Natural Polymer Based Mucoadhesive Hydrogel Beads of Nizatidine: Preparation, Characterization and Evaluation

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ABSTRACT

Background: Dosage forms which precisely control the release rates and targets drugs to a specific body site have made enormous impact in the formulation and development of novel drug delivery systems. Methods: A prolonged release mucoadhesive hydrogel system of nizatidine was prepared by ionotropic gelation and polyelectrolyte complexation technique using natural, biodegradable polymers with or without chitosan. Prepared formulations were subjected to in vitro evaluation and several characterization studies. Results: Formulations with chitosan showed good drug content, swelling index and mucoadhesive strength when compared to batches containing alginate alone. The drug in formulations found to be intact and compatible with polymers used and surface morphology of prepared beads were found satisfactory. Two optimized formulations containing alginate-chitosan shows Higuchi model and perfect zero order release. All the formulations with copolymer showed better sustained the drug release when compared with formulations without chitosan. Conclusion: Alginate-chitosan beads prepared by ionotropic gelation and polyelectrolyte complexation method found to be better than ionically cross linked alginate beads alone. Therefore, dual cross-linked beads are promising carrier for oral control release.

Key words: Chitosan, ionotropic gelation, Mucoadhesion, Nizatidine, Sodium alginate.

INTRODUCTION

Multiparticulate systems have been paid considerable attention since several years in controlling and sustaining of release rate of many active pharmaceutical ingredients. And use of natural biodegradable polymers as rate controlling agents also has been enormously increased. Recently, dosage forms that can precisely control the release rates and targets drugs to a specific body site have made enormous impact in the formulation and development of novel drug delivery systems. Oral multiunit dosage forms such as microcapsules and microspheres have received much attention as modified/controlled drug delivery systems for the treatment of various diseases without major side effects. Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic levels are reached at the target site while reducing the side effects by keeping systemic concentration low. It will therefore be advantageous to have means for providing an intimate contact of the drug delivery system with microbeads.¹

The suppression of gastric acid secretion with anti-secretary agents has been the mainstay of medical treatment for patients with acid-related disorders. The suppression of gastric acid secretion achieved with H₂ receptor antagonists has, however, proved to be suboptimal for effectively controlling acid-related disorders, especially for healing erosive oesophagitis and for the relief of reflux
Nizatidine is a competitive inhibitor of $H_2$ receptor for gastric acid secretion and is used for the treatment of acid-reflux disorders, peptic ulcer, active benign gastric ulcer and active duodenal ulcers. It is having an oral bioavailability of 70% with a very short biological half life of 1-2 h. Moreover it is reported that nizatidine stimulates gastrointestinal motility. Hence, to increase the duration of GIT retention and drug release, sustained mucoadhesive hydrogel beads are an appropriate dosage form for drugs like nizatidine. Nizatidine does not have any demonstrable anti-androgenic effects and drug interactions compared to any other class of $H_2$ receptor antagonists. It also finds applications in the field of local delivery of drug to the stomach and proximal small intestine and importantly in treating microorganisms ($H. pylori$) which colonize the stomach because the major factors governing reduced luminal drug delivery are gastric acidity, gastric emptying and the epithelial mucus layer and therefore it helps to provide better availability of new products with new therapeutic possibilities and increased patient compliance,

Table 1: Formulation details, bead size and percent drug content of hydrogel beads

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Drug (parts)</th>
<th>Sodium Alginate (parts)</th>
<th>Chitosan (parts)</th>
<th>Calcium Chloride (% w/v)</th>
<th>Average size(µm)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>728 ± 2.12</td>
<td>72.11 ± 0.14</td>
<td>72.11 ± 0.14</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>781 ± 3.23</td>
<td>76.38 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>801 ± 1.99</td>
<td>73.48 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>640 ± 2.74</td>
<td>74.33 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>699 ± 3.64</td>
<td>78.93 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>623 ± 3.21</td>
<td>74.85 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>612 ± 2.51</td>
<td>91.61 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>681 ± 2.62</td>
<td>76.53 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>624 ± 3.72</td>
<td>73.75 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>1</td>
<td>1</td>
<td>--</td>
<td>695 ± 2.91</td>
<td>68.13 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>F11</td>
<td>1</td>
<td>2</td>
<td>--</td>
<td>723 ± 2.56</td>
<td>69.33 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>F12</td>
<td>2</td>
<td>1</td>
<td>--</td>
<td>699 ± 3.12</td>
<td>69.93 ± 0.17</td>
<td></td>
</tr>
</tbody>
</table>
chloride cross-linked alginate core with outer chitosan-alginate complex membrane and loaded in capsules. The one-stage procedure for the preparation of cross-linking reinforced chitosan-alginate beads was examined by dropping alginate solution into chitosan solution containing calcium chloride cross-linking agent.

**MATERIALS AND METHODS**

**Materials**

Nizatidine and chitosan were obtained as a gift sample from Dr. Reddy’s Laboratory (Hyderabad, India) and Central Institute of Fisheries and Technology (Cochin, India), respectively. Sodium alginate was purchased from Loba Chemicals (Mumbai India). All other chemicals, reagents and solvents used were of pharmaceutical or analytical grade.

**Methods**

**Preparation of hydrogel beads**

Ionotropic gelation technique has been widely used for microbeads preparation purpose. The natural polyelectrolytes are having a property of coating on the drug core and act as release rate retardants contains certain anions on their chemical structure. These anions forms meshwork structure by combining with the polyvalent cation ions and induce gelation by binding mainly to the anion blocks. Hydrogel beads of nizatidine were prepared as per our previously reported article on hydrogel beads. Briefly, weighed quantity of nizatidine was dissolved in 15 ml of deionized water in a beaker.

In another beaker sodium alginate was soaked for 3 h, in measured amount of distilled water. Prepared nizatidine solution was slowly added to the beaker containing sodium alginate with continuous stirring. The stirring was continued to obtain uniform dispersion of nizatidine in sodium alginate. The resultant homogeneous bubble free slurry dispersion was dropped through a 21G syringe needle into 100 ml of calcium chloride solution with or without containing chitosan (Table 1), which was kept under stirring to improve the mechanical strength of the beads and to prevent aggregation of them. After 15 min, the formed beads were collected by filtration and dried at 40°C overnight. The dried beads were doubly wrapped in an aluminum foil and kept in a desiccator till further use.

**Evaluation Parameters of Microbeads**

**Particle size measurement**

All the particulate formulations were subjected for particle size analysis using a digimatic micrometer (MDC-258 Mitutoyo, Tokyo, Japan) having an accuracy of 0.001 mm. The average diameter of randomly selected 100 particles from all the formulations was measured.

**Drug content estimation**

Known amount of beads (10 mg) were added to 10 ml phosphate buffer (USP) of pH 7.4 and pH 1.2 solutions separately for complete swelling at 37°C. The beads were crushed in a glass mortar with pestle the solution was than kept for 2 h to extract the drug completely and centrifuged to remove polymeric debris. The clear supernatant solution was analyzed for drug content at 317.6 nm using UV-visible spectrophotometer (Pharmaspec-UV/Visible spectrophotometer-1700, Simadzu, Japan). The amount of nizatidine present in microbeads was determined using calibration curve and the following formula:

\[
\text{Percent drug content} = \left( \frac{\text{Practical concentration}}{\text{Theoretical concentration}} \right) \times 100
\]

**Dynamic swelling study**

Hydrogels exhibit dynamic swelling property. This unique nature enables to release the entrapped drug from hydrogels, because swelling is directly proportional to drug release. The pores of matrix network opens due to swelling of hydrogel beads and release of the entrapped solute occurs. Therefore, the dynamic swelling study of the prepared beads was carried out by mass measurement as a function of pH. The degree of swelling was measured gravimetrically by weighing the particles prior and after swelling. Weighed quantity of the dried microbeads dose equivalent to marketed nizatidine formulations were immersed for 2 h, in pH 1.2 buffer as swelling medium. Then swelling medium was replaced with pH 7.4 phosphate buffer and kept until to reach equilibrium. Subsequently, microbeads were removed from the buffer solution, carefully blotted with tissue paper and weighed. The degree of swelling (swelling index) was calculated using the formula:

\[
Q = \left( \frac{W_2}{W_1} \right) \times 100
\]

Where, \(W_1\) is mass of the dry beads and \(W_2\) is the mass of swollen beads. Each swelling experiment was repeated three times, and the average value was taken as the percentage swelling value.

**Mucoadhesive strength determination**

The mucoadhesive property of microbeads was evaluated by an in vitro adhesion testing method known as the wash-off test. freshly excised pieces of sheep intestinal mucosa obtained from local slaughter house were
mounted onto glass slides. About 100 microbeads were spread onto wet rinsed tissue specimen and immediately thereafter the slides were suspended onto the arm of a tablet disintegrating machine containing 7.4 pH phosphate buffer solutions at 37°C. The tissue specimen was given a slow, regular up and down movement in the test fluid for 8 h. At the end of 1, 2, 3, 4, 5, 6, 7, 8 h the machine was stopped and the number of microbeads still adhering to the tissue were counted. The percent mucoadhesive strength was calculated using the equation:

\[
\% \text{ mucoadhesive strength} = \frac{(Na-Nl)}{Na} \times 100
\]

Where, \(Na\) = number of microspheres applied; \(Nl\) = number of microspheres leached out

**In vitro drug release study**\(^{15-17}\)

*In vitro* drug release studies were performed on the beads, using a dissolution apparatus (USP-XXIII, Electro lab, Mumbai). A weighed amount of individual bead formulations were added to muslin cloth, placed in a basket and dipped indissolution vessel containing 900 ml of pH 1.2 phosphate buffer for 2 h and followed by pH 7.4 phosphate buffer till end of the study at 37.0 ± 0.5°C with 50 rpm paddle speed. At set times, 5 ml aliquots were withdrawn, filtered and amount of drug released was assayed spectrophotometrically at 317.6 nm. The same amount of medium was replaced with fresh buffer solution. The release data were fitted to various mathematical models to know which model is best fitting the obtained release profile.

**FTIR Spectroscopy**\(^{18}\)

The spectra of nizatidine and its different formulations were recorded on FTIR spectrometer (Bruker-Alpha) and evaluated for compatibility within the formulations.

**Differential Scanning Calorimetry (DSC)**\(^{18}\)

DSC allows the fast evaluation of drug-polymer compatibility because it shows changes in the appearance, shift of melting endotherms and exotherms and/or variation in the corresponding enthalpies of reaction. Thermal behavior of the beads was examined by using a thermal analyzer (DSC-60 Shimadzu, Japan). The DSC thermograms of pure nizatidine and the formulations were recorded on a thermal analyzer. The thermal analysis was performed at a heating rate of 10°C/min over temperature range of 50-400°C under a nitrogen atmosphere in a micro calorimeter and then thermograms were obtained.

**Scanning Electron Microscopy (SEM)**\(^5\)

For visualizing surface morphology of beads SEM is promising technique. Also by taking microphotographs at time intervals from dissolution study we can predict about possible release pattern of particulate drug delivery system. The surface morphology of the beads was investigated using scanning electron microscope (JEOL,
JSM-35CF, Japan). The beads were mounted onto stubs using double sided adhesive tape and sputter coated with platinum using a sputter coater. The coated beads were observed under SEM instrument at the required magnification at room temperature. The acceleration voltage used was 10 kV with the secondary electron image as a detector.

RESULT AND DISCUSSION

The details of formulation composition, results of bead size and percent drug content of various batches are shown in Table 1. The prepared hydrogel microbeads were smooth and free flowing with light brownish in colour. The percent drug content was found to be in the range of $68.13 \pm 0.21$ to $91.61 \pm 0.35\%$. The formulations coated with chitosan showed higher drug con-
Figure 4: *In vitro* drug release profile of hydrogel formulations
A: Batches F1 to F6, B: Batches F7 to F12

Figure 5: FTIR Spectra of pure drug and formulations
A: Nizatidine, B: Sodium alginate, C: Chitosan, D: Drug loaded beads with alginate and chitosan, E: Drug loaded beads with sodium alginate

Table 2: Kinetic values of nizatidine release from optimized microbead formulation.

<table>
<thead>
<tr>
<th>Formulations code</th>
<th>Zero order Equation</th>
<th>Korsemayer Peppas Equation</th>
<th>Higuchi Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>y=8.3913x + 4.2127</td>
<td>y=-0.12x + 1.9537</td>
<td>y=30.926x - 14.624</td>
</tr>
<tr>
<td></td>
<td>R²=0.9448</td>
<td>R²=0.8915</td>
<td>R²=0.9489</td>
</tr>
<tr>
<td>F7</td>
<td>y=9.0354x + 5.7378</td>
<td>y=0.0628x + 2.1273</td>
<td>y=33.478x - 14.864</td>
</tr>
<tr>
<td></td>
<td>R²=0.9838</td>
<td>R²=0.9812</td>
<td>R²=0.9477</td>
</tr>
</tbody>
</table>
tent when compared to the formulations which are not coated with chitosan. We analyzed the particle size of prepared hydrogel beads, which was found in the range of $612 \pm 3.51$ to $801 \pm 1.99 \mu m$ for different batches. The results indicated that the size of hydrogel beads found to be increased proportionally as the amount of alginate concentration increased. This could be attributed to the increase in micro-viscosity of the polymeric...
dispersion due to increased alginate concentration, which eventually led to formation of bigger beads.

The swelling ability of alginate-chitosan complex hydrogel beads is dependent on pH value of the swelling medium. Formulations were allowed to swell for initial 2 h in acid buffer pH 1.2 solution, followed by in phosphate buffer pH 7.4 solution up to 12 h. The details of swelling profile are presented in Figure 1. Formulation F7 with alginate-chitosan complex showed highest swelling index of 95%, while formulations F10 and F11 without chitosan showed lowest swelling index of 50%. Cross-linked hydrogel beads showed obviously the higher swelling ability at pH 1.2 and slightly higher swelling ability at pH 7.4. The protonation of outer chitosan coat in presence of acidic medium could be attributed to the increased swelling ability of formulations and on other hand the slightly increased swelling degree of hydrogel beads at alkaline pH is attributed to the ionization of carboxyl groups of alginate in the inner complex layer of the beads. Swelling ability of hydrogel beads not only depends on pH of medium used but also on the concentration of matrix forming polymers and the strength of cross linking agent used.

Mucoadhesive strength study or In vitro wash off test was carried out by using sheep intestinal mucosa. The details of procedure followed and data obtained are presented in Figure 2 and 3 respectively. Alginate-chitosan complex hydrogel beads exhibited good mucoadhesive strength, which might be due to ionization of chitosan outer coat, increased the mucoadhesion ability of beads, whereas, the formulations without chitosan showed very poor mucoadhesion ability due to absence of chitosan coat on their outer surface. The formulation F7 showed highest mucoadhesive strength of 91%, while F12 batch showed lowest mucoadhesive strength of 16%. The in vitro wash off results revealed that the hydrogel beads are able to adhere to the mucous membrane for longer time and release drug from the microbeads slowly for an extended period of time up to 12 h. The in vitro drug release profiles of all the batches are shown in Figure 4. The formulation F1 and F7 showed highest drug release of 95.79% and 98.87% respectively. All the alginate-chitosan complex formulations showed slower drug release might be due to rate retarding property of outer chitosan coat and slower erosion diffusion of drug from the beads. The results of drug release profile revealed that the rate and extent of drug release from-Preparation of hydrogel beads significantly decreased with an increase in polymer concentration. This could be attributed to the increase of alginate matrix density and in the diffusion path length which the drug molecules have to traverse. The burst effect in drug release was characterized by an initial phase of high release from these beads. However, as gelation proceeded through cross-linking of alginate with calcium ions, the remaining drug was released at a slower rate followed by a phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with the increase in alginate gum concentration. The initial burst effect from batches of chitosan-coated beads was considerably reduced when compared to the corresponding batches of non-coated beads. The fact is that chitosan coating over the beads resulted in better incorporation efficiency and formed a
thick coating layer around the beads. This could be the reason for the observed decrease in the burst effect. The drug release found to be slow and extended up to 12 h as increase in the concentration of cross-linking agent calcium chloride. Low concentration of calcium chloride leads probably to a loose gel. As a consequence, the drug can be easily released from the beads, as the steric entanglements do not constitute a strong barrier. Further increase in the concentration of calcium chloride gives more structured gel and the drug is more retained inside the beads due to steric reason, since the existence of physical entanglements of cross-linked alginate-calcium chloride complex of lower dimensions controlling the drug diffusion flow within the beads.\textsuperscript{20} At high concentration of calcium chloride, as in formulation F7, strong and rigid gel is formed around the matrix and this strong gel does not allow the dissolution medium to penetrate into the matrix at a high speed, resulting in a reduction in the release rate.

In order to describe the kinetics of drug release from SR preparations, various mathematical equations have been proposed. The zero order models describe the system, where the drug release is independent of its concentration. According to Higuchi model, the drug release from matrix is directly proportional to square root of time and is based on the Fickian diffusion. A more comprehensive, but still very simple, semi-empirical equation to describe drug release mechanism from polymeric systems more precisely is the so-called Korsmeyer-Peppas power law. To study the mechanism of drug release from the randomly selected formulation F5 and F7 the release data was fitted to above mentioned models.\textsuperscript{20} The details of data obtained are presented in Table 2. The formulation F7 showed quasi Fickian release pattern with $n=0.0628$ and regression of $R^2=0.9812$. The Higuchi plot of F5 formulation showed good release profile with $R^2=0.9489$. The embedded drug within the microbeads showed matrix and SR mechanism, from this matrix drug was diffused as indicated by Higuchi plot.\textsuperscript{21-24} In an effort to investigate the possible incompatibilities between drug and polymer, we have carried out FTIR and thermal analysis of pure nizatidine, polymers and drug-loaded beads. The FTIR spectra of formulation (Figure 5) revealed that the characteristic peaks of drug were retained in the formulation containing polymers indicated that the drug is intact in the formulation. The DSC thermogram (Figure 6) showed sharp endothermic peak at 135.91°C which corresponds to the melting point of pure drug. The peak starts at 130.42°C and end at 145.9°C which indicates the higher melting rate of nizatidine. The sodium alginate and chitosan showed characteristic peak at 249.02 and 100.02°C respectively, which corresponds to their melting point. The absence of detectable peak of nizatidine in the formulation F7 and F12 clearly indicates that nizatidine was dispersed completely in the formulation, thus modifying the microbeads to an amorphous, disordered crystalline phase. The formulation F7 showed endothermic peak at 140.11°C which reveals slight shifting of melting point of nizatidine within the formulation. So nizatidine found to be compatible with the polymers in this formulation.

The surface morphology was examined by SEM studies (Figure 7 and 8). SEM microphotograph of pure nizatidine showed crystalline nature. The SEM microphotographs revealed that the beads were irregular in shape having smooth and dense surface with inward dent and shrinkage due to the collapse of the wall of the beads during dehydration. The fibrous network was also found on the surface of the beads. The microbeads from formulation F7 contain nizatidine, sodium alginate and chitosan (Figure 7), showed debris or dimple like structure on their surface. This might be due to chitosan undergo less surface deformation due to sodium alginate shrinkage during drying. Exposure of sodium alginate to heat during drying evaporates excessive interstitial solvent that leaves debris. In Figure 7 (B), microbeads from same formulation showed a rough surface morphology that indicates formation of micropores due to solvent evaporation, which was also observed in Figure 7 (C). The microbeads from batch F12 contain nizatidine and sodium alginate without chitosan (Figure 8 (A)), showed smooth surface and debris due to shrinkage of sodium alginate during drying. The Figure 8 (B), showed smooth surface and less micropores as compared to microbeads coated with chitosan. The absence of chitosan in the formulation showed smooth surface and fewer micropores on the surface.

**CONCLUSION**

From the experimental results it can be concluded that FTIR and thermal studies of formulations revealed that nizatidine was compatible with all the polymers used. From swelling index study it was proved that alginate-chitosan complex formulation showed better swelling effect. From *in vitro* mucoadhesive strength it was evident that the alginate-chitosan complex formulation showed better mucoadhesive strength when compared to formulations without chitosan coating. *In vitro* drug release study of alginate-chitosan complex formulation indicated that nizatidine was released in sustained manner up to 12 h with best zero order profile and quasi Fickian release pattern. The embedded drug within the
microbeads showed matrix and controlled release mechanism, from this matrix drug was diffused as indicated Higuchi plot. Hence, it can be concluded from the study that, among the prepared formulations with respect to percentage drug content, swelling studies and in vitro drug release, the alginate-chitosan beads prepared by ionotropic gelation and polyelectrolyte complexation method found to be better than ionically cross linked alginate beads alone. Therefore, dual cross-linked beads are promising carrier for oral control release.

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CONFLICTS OF INTEREST

Authors disclose that there is no Conflict of Interest in this work.

ABBREVIATION

DSC : Differential scanning calorimetry
FTIR : Fourier transforms infrared spectroscopy
GIT : Gastro intestinal tract
SEM : Scanning electron microscopy

SUMMARY

- Mucoadhesive hydrogel beads of nizatidine were prepared with or without chitosan by using sodium alginate as polymer
- Formulations with chitosan showed good drug content, swelling index and mucoadhesive strength when compared to batches containing alginate alone.
- The drug in formulations found to be intact and compatible with polymers used which was confirmed by DSC and FTIR analysis. Surface morphology of prepared beads were found satisfactory which was confirmed by SEM
- Two optimized batches containing alginate-chitosan shows Higuchi model and perfect zero order release. All the batches with copolymer showed better sustained the drug release more than 12 h when compared with batches prepared with alginate alone.

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