RESEARCH ARTICLE

PREPARATION AND IN-VITRO EVALUATION OF LANSOPRAZOLE MUCOADHESIVE MICROSPHERES

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ABSTRACT:

Lansoprazole is a proton pump inhibitor prodrug used in the treatment of gastric ulcers and gastroesophageal disease. Lansoprazole must be absorbed in gastrointestinal tract and because it is unstable under acidic conditions, enteric delivery systems are required. The purpose of this study was to prepare lansoprazole mucoadhesive microspheres by ionotropic gelation technique using sodium alginate and mucoadhesive substance chitosan followed by a enteric coating with cellulose acetate phthalate. The microspheres have been characterized in vitro in the terms of their surface morphology, particle size, encapsulation efficiency, swelling ratio, mucoadhesivity and ability of stabilizing lansoprazole in acidic media. Drug entrapment efficiency of lansoprazole was evaluated at 285nm. Different formulation variables like polymer-polymer ratio, drug-polymer ratio and coating concentrations were considered. Almost spherical microspheres were obtained with sufficient swelling, Mucoadhesive property and acid resistance. Dissolution study was followed at phosphate buffer (pH-7.4) for 8 hr.

KEYWORDS: Lansoprazole, sodium alginate, ionotropic gelation, chitosan, cellulose acetate phthalate.

INTRODUCTION AND EXPERIMENTAL

Despite tremendous advancement in drug delivery, oral route remains the preferred route for the administration of therapeutic agents, low cost of therapy and ease of administration leads to higher levels of patient compliance. Conventional oral dosage forms such as tablets and capsules provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels.

These have a disadvantage of a release all or nothing emptying process while the multiple unit particulate system pass through the GIT to avoid the vagaries of gastric emptying and thus release the drug more uniformly. Various approaches have been worked out to improve the retention of oral dosage form in the stomach. e.g.,bio-adhesive systems, floating systems, swelling or expanding systems and high density systems. Microsphere1 carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery2. However,
the success of these microspheres is limited owing to their short residence time at the site of absorption. This can be overcome by coupling bio-adhesive characteristics to microspheres and developing bio-adhesive microspheres.

The term bio-adhesion describes materials that bind to the biological substrates such as mucosal membranes. Adhesion of bio-adhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of absorption. This prolonged residence time can result in the enhanced absorption and in combination with a controlled release of drug also improved patient compliance by reducing the frequency of administration. The epithelial adhesive properties of mucin have been applied in the development of gastroretentive drug delivery systems.

Alginate\(^5\) is a naturally occurring biopolymer that is finding increasing applications in the biotechnology industry. Alginate has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Alginic acid is a linear copolymer of p-O-mannuronic acid and \(\alpha\)-L-glucuronic acid linked by \((1-4)\) - glycosidic bonds. Alginate gelation takes place when divalent cations (usually \(\text{Ca}^{+2}\)), interact ionically with blocks of glucuronic acid residues, resulting in formation of three-dimensional network which is usually described by 'Egg-box' model.

The natural mucoadhesive substance\(^6\) (NMS) used in the present work was obtained from the local market e.g. Chitosan\(^7\). The NMS has considerable swelling behavior in water particularly in buffer 7.4. This may be considered as significant for its use in mucoadhesive drug delivery, particularly for controlled release.

The phthalates are widely used as film-coating materials in oral pharmaceutical formulations; cellulose acetate phthalate is insoluble below pH 5 and thus resistant to gastric fluid. By salt formation in the neutral to weakly alkaline medium of the intestinal fluid, the polymer dissolves step-wise at pH values above 5.5. For this study, an acid-labile drug, lansoprazole, was chosen to be microencapsulated by the ionotropic gelation technique using sodium alginate and chitosan blend.

Lansoprazole is a proton pump inhibitor, used in the treatment of digestive ulcers. It is a prodrug that degrades once protonated in acidic media. So, the drug protonation for activation must occur inside the gastric parietal cells, and the tetra cyclic form of lansoprazole binds irreversibly to cysteine residues of the proton pump (\(\text{H}^+ / \text{K}^+\) ATPase). In this way, lansoprazole must be absorbed intact before activation and, because of this it requires an enteric drug delivery system.

The proposed system is expected to provide several advantages. Firstly, gelation of the aqueous solution of alginate/chitosan blend renders oral sustained drug delivery. Secondly, CAP-coating prevents the solvation of beads and acid labile drug leakage in the stomach, leading to intestinal drug release. Gastric retention time of enteric coated microspheres enhances with the addition of mucoadhesive agent, resulting in the delivery of drug across the mucous membrane for an extended period of time in intestine.

The main aim of the present work was to prepare by ionotropic gelation\(^8\) technique and to characterize the controlled-release enteric microspheres containing lansoprazole.
MATERIALS AND METHODS

Materials:

Lansoprazole\(^9\),\(^10\) was obtained from Enal drugs Pvt. Ltd (Hyderabad, India). The polymers cellulose acetate phthalate (CAP), sodium alginate and chitosan were obtained from Chemi Pvt Ltd (Bangalore, India).

Methods:

PREPARATION OF MICROSPHERES:

Accurately weighed 2g of sodium alginate was dispersed in 100ml of distilled water using a magnetic stirrer at 40°C. After complete dispersion, added 1g of lansoprazole while the stirring was continued until complete and uniform dispersion was obtained. The chitosan\(^11\) solution was prepared by dispersing 10mg of chitosan powder in 10 ml of distilled water by heating at 40°C. Then the above prepared chitosan solution was added to the homogenous dispersion of sodium alginate containing 1g of drug (lansoprazole) which was homogenized thoroughly with the help of magnetic stirrer.

The resulting bubble free dispersion was added manually drop wise with a 5 ml syringe (22 gauze needle) into 100ml of (10%w/v) calcium chloride solution (CaCl\(_2\)) stirred in a 250ml beaker. The gelation time of 15 min was allowed to complete the curing reaction and produce spherical and rigid microspheres. The beads so prepared were collected by decantation, washed with water and dried in hot air oven at 60°C for 2 hours. The process was applied to 3 different formulations by using varying proportions of chitosan and sodium alginate\(^12\) (i.e., F1, F2, and F3).

Formulations with different formulation variables:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lansoprazole (%)</th>
<th>Sodium Alginate (%)</th>
<th>NMS (Chitosan)</th>
<th>Stirring Speed (rpm)</th>
<th>Cross linking Agent CaCl(_2) (%w/v)</th>
<th>Curing Time</th>
<th>Coating material (CAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1g</td>
<td>2g</td>
<td>10mg</td>
<td>200</td>
<td>10</td>
<td>15min</td>
<td>10g</td>
</tr>
<tr>
<td>F2</td>
<td>1g</td>
<td>1.8g</td>
<td>20mg</td>
<td>200</td>
<td>10</td>
<td>15min</td>
<td>10g</td>
</tr>
<tr>
<td>F3</td>
<td>1g</td>
<td>1.5g</td>
<td>30mg</td>
<td>200</td>
<td>10</td>
<td>15min</td>
<td>10g</td>
</tr>
</tbody>
</table>

Table No. 1: Formulations with different formulation variable

Preparation of enteric coated microspheres\(^13\):

The above prepared spheres were transferred into acetone solution of CAP (cellulose acetate phthalate) at concentration of 10%, and coated for 15min under uniform stirring. The resulting coated beads were filtered and air dried. The coating process was continued for 3 times, and the above process was applied to 3 different formulations i.e., F1, F2 and F3.

CHARACTERIZATION OF MICROSPHERES\(^6\),\(^14\):

Determination of particle size:

The prepared microspheres were mounted in light liquid paraffin, and the diameters of 50 particles were measured by means of an optical microscope equipped with a calibrated ocular micrometer. The mean diameter was then calculated. Mean particle size = \(\Sigma \text{n.d} / \Sigma n\)
**Entrapment efficiency:**

The drug entrapment efficiency of beads was estimated by dispersing the beads in 100 ml of phosphate buffer at pH 7.4 by vigorous shaking on mechanical shaker for 12 hr. Then, the solution was filtered, and the lansoprazole content was assayed by a UV-spectrophotometer at 285 nm. The entrapment efficiency of micro beads was calculated using the following formula:

\[
\text{Estimated percentage drug loading} = \left( \frac{\text{Theoretical percentage drug loading}}{\text{Actual percentage drug loading}} \right) \times 100
\]

**Swelling study:**

The swelling studies of uncoated beads were performed in aqueous swelling media with pH 7.4 buffer at 37.5 ± 0.5°C. The swelling ratio, S_{sw}, was calculated from the following expression.

\[
S_{sw} = \left( \frac{W_t - W_0}{W_0} \right) \times 100
\]

Where, W_t and W_0 are weight of sample swollen at time ‘t’ and weight of the original sample respectively.

**In vitro drug release study:**

The release of lansoprazole from the microbeads was studied in phosphate buffer pH 7.4 as medium using dissolution test apparatus paddle type at 37 ± 0.2°C with a rotating speed of 50 rpm. A sample of microbeads equivalent to 40 mg of lansoprazole was used in each test. At present time intervals 5ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. The samples were withdrawn through a membrane filter and were analyzed for lansoprazole content spectrophotometrically at 285 nm using the UV-visible spectrophotometer.

**Determination of Gastro-Resistance of enteric coated beads:**

The gastro resistance of coated beads was determined by the following method.

- The samples (F_1, F_2 and F_3) were placed in dissolution bath containing 0.1N HCl at 37 ± 0.2°C for 1 hour. During the acid step, no sample was collected for quantification because any amount of lansoprazole released at this pH is quickly degraded.
- After the acid step, the HCl solution was replaced by phosphate buffer pH 7.4. Then, samples were collected at predetermined time intervals and analyzed spectrophotometrically at 285 nm.

**EVALUATION OF MUCOADHESIVE PROPERTY:**

**Apparatus used:**

Chicken intestine (2x2cm), glass slides, USP tablet disintegration apparatus, phosphate buffer pH 7.4.

**Method:**

The mucoadhesive property of uncoated beads was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of chicken intestinal mucous were mounted on to glass slides with cotton thread.

About 20 micro beads were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test (tab. Machines, Mumbai, India). When the test apparatus was operated, the sample is subjected to
slow up and down movement in the test fluid at 37°C contained in a 1-litre vessel of the apparatus. At an interval of 30min up to 8 hours the machine is stopped and number of beads still adhering to mucosal surface was counted. The test was performed at intestinal (phosphate buffer pH 7.4) condition.

RESULTS AND DISCUSSION

Determination of particles size:

<table>
<thead>
<tr>
<th>Particle size (in µm)</th>
<th>No. of Particles</th>
<th>Mean particle size (in µm)</th>
<th>Average Particle Size (micrometers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>10-20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>41-50</td>
<td>11</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>51-60</td>
<td>12</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>61-70</td>
<td>16</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>71-80</td>
<td>23</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>81-90</td>
<td>19</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>91-100</td>
<td>13</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

Table No.2: Particles Size Analysis

Observation:

The effect of different parameters on particle size of micro beads has been summarized in table No. 2. Increase in gel concentration increases the mean particle size of the beads. This is due to the increase in viscosity, which in turn increases the droplet size during addition of the polymer solution to the cross-linking agent solution. Particle size also increases by increasing the drug load.

Entrapment efficiency:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Weight taken (g)</th>
<th>Media Qty (mL)</th>
<th>Absorbance</th>
<th>Entrapment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.21</td>
<td>100</td>
<td>0.272</td>
<td>67.50%</td>
</tr>
<tr>
<td>F2</td>
<td>0.21</td>
<td>100</td>
<td>0.298</td>
<td>75.02%</td>
</tr>
<tr>
<td>F3</td>
<td>0.21</td>
<td>100</td>
<td>0.324</td>
<td>82.52%</td>
</tr>
</tbody>
</table>

Table No.3: Entrapment efficiency
Observation:
- The drug entrapment efficiency of different formulations has been summarized in the Table No. 5.
- Lansoprazole being highly soluble in water has a tendency to diffuse out to the aqueous medium despite this sufficiently high drug entrapment to the gel beads prepared with chitosan could be achieved that might be resulted due to hindered diffusion of the medicament through the gel barrier formed by the chitosan. It was observed that, as the concentration of chitosan increases, viscosity of resulting gel increases and thereby increases entrapment efficiency.
- An increase in drug load was also observed by increasing the concentration of drug. The decrease in entrapment efficiency in case of coated beads maybe due to leakage of drug into coating solution during coating.

Swelling factor:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Wt. of spheres taken (mg)</th>
<th>Initial wt. (mg)</th>
<th>Final wt. (mg)</th>
<th>Swelling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>F₂</td>
<td>10</td>
<td>10</td>
<td>16.2</td>
<td>62</td>
</tr>
<tr>
<td>F₃</td>
<td>10</td>
<td>10</td>
<td>17.5</td>
<td>75</td>
</tr>
</tbody>
</table>

Table No.4: Swelling factor

Observation:
- The swelling behavior of uncoated microbeads was determined gravimetrically. The result indicated that as the amount of polymer (formulations F₁, F₂, F₃) in microbeads increased the swelling ratio also proportionately increased. Results given in Table No.4.
- The higher percentage of chitosan in micro beads renders high swelling and gel formation. So the inclusion of chitosan in alginate gel opens an option for the manufacture of cross linked matrix devices for gastrointestinal delivery.
**In-vitro drug release study:**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TIME (hrs)</th>
<th>ABSORBANCE(285nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.102</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.102</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.205</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.255</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.305</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>0.398</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>0.425</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>0.479</td>
</tr>
</tbody>
</table>

**Table No. 5: In-vitro drug release study**

**Observation:**

- The drug-polymer ratio was found to affect the drug entrapment, particle size and ultimately the drug release characteristics of the prepared micro beads. At higher drug-polymer ratio the drug release from the micro beads was faster as compared to lower drug/polymer ratio.

- This may be due to the increase in the drug/polymer ratio with an increase in the amount of drug loaded in the polymer, suggesting that higher amount of drug is released per unit area of exposed surface of the polymer matrix.

- A significant decrease in rate and extent of drug release was observed with the increase in
polymer concentration in micro beads and is attributed to an increase in the density of polymer matrix and in the diffusion path length that the drug molecules have to traverse.

- The prolongation of the release rate from the hydro gel beads with increase of chitosan concentration reflects the concomitant increases in gel strength which is a determining factor in this case since the release of drugs in polymer matrices are mainly through the diffusion of the drug through the pores of the polymer network which can be significantly reduced in size by increasing the polymer concentration.

- The initial higher release from the uncoated beads reflects the lower diffusional resistance of these core beads compared with that of the coated beads caused by the absence of a barrier against drug diffusion.

- The results demonstrated that the enteric-coated beads provide a system of low permeability and a good barrier against drug diffusion under pH conditions, at which protection is required.

**Mucoadhesion test:**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>No. of microspheres</th>
<th>Percentage of adhesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>F1</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>F2</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>F3</td>
<td>20</td>
<td>16</td>
</tr>
</tbody>
</table>

Table No.6: Mucoadhesion test

**Observation:**

- The adhesion of microspheres to the intestinal mucosa of chicken was evaluated as the mean percent of microspheres remain adhered after a defined period of washing.
- Results indicating that the polymer to drug ratio had a significant effect on mucoadhesive property.
- The greater the concentration of the polymer associated with chitosan-alginate matrix, greater will be the adhesion.
- An increase in drug load has no such effect on mucoadhesive property.

**CONCLUSION:**

- Lansoprazole release from these enteric coated mucoadhesive micro-spheres was slow and extended over a longer period of time and dependent on ratio of polymers.
- The acid resistance experiment carried out with microspheres showed that 68%, 74%, 82% of lansoprazole remained stable for F1, F2 and F3 respectively, presenting drug protection.
- These studies demonstrated that lansoprazole can be encapsulated into microspheres having chitosan and sodium alginate backbone by micro orifice syringe gelation technique. Presence of enteric coating has little effect on drug release, but efficiently protects the acid labile drug from highly acidic environment of stomach.
In conclusion, the performed studies suggested that chitosan may be a promising candidate for oral controlled drug delivery system because of its gel forming ability and sustaining the release of drug.

Among F1, F2, F3 formulations, F3 formulation had better controlled drug release profile because F3 formulation contained more mucoadhesive polymer concentration compared to other formulations.

ACKNOWLEDGEMENT

The authors would like to express sincere thanks to our guide Dr K S Chandraprakash Mpharm, PhD (HOD), MES College of pharmacy, Bangalore, India.

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