Algino-Losartan Mucoadhesive Microspheres: An Unique Device for Prolonged Drug Delivery

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ABSTRACT

The purpose of this research was to develop and evaluate algino-losartan multiparticulate system in order to obtain a unique drug delivery device to sustain the drug release for prolong period of time and thereby increase the bioavailability as well as patient compliance and reduce the dosage frequency. Various polymer concentrations potentially influencing the entrapment efficiency, particle size and drug release were investigated. Micromeritic study suggested that flow property and compressibility of the pure drug could be improved by prepared microspheric formulations. It was also found that by increasing the polymer concentration, mean particle size as well as encapsulation efficiency also increased. All the formulations exhibited excellent swelling and mucoadhesive properties in distilled water. In-vitro drug release study revealed that alginate microspheres could able to sustain the drug release for prolong period of time. Kinetics of drug release proposed a combined effect of diffusion and erosion mechanism for drug release from the microspheres. FTIR and DSC study suggested that there was no interaction between drug and polymer. Holm-Sidak multiple comparison analysis suggested a significant difference with respect to in-vitro drug release among all the formulations. So, it is concluded from the present research that losartan potassium loaded alginate microspheres is a unique drug delivery device to improve the patient compliance, reduce the dosage frequency by sustaining and prolonging the systemic absorption of losartan potassium.

Keywords: Algino-losartan microspheres, prolong release, mucoadhesion, multiple comparison analysis, interaction study.

INTRODUCTION

A number of research works are involved using different categories of drugs to be delivered as microsphere formulation, but among all the drugs, losartan potassium has its own importance. Losartan potassium is an imidazole derivative [2-n-butyl-4-chloro-5-hydroxymethyl-1-((2′-(1H-tetrazol-5-yl)(biphenyl-4-yl)methyl)imidazo1, 5-α-potassium salt], is a potent, highly specific angiotensin II type 1 receptor antagonist with anti hypertensive activity1. It is readily absorbed from the gastrointestinal tract with oral bioavailability of about 33% and a plasma elimination half life of about 1.5 to 2.5 h.2 The main drawbacks of losartan potassium conventional dosage form are its short biological half life, frequent administration and low bioavailability. These criteria’s makes losartan potassium an ideal candidate for the development of controlled release microsphere formulation to release the drug at a sustain manner as well as reduce the dosage frequency.

Sodium alginate is a biodegradable, biocompatible and bioadhesive polymer is gaining attention in the pharmaceutical field for a wide range of drug delivery3. Alginites are linear, anionic block copolymer heteropolysaccharides consisting of monomers of (d-mannuronic acid) (M) and its C-5 epimer, (l-guluronic acid) (G), residues joined together by 1, 4-glycosidic linkages. Sodium alginate is converted to aqueous-based gel beads in presence of divalent alkaline metals such as Ca2+ and Ba2+ or trivalent Fe3+ and Al3+ due to an ionic interaction and intramolecular bonding between the carboxylic acid groups located on the polymer backbone and these cations4. Microparticulate system is a potential drug delivery devices widely used for targeted and controlled release drug delivery. But if microparticulate system has coupling with mucoadhesive properties shows additional advantages, such as efficient absorption, increase the intimate contact time with the mucus layer and targeting of the drug to the particular site5.

So, the aim of the present research work was to develop and evaluate mucoadhesive algino-losartan multiparticulate system by ionotropic gelation method in order to obtain a unique drug delivery device to sustain the drug release for prolong period of time and increase the patient compliance by reducing the dosage frequency.

MATERIALS AND METHODS

Materials

Losartan potassium (LP) was a gift sample from Alkem Pvt. Ltd., Mumbai, India. Sodium alginate (viscosity ≈ 3500 cps) was a gift from Signet Chemical Co. Mumbai, India. Calcium chloride was a gift from Loba Chem., India. Other materials and solvents used were of analytical grade.
Method

Fabrication of algin-losartan microspheres

Algin-losartan microspheres were formulated employing the method described by Khandai et al. Microspheres containing losartan potassium were prepared employing sodium alginate by ionic gelation method. Sodium alginate was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution. Then core material losartan potassium was added to the polymer solution and mixed thoroughly to form a smooth viscous dispersion. The resulting dispersion was then added drop wise using a 24 G needle in 500 ml of 5% calcium chloride solution (Formulation LA1 to LA6) at a constant rate and under continuous stirring at 200 rpm. The stirring was continued for 1 hour for complete reaction and then the microspheres were collected by filtration, washed extensively with distilled water and dried overnight at 40°C. Dried microspheres were kept in a desiccator for further use. The compositions of the microspheres are listed in Table-1.

% Yield

The percentage yield of microspheres of various formulations were calculated using the weight of final product (microspheres) after drying with respect to the initial total weight of the drug and polymers used for preparation of microspheres. The percentage yields were calculated as per the following formula,

\[
\text{Percentage Yield} = \frac{\text{weight of the initial raw materials used in the formulation}}{\text{weight of the final product (microspheres)}} \times 100
\]

Entrapment efficiency

The entrapment efficiency was determined by the reported method. The microspheres (100 mg) were allowed to disintegrate in 50 mL of distilled water for 4 h. Dispersion of microspheres was sonicated at 125 W for 30 min (Imeco Sonifier, Imeco Ultrasonics, India) and the solution was filtered through Whatman filter paper (0.45 mm). Then, the polymeric debris was washed twice with fresh solvent (distilled water) to extract any adhering drug. The drug content of the filtrate was determined spectrophotometrically at 252 nm (UV-2450, Shimadzu, Japan). Each determination was made in triplicate and encapsulation efficiency was calculated as follows:

\[
\text{Entrapment efficiency(\%)} = \frac{\text{total amount of drug in microspheres}}{\text{total amount of drug added initially}} \times 100
\]

Table 1: Formulation and characterization of the aceclofenac-loaded alginate microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug : Polymer</th>
<th>Rigidizing agent/level (5% w/v)</th>
<th>Reaction time (min)</th>
<th>Stirring Speed (rpm)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA1</td>
<td>1 : 1</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>91.32 ± 2.29</td>
</tr>
<tr>
<td>LA2</td>
<td>1 : 2</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>90.49 ± 3.65</td>
</tr>
<tr>
<td>LA3</td>
<td>1 : 3</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>93.71 ± 3.38</td>
</tr>
<tr>
<td>LA4</td>
<td>1 : 4</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>96.99 ± 2.87</td>
</tr>
<tr>
<td>LA5</td>
<td>1 : 5</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>94.30 ± 4.39</td>
</tr>
<tr>
<td>LA6</td>
<td>1 : 6</td>
<td>CaCl₂</td>
<td>60</td>
<td>200</td>
<td>96.13 ± 2.15</td>
</tr>
</tbody>
</table>

Characterization of Microspheres

Physicochemical characterization of the algin-losartan microspheres

Particle size analysis

Size and size distribution of microspheres were measured by sieve analysis. The microspheres were separated into different size fractions (% weight fraction) by sieving using standard sieves (sieve no. 12, 14, 16, 18 and 22). The sieve set was fixed to the mechanical shaker and shaken for 5 min. Then the microspheres retained on each sieve were collected separately and weighed. The study was conducted in triplicate and mean particle size of microspheres was calculated using the following formula:

\[
\text{Mean particle size} = \frac{\sum (\text{mean particle size of the fraction} \times \text{weight fraction})}{\sum \text{weight fraction}}
\]

Micromeritic properties of microspheres

The different micromeritic properties such as angle of repose, Carr’s index and Hausner ratio of the microspheres and pure drug were calculated and shown in Table-2. The angle of repose was used to estimate the flowability of the microspheres and pure drug. It was measured by fixed funnel method. Carr’s index and Hausner ratio are the two simple testing methods calculated from the bulk density and tapped density of the samples to analyze the flowability and compressibility of a powder. Each experiment was conducted in triplicate and values are shown in Table-2.

Fluid sorption isotherm studies

An accurately weighed quantity of the microsphere was placed in distilled water at 37 ± 0.5°C and allowed to swell. At regular time intervals, the swollen microspheres were carefully withdrawn from the medium and excess amount of solvent was removed by using blotting paper.
from the surface of the microsphere. Then the swollen microspheres were weighed on a single pan balance. Fluid sorption was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination. Each determination was made in triplicate for each individual experiment and percentage fluid sorption was calculated using the following formula:

\[
\text{Fluid sorption (\%) } = \frac{(\text{weight of microspheres after swelling} - \text{initial weight of microspheres})}{\text{initial weight of microspheres}} \times 100
\]

**Mucoadhesion studies of microspheres**

The mucoadhesion property of the microsphere was assessed by *in-vitro* wash-off test\(^8\) using distilled water as a solvent. The freshly excised pieces of goat intestinal mucosa (2×3 cm) were mounted onto glass slides and about 50 no. of microspheres were spread onto each wet rinsed tissue specimen. Then the slides were hung onto the arm of a USP tablet disintegrating test apparatus and the tissue specimen were placed in a vessel containing one liter distilled water at 37 ± 0.5°C. At predetermined time interval, the number of microspheres still adhering to the tissue was counted. Each determination was made in triplicate and the adhering percent was calculated by the following formula,

\[
\text{Adhesion (\%) } = \frac{\text{number of microspheres adhered}}{\text{number of microspheres applied}} \times 100
\]

**In-vitro drug release studies**

*In-vitro* drug release study of losartan potassium was determined using USP dissolution rate test apparatus II (DISSO 2000, LABINDIA, India). An accurately weighed quantity of microspheres (equivalent to 50 mg of pure losartan potassium) was suspended in dissolution flask containing 900 mL of distilled water. The dissolution medium was stirred at 50 rpm and maintained to a constant temperature 37 ± 0.5°C for throughout the study. 5 mL samples were withdrawn at predetermined time interval and replaced by an equal volume of fresh pre-warmed dissolution medium. After suitable dilutions, samples were analyzed for losartan potassium content using UV-Visible double beam spectrophotometer (UV-2450 Shimadzu, Japan) at 252 nm. The release studies were conducted in triplicate shown in Figure-4.

**Kinetic modeling**

The kinetics of drug release from the prepared microspheres was analyzed by fitting the dissolution data into different rate equations such as:

Zero order equation\(^{10}\),

\[
Q = k_0 t
\]

Where, \(Q\) is the amount of drug released at time \(t\) and \(k_0\) is the zero order release rate constant.

First order equation\(^{11}\),

\[
\ln(100 - Q) = \ln 100 - k_1 t
\]

Where, \(k_1\) is the first order release rate constant.

Higuchi’s square root model\(^{12}\),

\[
Q = k_H t^{1/2}
\]

Where, \(Q\) is the percentage of drug released at time \(t\), \(k_H\) is the Higuchi release rate constant.

Peppas equation\(^{13}\),

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

Where, \(n\) is the release exponent; indicative of the mechanism of release, \(M_t/M_\infty\) is the fraction of the drug at time \(t\), \(k\) is the release rate constant.

The criteria for selecting the most appropriate model were based on the highest values of the coefficient of determination (\(r^2\)).

**Surface morphology study**

Shape and surface morphology of microspheres were studied by using scanning electron microscope (SEM)\(^{14}\). The sample was placed in the scanning electron microscope (S 3700 VP FE-SEM, Hitachi High-Technologies, Europe) chamber (chamber pressure of 0.6 mm Hg) at acceleration voltage of 15 kV and photographs were taken.

**FTIR analysis**

Fourier Transform Infrared Analysis (FTIR) of pure drug (Losartan Potassium), pure polymer and drug-loaded optimized microsphere formulations were obtained using FTIR analyzer (Prestige-21, Shimadzu FT-IR, Japan). The samples were scanned over the wave number ranges between 4000 to 400 cm\(^{-1}\) at the ambient temperature and shown in Figure-6.

**Differential scanning calorimetric analysis**

Differential scanning calorimetric (DSC) thermograms of pure drug (Losartan Potassium), pure polymer and algino-losartan optimized microsphere formulation were obtained using a Differential Scanning Calorimeter (Diamond DSC, PYRIS, Perkin Elmer, USA). The samples were heated at constant rate of 10°C/min over a temperature range of 50°C to 400°C. The system was purged with nitrogen gas at the rate of 100 mL/min to maintain inert atmosphere. The DSC thermograms were shown in Figure-7.

**Statistical analysis**

In-vitro drug release data of all the formulations were subjected to one way analysis of variance (one way ANOVA) followed by multiple comparison analysis study to find out whether any significant difference was present among the formulations or not. Statistical analysis of the data was performed using the PRISM software (Graph pad, San Diego, CA). A confidence limit of \(P < 0.05\) was fixed for interpretation of the results.
RESULTS AND DISCUSSION

Physicochemical characterization of the algino-losartan microspheres

The percentage yield of the different algino-losartan microsphere formulations was found to be 90.49 ± 3.65 % to 96.99 ± 2.87 % (Table-1). The entrapment efficiency and particle size were found within the range of 68.63 ± 3.27 % to 92.10 ± 4.01 % and 578 ± 11.3 µm to 797 ± 6.7 µm respectively (Table-2, Figure-1). It was found that by increasing the polymer concentration, mean particle size as well as encapsulation efficiency was also increased (Figure-1). This may be due to the fact that higher polymer concentration producing much larger particles as compare with lower concentration. Higher concentration of the polymer also increases the viscosity of the medium as well as greater availability of calcium binding sites in the polymeric chains. As a result degree of cross-linking was increases15 and larger droplets were formed which entrapping greater amount of drug.

Micromeric properties of microspheres

The micromeric properties of the prepared microspheres were found to be within the desired theoretical level (Table-2). The angle of repose and Carr’s index of the pure drug was found to be 40.9 ± 3.2 degrees and 35.3 ± 2.9 % respectively, indicates the poor flow nature of the pure drug. In case of microsphere formulations, the angle of repose and Carr’s index were found to be within 16.9 ± 2.1to 23.5 ± 2.1 degrees and 12.4 ± 1.8 % to 20.6 ± 3.1 % respectively. The micromeric study shows that by these microsphere formulations, helps to improve the flow properties and compressibility of the pure drug. The improvement of flow property was further confirmed by Hausner ratio. The Hausner ratio was found to be 1.13 ± 0.11 to 1.32 ± 0.21 of the prepared formulations whereas in case of pure drug, the value was 1.57 ± 0.26. This suggests that all alginate microsphere formulations exhibit good flow property and excellent compressibility as compare to pure drug.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Angle of Repose (°)</th>
<th>Carr’s index (%)</th>
<th>Hausner’s ratio</th>
<th>Encapsulation efficiency (%)</th>
<th>Particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>40.9 ± 3.2</td>
<td>35.3 ± 2.9</td>
<td>1.57 ± 0.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LA1</td>
<td>23.5 ± 2.1</td>
<td>20.6 ± 3.1</td>
<td>1.32 ± 0.21</td>
<td>68.63 ± 3.27</td>
<td>578 ± 11.3</td>
</tr>
<tr>
<td>LA2</td>
<td>21.8 ± 2.9</td>
<td>19.3 ± 3.4</td>
<td>1.28 ± 0.26</td>
<td>76.12 ± 4.03</td>
<td>601 ± 9.5</td>
</tr>
<tr>
<td>LA3</td>
<td>17.6 ± 3.1</td>
<td>18.1 ± 2.1</td>
<td>1.16 ± 0.27</td>
<td>79.93 ± 2.18</td>
<td>644 ± 10.7</td>
</tr>
<tr>
<td>LA4</td>
<td>18.3 ± 1.3</td>
<td>19.7 ± 1.4</td>
<td>1.18 ± 0.19</td>
<td>83.29 ± 3.36</td>
<td>727 ± 14.8</td>
</tr>
<tr>
<td>LA5</td>
<td>17.2 ± 1.7</td>
<td>13.7 ± 2.8</td>
<td>1.15 ± 0.09</td>
<td>92.10 ± 4.01</td>
<td>739 ± 12.1</td>
</tr>
<tr>
<td>LA6</td>
<td>16.9 ± 2.1</td>
<td>12.4 ± 1.8</td>
<td>1.13 ± 0.11</td>
<td>87.33 ± 2.98</td>
<td>797 ± 6.7</td>
</tr>
</tbody>
</table>

Mean ± SD, n = 3; PD: Pure drug, LA: Algino-losartan formulation.

Fluid sorption isotherm studies

In the fluid sorption isotherm studies, the microspheres were subjected to swell up in presence of distilled water at 37 ± 0.5 °C. The amount of media up take by the prepared microspheres at different time intervals was shown in Figure-2 (Formulation LA1 to LA6).

It was observed that, all the formulations exhibited excellent swelling property in distilled water. This may be due to the fact that when the microspheres came in contact with dissolution media, they absorbed the media.
and swelled up. As a result they form a gel layer around the microsphere matrix system. It was also observed that the fluid sorption capacity of the prepared formulations was enhanced by increasing the polymer concentration and LA6 shows maximum sorption in comparison to other formulations. As polymer concentration was maximum in the formulation (LA6), maximum viscous gel was produced around it, which helps in absorbing maximum fluid.

**Mucoadhesion studies of microspheres**

The mucoadhesive properties of prepared algino-losartan microspheres being studied with *in-vitro* wash-off test. It was found that all the microspheres have excellent mucoadhesion property in distilled water. It was observed that all the formulations exhibited 52.80 ± 3.89 % to 86.62 ± 2.21 % mucoadhesion up to 6 hours (Figure-3). The results indicate that the solubility, hydration and mucoadhesivity property of sodium alginate was increased in distilled water. The increase in polymer solubility in turn to produce a viscous gel which increases the mucoadhesion property.

*Figure 3: Mucoadhesion behavior of algino-losartan microsphere formulation in distilled water (mean ± SD, n = 3).*

**In-vitro drug release studies**

The effect of polymer concentration on the release of losartan potassium from alginate microspheres was studied using USP dissolution rate test apparatus II (Figure-4). It was found that the polymer concentration of the prepared microspheres increased; result in decrease the drug release proportionately. It may be due to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increases the diffusion path length, which the drug molecules have to traverse. Algino-losartan microspheres (LA1 to LA6) were able to sustain the drug released from 3 to 8 hours. Formulation LA1, LA2, LA3 and LA4 (drug: polymer ratio 1:1, 1:2, 1:3 and 1:4 respectively) were able to sustain the drug release up to 3, 4, 5 and 6 hours respectively whereas formulation LA5 was able to sustain the drug release for 8 hours.

*Figure 4: Effect of sodium alginate concentrations on release characteristics of losartan potassium in distilled water (mean ± SD, n = 3).*

It has been observed that formulations LA1 and LA2 shows erosion type release behavior. Subsequently increasing the concentration of sodium alginate (formulation LA5), prolonged the drug release was observed (Formulation F5 shows 91.14 ± 1.19 % drug release at 8 hours). On increasing the quantity of sodium alginate up to 6 % w/w (formulation LA6), the release of the drug was too slow and only 73.33 ± 1.92 % of the drug was released after 8 hours. It has been observed that algino-losartan microsphere could sustain the drug release due to the fact that calcium (divalent) could form a bonding structure with alginate inside the microspheres. This bonding results in extended cross-linking through the whole microsphere producing hard calcium-alginate microspheres and thus leading to slow removal of Ca$^{2+}$ due to ion-exchange with Na$^+$ in water. As a result, the swelling of the microspheres were delayed leading to slow disintegration as well as slow dissolution.

**Kinetic modeling**

The in vitro dissolution data were analyzed by different kinetic models in order to find out the n value, which describes the drug release mechanism. The kinetic data of all the formulations shows best fit in Korsmeyer model followed by Fickian diffusion mechanism which indicated the combined effect of diffusion and erosion mechanism for drug release. The coefficient of determination ($r^2$) values of all the formulations was shown in Table-3.

One way analysis of variance suggested a significant difference in *in-vitro* drug release at P<0.05 among all the formulations. Holm-Sidak multiple comparison analysis suggested a significant difference among all the formulations with respect to *in-vitro* drug release. Formulation LA5 shows the best dissolution profile (more than 90 % drug was released in 8 hours) among all the formulations with respect to *in-vitro* drug release. So, Formulation LA5 has been selected as an optimized formulation for further studies.
**Surface morphology study**

The scanning electron micrograph (SEM) of the microspheres is shown in Figure-5.

**Figure 5:** Scanning electron photomicrographs of optimized algino-losartan microspheres of formulation (LA5; 50 X).

The SEM results revealed that the microspheres were discrete and almost spherical in shape with rough outer surface. The dense network of drug-polymer increases the tortuisity, thus delaying the release of the drug and retarding the penetration of water (penetration of medium) required to make the sphere swell for disintegration.

**FTIR analysis**

To study the drug-excipient interactions, the infrared spectrum was taken in the Prestige-21, Shimadzu FT-IR, Japan by scanning the samples in potassium bromide discs. The sample of pure drug (losartan potassium), pure polymer (sodium alginate) and the optimized formulation containing both the drug and polymer were scanned and shown in Figure-6. FTIR spectra shows that pure losartan potassium shows different peaks at 1422.53 and 1459.54 for CH bending vibration of CH$_3$ group, 1259.75 for stretching vibration of C-N, 1580.15 for stretching vibration of C-C, 2956.01 for stretching vibration of CH of aromatic hydrocarbon chromophore. FTIR spectra shows that the characteristic peaks of pure losartan potassium were almost intact in microsphere formulation indicating no interaction between the drug and polymer.

**Table 3:** Kinetic modeling of drug release from algino-losartan microspheres

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug release kinetics, Coefficient of determination ($r^2$)</th>
<th>Release exponent (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero Order</td>
<td>First Order</td>
</tr>
<tr>
<td>LA1</td>
<td>0.769</td>
<td>0.899</td>
</tr>
<tr>
<td>LA2</td>
<td>0.791</td>
<td>0.902</td>
</tr>
<tr>
<td>LA3</td>
<td>0.765</td>
<td>0.914</td>
</tr>
<tr>
<td>LA4</td>
<td>0.810</td>
<td>0.955</td>
</tr>
<tr>
<td>LA5</td>
<td>0.772</td>
<td>0.937</td>
</tr>
<tr>
<td>LA6</td>
<td>0.759</td>
<td>0.964</td>
</tr>
</tbody>
</table>

*Analyzed by the regression coefficient method.

**Figure 6:** FTIR spectra of losartan potassium (LP), sodium alginate (SA), optimized formulation (OF).
**Differential scanning calorimetric analysis**

In the present investigation, DSC thermograms of pure drug, pure polymer and optimized microspheres (formulation LA5) were taken and shown in Figure-7. It was observed that the DSC thermogram of losartan potassium shows that the drug was typical crystalline substance, exhibiting a sharp peak at 271.47°C corresponding to its melting and decomposition whereas optimized microsphere formulation (FS) shows a melting endotherm at 269.87°C for losartan potassium, indicating no considerable change in melting endotherm of the drug.

![Figure 7: DSC thermogram of losartan potassium (LP), sodium alginate (SA), optimized microsphere formulation (OF).](image)

**CONCLUSION**

It was concluded from the above research work that algino-losartan microspheres can be used as an unique drug delivery device to deliver losartan potassium for prolong period of time to improve the patient compliance and reduce the dosage frequency by sustaining and prolonging the systemic absorption of losartan potassium.

**REFERENCES**


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