in Figure VII.6 to investigate the extent of crosslinking on the in vitro release profiles. The F1 showed a higher release rate than F7 and similarly, F2 and F3 showed higher release rates than F8 and F9. This is attributed to an increase in the extent of crosslinking, leading to the formation of a denser network structure. Effects of MC content in formulations F7, F8, F9 and control formulation (CF) on the release rates are presented in Figure VII.7. The % cumulative release is higher in case of F8 than F7 and similarly, F9 shows higher release rates than F8 (i.e., the trend is: F9 > F8 > F7). All the
formulations showed higher release rates than CF, because, with an increasing MC content of the matrix, swelling of the matrix also increased due to more hydrophilic nature of MC.

The effect of drug loading on in vitro release profiles for formulations F1, F4, F2, F5, F3 and F6 are displayed in Figure VII.8, wherein it was observed that formulation F4 exhibited higher release rate than F1. Similarly, F5 and F6 formulations showed higher release rates than F2 and F3 indicating that the release rates vary depending upon the amount of drug loaded in the matrices. The release rate is higher in case of formulations containing higher amount of drug and similarly, drug release was lower for formulations having a lower amount of drug. Drug in the microspheres might also act as inert filler by occupying the available free volume of the swollen hydrogel. This might have created a tortuous path for water molecules to permeate through, but the degree of tortuosity depends upon the volume fraction of the filler. During the first two hours of release, the dissolution was performed in 0.1 N HCl, wherein we observed a burst release and the release of drug was extended up to 24 h.

![Graph showing release profile of formulations](image)

**Figure VII.6.** Effect of crosslinking on in vitro release profile of formulations.
Figure VII.7. Effect of polymer ratio on \textit{in vitro} release profile of formulations.

Figure VII.7. Effect of % drug loading on \textit{in vitro} release profile of formulations.
The \textit{in vitro} drug release rates were correlated to diffusion coefficients presented in Table VII.1. Diffusion coefficients are higher for formulations containing a higher amount of MC as compared to formulations containing a lower amount of MC. A similar trend was observed in drug release profiles i.e., formulations having a higher amount of MC showed a faster release rate as compared to formulation with a lower amount of MC. Also, the crosslinking agent exhibited a perfect correlation between diffusion coefficient and the drug release behavior. It was found that formulations crosslinked with 5 mL of GA showed higher diffusion coefficients as compared to formulations crosslinked with 10 mL of GA. Similarly, the drug release was fast in those formulations that were crosslinked with 5 mL of GA as compared to formulations crosslinked with 10 mL of GA. In all the formulations, we observed the burst release, which varied depending upon the amount of MC, extent of drug loading and amount of GA present in the matrix. The drug release was continued up to 24 h.

The diffusion coefficient values were calculated using an empirical equation of the type:

\[
D = \left( \frac{r \theta}{6M_\infty} \right)^2 \pi 
\]  \ (VII.3)

where $\theta$ is slope of the linear portion of the plot of $M/M_\infty$ vs. $t^{1/2}$, $r$ is initial radius of the microspheres and $M_\infty$ is the maximum value of drug release. It is noticed that diffusion coefficient values showed an increase with increasing content of MC from 10 to 20 \%. The diffusion coefficient values were higher in case of microspheres crosslinked with 5 mL of GA than those crosslinked with 10 mL of GA. This could be due to the hydrophilic nature of MC as well.
as its loose network structure at lower crosslinking concentration, leading to higher matrix swelling.

Drug release and molecular transport parameters were correlated using an empirical equation [25]:

\[ \frac{M_t}{M_\infty} = k t^n \]  

(VII.4)

Here, \( k \) is rate constant and \( n \) is an exponent parameter that represents the type of transport. The \( n \) values calculated by Equation (VII.4) included in Table VII.1 range from 0.363 to 0.563, indicating a slight deviation from the Fickian transport [6,26,27]. It is evident from Table VII.1 that the correlation coefficient \( (r) \) values approached unity, suggesting a best fit to the Fickian model.
VII.3. Conclusions

This work demonstrates the successful use of two carbohydrate polymers viz., chitosan and methylcellulose to produce in the form of IPN microspheres for the effective encapsulation of THP by emulsification method. The encapsulation efficiency was obtained up to 82%. The IPNs of this demonstrated better mechanical properties than pure CS, indicating the suitability of IPNs for microsphere preparation. The cross-link density was significantly affected by the content of GA and MC in the formulations. FTIR confirmed the formation of IPN as well as chemical stability of THP in the microspheres. Microspheres with spherical shapes having smooth surfaces were produced. Microspheres with a narrow size distribution of sizes in the range of 119-318 μm were obtained. Swelling kinetics was dependent on the extent of crosslinking and the amount of MC used. The release of THP was found to depend on the extent of matrix crosslinking, amount of drug loading and MC content of the matrix. The release of THP was extended up to 12 h. The release mechanism showed a slight deviation from the Fickian behavior. The microspheres of this study could be used as controlled release devices for the release of THP.
VII.4. Literature Cited


