Chapter 7

Chitosan-Sodium Alginate Biodegradable Interpenetrating Polymer Network (IPN) Beads for Delivery of Ofloxacin Hydrochloride
7.1. INTRODUCTION

Controlled drug delivery systems offer some advantages compared to the conventional dosage forms, which include reduced adverse reaction, toxicity and frequency of dosing, improved efficacy, patient compliance and convenience.\textsuperscript{1-2} Using the novel microencapsulation techniques and varying the polymer ratio and molecular weight, microspheres can be developed as an optimal drug delivery system which provide the desired release profile. The use of microsphere-based therapy allows drug release to be carefully observed to the specific treatment site through the choice and formulation of various drug-polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired results. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents.\textsuperscript{3}

Microencapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature capsules that can release their contents at controlled rates under specific conditions.\textsuperscript{4} The choice of materials and the methodology used for encapsulation are dependent on the active agent in question and the target application.\textsuperscript{5} Microencapsulation has been applied to enhance the viability of probiotic bacteria during processing and also for targeted delivery to the GIT.\textsuperscript{6-7} The IPNs have found astonishing applications in the CR of bioactive molecules.\textsuperscript{8}

The importance of polymeric blends has increased in recent years because of the preparation of the polymeric materials with desired properties, low basic cost, and improved processability.\textsuperscript{9} The use of natural polymers as drug carriers has received much attention in the pharmaceutical field due to their safety. In particular, the polysaccharides such as sodium alginate and chitosan have been studied for application in the design of dosage forms for controlled release.\textsuperscript{10} The use of natural polymers is valuable based on
proven biocompatibility. The emulsion-polymerization method has been reported earlier for preparation of chitosan microspheres because it is easy and requires simple equipment.\textsuperscript{11} Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin.\textsuperscript{12-13} Properties of chitosan make the polymer suitable for use in biomedical and pharmaceutical formulations.\textsuperscript{14-15} It has also been used for the encapsulation of drugs.\textsuperscript{16-21}

Sodium alginate was chosen to prepare the carrier matrix from the galaxy of polymers, because it is a natural and hydrophilic polymer suitable for the entrapment of water soluble drugs.\textsuperscript{22-24} Formation of interpolymer complexes of chitosan with sodium alginate and development of chitosan IPN beads by crosslinking using glutaraldehyde (GA) has been discussed in this study.

Ofloxacin hydrochloride was chosen as a model drug for encapsulation in the polymer matrix and to study the \textit{in vitro} release. Although many drugs have been extensively investigated using natural polymeric carriers, the studies on the release of antibiotic drugs are limited. Ofloxacin HCl is a synthetic antimicrobial having a quinolone structure which is active primarily against gram-negative bacteria, but is comparable to Ciprofloxacin for gram-positive organisms and certain anaerobes.\textsuperscript{25}

\textbf{Drug data}

\textbf{Ofloxacin Hydrochloride}\textsuperscript{25}

<table>
<thead>
<tr>
<th>Name</th>
<th>Ofloxacin hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action</td>
<td>A fluoroquinolone antibiotic</td>
</tr>
<tr>
<td>Synonyms</td>
<td>(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hydrochloride</td>
</tr>
</tbody>
</table>
Molecular formula : $\text{C}_{18}\text{H}_{20}\text{FN}_3\text{O}_4 \cdot \text{HCl}$

Molecular weight : 397.83

Chemical structure :

\[
\begin{array}{c}
\text{F} \\
\text{N} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{HCl}
\end{array}
\]

Dose : Orally 400 mg daily, 200 mg/100 ml iv infusion

Appearance : Crystalline powder

Color : Pale yellow or bright yellow

Solubility : Ofloxacin is freely soluble in acetic acid, soluble in water, methanol, ethanol or acetone

Melting point : 270 °C - 273 °C

7.2. EXPERIMENTAL

7.2.1. Materials

The gift sample of Ofloxacin.HCl was supplied by Sun Pharmaceuticals Pvt. Ltd., Mumbai, India. The 75-85 % deacetylated chitosan with a viscosity of 200-800 cP measured by Brookfield viscometer in 1% w/v of chitosan solution, in 1 % v/v acetic acid solution was purchased from Aldrich Chemical Company, USA. Sodium alginate, glutaraldehyde solution (25 % v/v) (GA) analytical grade chemicals were purchased from s.d. Fine-Chemicals Ltd. Mumbai, India and used as received. Distilled water was used throughout the study.
7.2.2. Preparation and evaluation methods for IPN beads

7.2.2.1. Preparation of IPN beads

The IPN beads of chitosan and sodium alginate crosslinked with GA were prepared by using the documented procedure with some modification. Chitosan and sodium alginate were separately dissolved in 1 % v/v acetic acid solution and distilled water respectively; by stirring on a magnetic stirrer at 40 °C. Varying proportions of polymers (as shown in Table 7.3) were mixed. The final concentration of mixture of polymers in each solution was 3 % w/v. Weighed quantity of Ofloxacin. HCl was added and stirred further for 30 min. The beads were prepared by extruding a solution of chitosan alone, and in combination with sodium alginate dropwise through a syringe into a solution of methanol containing 1% GA and a small amount of (0.1 ml) conc. HCl at 40 °C. The speed was maintained at 2000- rpm. After 15 min and 30 min of contact time, the beads were recovered by filtration, washed with methanol, then with distilled water and dried in an oven at 40 °C. The yield of beads thus formed was calculated and different formulation parameters were studied to find the effect of IPN formation on drug release.

7.2.2.2. Measurement of bead size

Particle size of the beads was determined by optical microscopy. Average of 100 beads were used for the study and the mean particle size (arithmetic mean diameter) was considered to be the deciding factor in selecting optimum formulation conditions for each variable parameter studied.

7.2.2.3. Swelling study

Randomly selected samples of beads were soaked in water and at different time intervals (every 30 min), they were taken out and blotted carefully (without pressing hard) to remove the surface adhered water. These swollen beads were weighed using an
electronic balance until a constant weight was achieved and the rate of water uptake was calculated with respect to the initial dry weight of the samples. The experiments were carried out in triplicate.

7.2.2.4. Drying study

The beads formed (about 2g) were allowed to dry in an oven maintained at 40 °C. Initial mass of the beads should be nearly equal for easy comparison. The beads were weighed at an hourly interval of time, until a constant weight was achieved. Mass measurements were done on a single pan analytical balance. In order to maintain the accuracy, experiments were carried out in triplicate.

7.2.2.5. Encapsulation efficiency

The drug content was determined by incubating the known mass of beads with 5 ml of water, till the beads were swollen completely. The swollen beads were crushed using a mortar and pestle and the solution thus formed was sonicated for 2 min using 60 MHz frequencies. The above solution was concentrated using a rotary flash evaporator to form a thick paste, to which about 10 ml of methanol was added to extract the entire drug. The precipitated polymer was separated by centrifugation. After appropriate dilution, the absorbance of the solution was measured at 280 nm and the drug content was determined.

7.2.2.6. In vitro dissolution study

Dissolution studies were carried out in 900 ml SGF (0.1 M HCl, pH 1.2) and SIF, (phosphate buffer, pH 7.5), both without enzymes, prepared according to the US Pharmacopeia using the dissolution tester equipped with eight pedals. The dissolution rates were measured at 37 °C and 100-rpm speed. A 10 ml of aliquot was withdrawn from the vessel at predetermined time intervals (every 30 min) and replaced
with an equal volume of corresponding dissolution medium. The samples were diluted appropriately before the assay. The amount of drug released was monitored by measuring the UV absorbance at 280 nm\textsuperscript{31} and concentration of drug was determined using the calibration curves constructed from reference standards.\textsuperscript{32}

7.2.2.7. Stability study

The beads were placed in screw capped glass containers and stored at 60 °C, 37 °C, room temperature and 5 °C for a period of 3 months. The samples were analyzed for the drug content and observed for any change in physical appearance at weekly intervals of time.\textsuperscript{33}

7.3. RESULTS AND DISCUSSION

The chitosan-alginate IPN beads were prepared at ambient temperature; preparation is simple, rapid and reliable. The beads were obtained under very mild conditions. The effects of formulation variables (polymer concentration, and contact time with crosslinking agent) on preparation of beads were investigated. The crosslinking of polymers with GA has been shown by scheme 7.1.
Scheme 7.1. Chitosan crosslinked with sodium alginate by glutaraldehyde
7.3.1. FTIR Spectral study

Polymer, drug as well as the formulations were characterized by FTIR spectroscopy to know any possible interaction between drug, polymer and the crosslinking agent (Fig. 7.1). The FTIR spectrum of chitosan showed peaks corresponding to O-H stretching at 3431 cm\(^{-1}\) and amine group (NH\(_2\)) at 2925 cm\(^{-1}\) respectively. Sodium alginate showed peaks at 3427 cm\(^{-1}\) due to O-H stretching and 1742 cm\(^{-1}\) corresponding to C=O stretching of carboxylic group respectively. The drug ofloxacin showed a broad band at 1633 cm\(^{-1}\) due to aromatic keto group and C=O stretching of carboxylic group. The aromatic C-H stretching band was observed at 2844 cm\(^{-1}\). The spectra of drug loaded crosslinked polymer matrix displayed peaks due to imine (C=N) formation (crosslinking of chitosan with GA) at 1579 cm\(^{-1}\) and acetal formation (crosslinking of sodium alginate with GA) at 1096 cm\(^{-1}\), along with peaks due to the presence of drug at 1628 cm\(^{-1}\) (aromatic keto group) and 1728 cm\(^{-1}\) (C=O stretching of carboxylic group) respectively. This denotes the drug was intact in the formulation and did not react either with the polymer or the crosslinking agent.

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>O-H</th>
<th>-NH(_2)</th>
<th>C=N</th>
<th>OC-O</th>
<th>C=O (acid)</th>
<th>C=O (keto)</th>
<th>C-H (aromatic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>3431</td>
<td>2925</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>3427</td>
<td></td>
<td></td>
<td></td>
<td>1742</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ofloxacin.HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1633</td>
<td></td>
<td>2844</td>
</tr>
<tr>
<td>Chitosan + Sodium alginate + GA + Ofloxacin. HCl</td>
<td></td>
<td>1579</td>
<td>1096</td>
<td>1728</td>
<td>1628</td>
<td></td>
<td>2851</td>
</tr>
</tbody>
</table>
Fig. 7.1. FTIR spectra of (1) chitosan, (2) sodium alginate, (3) Ofloxacin.HCl, and (4) Ofloxacin.HCl loaded Chitosan - sodium alginate IPN crosslinked by GA
7.3.2. DSC Study

DSC was employed for the determination of glass transition temperatures (Tg) of pure drug, polymer and drug loaded IPN. The Tg and the melting/decomposition temperatures are listed in Table 4.2 and the corresponding thermograms are shown by Fig. 7.2. Chitosan showed an endothermic peak at 50 °C, which corresponds to its Tg and a peak at 261 °C corresponding to its melting point; above 360 °C it undergoes decomposition. Sodium alginate exhibits the transition temperature at 90 °C and a melting endotherm at 290 °C. The drug showed its melting endotherm at 280 °C. This peak was also observed in drug embedded chitosan-alginate matrix, indicating crystalline nature of the drug.

Table 7.2 Thermal analysis data

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>DSC peaks (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tg</td>
</tr>
<tr>
<td>Chitosan</td>
<td>50</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>90</td>
</tr>
<tr>
<td>Ofloxacin.HCl</td>
<td>--</td>
</tr>
<tr>
<td>Chitosan + Sodium alginate + GA + Ofloxacin.HCl</td>
<td>65</td>
</tr>
</tbody>
</table>
Fig. 7.2. DSC curves of – (1) chitosan, (2) sodium alginate, (3) Ofloxacin.HCl, and (4) Ofloxacin.HCl loaded chitosan- sodium alginate IPN crosslinked by GA
7.3.3. TGA Study

TGA thermograms of chitosan, alginate, drug and drug loaded IPN have been obtained as shown in Fig. 7.3. Chitosan decomposes in two steps: starts decomposing at 100 °C (moisture release) and the other weight loss was observed at 280 °C. It undergoes a weight loss of 59.1 % at 400 °C. The mass loss of pure sodium alginate occurs at two stages, one due to moisture loss at 105 °C; another starts at 229 °C and reach to maximum at 243 °C. At 400 °C it showed a mass loss of 62 %. Ofloxacin.HCl decomposes at a single step near 280 °C with a weight loss of 98 %. The drug embedded GA crosslinked IPN showed two transitions: one at 102 °C due to moisture release and the other between 276 °C -280 °C; with an increased weight loss (68 %) at 400 °C. This increase in weight loss may be due to complete destruction of drug moiety at higher temperature.

7.3.4. Measurement of bead size

The method of preparation allowed the preparation of beads smaller than 250 μm (Table 7.3). In order to prepare these beads, the effect of formulation variables such as polymer concentration and contact time with the crosslinking agent were investigated. It was observed that the particle size did not vary significantly either by increasing the exposure time to the crosslinking agent or by altering the polymer ratio.

The beads were further evaluated by SEM, to investigate the morphology of the beads. Ofloxacin was successfully encapsulated into chitosan beads; they were spherical in shape and smooth in surface. SEM view of sample is shown by Fig. 7.4.
Fig. 7.3. TGA curves of - (1) GA crosslinked chitosan-sodium alginate IPN containing Ofloxacin.HCl, (2) chitosan, (3) sodium alginate, and (4) Ofloxacin.HCl
Fig. 7.4. SEM view of chitosan-alginate IPN beads
7.3.5. Swelling study

Chitosan is a hydrophilic polymer. Transport of water through the polymer depends upon the rigidity of the polymer and extent of its crosslinking ability. The results of water uptake by the beads exposed to GA, for different time intervals are displayed in Fig. 7.5 and Fig. 7.6. Results of % water uptake indicate that all the beads absorb maximum amount of water during the first hour. The beads prepared by exposing to the crosslinking agent only for 15 min, absorbed more amount of water than those prepared by extended exposure time (30 min) to GA. Chitosan beads (crosslinked with GA for 15 min as well as 30 min) showed lesser extent of swelling compared to those of alginate incorporated beads; sodium alginate being a hydrophilic polymer. The increased porosity and decreased crystallinity of the polymer enhance the water uptake and swelling ability. Crosslinking occurs fast at higher temperature and highly crosslinked beads absorb very small amount of water.

7.3.6. Drying study

In order to optimize the drying conditions, some of the beads with different process variables were selected with approximately equal initial mass. The results of drying (Fig. 7.7 and Fig. 7.8) indicated that the time of exposure to GA influenced the drying rate of the beads.

The beads exposed to GA only for 15 min were dried within 20 hrs as compared to the beads exposed GA for 30 min (24 hrs). This may be due to an increased rigidity of the polymer by increased exposure to the crosslinking agent.
Fig. 7.5. Rate of water uptake by beads (exposed to GA for 15 min)

Fig. 7.6. Rate of water uptake by beads (exposed to GA for 30 min)
Fig. 7.7. Drying rate of beads (exposed to GA for 15 min)

Fig. 7.8. Drying rate of beads (exposed to GA for 30 min)
7.3.7. **Encapsulation efficiency**

The production yield was relatively high for all formulations and processing parameters did not affect the yield. The encapsulation efficiency of beads prepared in our study varied between 76 % - 86 % as shown in Table 7.3. Increased sodium alginate content and decreased contact time with the crosslinking agent has produced beads with higher entrapment efficiency. The chitosan-alginate crosslinking leads to a three dimensional lattice structure entrapping the drug. Lower encapsulation efficiencies were generally obtained for low molecular weight drugs.\(^34\) Chitosan beads entrapped 79 % of drug, and as the IPN was formed the entrapment efficiency increased up to 85 %.

Drug content is the amount of drug entrapped within the matrix with respect to the total drug introduced into the polymer solution. The drug content in the beads reflects the composition and rigidity of the beads, because % drug content depends upon the space available within the matrix. The porosity of alginate beads could be responsible for the fast release of small drugs such as during washes and could explain the low encapsulation efficiencies. Moreover, the encapsulation efficiencies of water soluble drugs are in general lower than for slightly soluble or insoluble drug.\(^35\)-\(^37\)

7.3.8. **In vitro dissolution study**

In order for the microencapsulated drug to elicit a response it must be released from the beads. Therefore the release profiles of ofloxacin from chitosan-sodium alginate IPN beads were evaluated. The release profile was characterized by an important initial burst effect followed by a continuous and fast release (from beads containing more amount of alginate). The highly porous structure of alginate beads explains this fast release pattern.\(^35\)
<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Polymer</th>
<th>Time of exposure to GA (min)</th>
<th>Yield %</th>
<th>Mean particle size (μ)</th>
<th>Encapsulation efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Chitosan</td>
<td>15</td>
<td>92.15 ± 1.72</td>
<td>212 ± 0.19</td>
<td>79.40 ± 1.7</td>
</tr>
<tr>
<td>F2</td>
<td>Chitosan</td>
<td>30</td>
<td>93.15 ± 1.66</td>
<td>216 ± 0.6</td>
<td>76.94 ± 1.3</td>
</tr>
<tr>
<td>F3</td>
<td>Chitosan + (10%) Sodium alginate</td>
<td>15</td>
<td>92.14 ± 2.14</td>
<td>222 ± 0.12</td>
<td>84.38 ± 1.73</td>
</tr>
<tr>
<td>F4</td>
<td>Chitosan + (10%) Sodium alginate</td>
<td>30</td>
<td>94.0 ± 1.6</td>
<td>196 ± 2.1</td>
<td>82.14 ± 0.9</td>
</tr>
<tr>
<td>F5</td>
<td>Chitosan + (30%) Sodium alginate</td>
<td>15</td>
<td>91.65 ± 2.19</td>
<td>156 ± 0.8</td>
<td>85.50 ± 0.96</td>
</tr>
<tr>
<td>F6</td>
<td>Chitosan + (30%) Sodium alginate</td>
<td>30</td>
<td>93.66 ± 2.3</td>
<td>222 ± 0.4</td>
<td>84.81 ± 2.06</td>
</tr>
<tr>
<td>F7</td>
<td>Chitosan + (50%) Sodium alginate</td>
<td>15</td>
<td>92.90 ± 1.7</td>
<td>219 ± 1.0</td>
<td>80.85 ± 0.6</td>
</tr>
<tr>
<td>F8</td>
<td>Chitosan + (50%) Sodium alginate</td>
<td>30</td>
<td>92.4 ± 2.8</td>
<td>236 ± 0.43</td>
<td>79.90 ± 0.8</td>
</tr>
</tbody>
</table>
In case of higher concentrations of chitosan the release was comparatively slow and sustained. Both the burst effect and the continuous release phase appeared highly dependent on the concentration of polymer used for encapsulation. Concentration of alginate was an influential factor: addition of alginate (10 %) and exposure to GA for 15 min and 30 min showed 84 % and 82 % entrapment respectively. On increasing the alginate concentration to 50 % the efficiency was reduced to 80 % and 79 % (exposed to GA for 15 min and 30 min) respectively. This might be due to loss of drug during washing from a hydrophilic polymer matrix.

The crosslinking procedure gives a more sustained release in the release medium because of the denser gel structure after the crosslinking process. According to in vitro dissolution studies, the IPN beads showed sustained effect up to 24 hrs. The beads (exposed to GA for 15 min) containing 10 %, 30 % and 50 % alginate showed drug release of 95 %, 85 % and 80 % respectively (Fig. 7.9). The IPN beads (exposed to GA for 30 min) containing 10 %, 30 % and 50 % alginate showed drug release of 88 %, 81 % and 77 % respectively (Fig. 7.10). The highest dissolution rate was obtained with F7 (95 %) whereas the lowest was F2 (73 %).

Early studies reported that the drug release from the polymeric microspheres was affected by the particle size and drug: polymer ratio. In this study formulation factors showed no significant effect on microsphere size. Two processes can explain the release of a drug from a particle: diffusion and erosion. Drug could diffuse out of the beads, following the water phase that fills the matrix. Drug could also be released from the beads through the erosion of the matrix. Erosion could occur through the reversal of the gelation reaction, thus resulting in the solubilization of polymer molecules, or through the degradation of the polymer backbone into smaller molecular weight components.
Figure 7.9. *In vitro* drug release of IPN beads (exposed to GA for 15 min)

Figure 7.10. *In vitro* drug release of IPN beads (exposed to GA for 30 min)
7.3.9. Stability study

Stability studies were carried out to predict the degradation that may occur over prolonged periods of storage at normal shelf condition for the formulations; Table 7.4 gives the data obtained from the stability studies.
Table 7.4 % Drug content after stability studies

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Drug content At 5 °C</th>
<th>% Drug content At 25 °C</th>
<th>% Drug content At 37 °C</th>
<th>% Drug content At 60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 30 Days</td>
<td>After 3 months</td>
<td>After 30 Days</td>
<td>After 30 Days</td>
</tr>
<tr>
<td>F1</td>
<td>99.00</td>
<td>98.21</td>
<td>99.11</td>
<td>99.06</td>
</tr>
<tr>
<td>F2</td>
<td>99.98</td>
<td>98.13</td>
<td>99.00</td>
<td>98.47</td>
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<tr>
<td>F3</td>
<td>99.32</td>
<td>98.93</td>
<td>99.23</td>
<td>98.86</td>
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<td>F4</td>
<td>99.22</td>
<td>99.01</td>
<td>99.14</td>
<td>99.00</td>
</tr>
<tr>
<td>F5</td>
<td>99.60</td>
<td>99.10</td>
<td>99.19</td>
<td>98.94</td>
</tr>
<tr>
<td>F6</td>
<td>99.80</td>
<td>99.20</td>
<td>99.16</td>
<td>99.03</td>
</tr>
<tr>
<td>F7</td>
<td>99.20</td>
<td>98.9</td>
<td>99.23</td>
<td>98.94</td>
</tr>
<tr>
<td>F8</td>
<td>99.60</td>
<td>99.23</td>
<td>98.79</td>
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7.4. REFERENCES


