FORMULATION AND IN VITRO EVALUATION OF FLOATING MICROSPHERES OF ATOMOXETINE HCL

S.Swathi*, Potlapally Laxmi, Azeez Mohammad, G.Shirisha

Department of Pharmaceutics, Venkateshwara Institute of Pharmaceutical Sciences, Cherlapally, Nalgonda-508001, Andhra Pradesh, India.

ABSTRACT

In Research work an attempt was made to prolong the gastric residence time of Atomoxetine Hcl and by fabricating it into floating sustained release microspheres. Atomoxetine Hcl is a non-stimulant drug used for the treatment of ADHD. Atomoxetine has good oral absorption profile, an oral bioavailability of 63% and a plasma elimination half-life of 5 hours. The floating microspheres were prepared by using bio compatible polymers like ethyl cellulose along with the drug in different proportions by Non-aqueous solvent evaporation method. The microspheres were characterized for micromeritic properties, percentage entrapment efficiency, percentage yield, Mean particle size, In-vitro buoyancy, In-vitro drug release studies. It was observed the increase in concentration of ethyl cellulose increases the entrapment efficiency and particle size of the microspheres. The data obtained in this study thus suggests that a micro particulate floating dosage form of Atomoxetine Hcl can be successfully designed to give prolonged release of drug and hence improved bioavailability.

Key words: Atomoxetine Hcl, floating microspheres, Ethyl cellulose.

INTRODUCTION

The aim of the present work is to formulate and evaluate floating microspheres of Atomoxetine Hcl which after oral administration could prolong gastric residence time and increase drug bioavailability. Atomoxetine Hcl mostly absorbed in the stomach and upper part of GIT. This narrow absorption window is responsible for its low bioavailability of about 63% and half-life of 6 hr. Attempts has been made to prolong the gastric residence time and to improve the bioavailability. Since many drugs well absorbed in the upper part of the
gastrointestinal tract, such high variability may lead to non-uniform absorption and makes the bioavailability unpredictable. Hence a beneficial delivery system would be one which possesses the ability to control and prolong the gastric emptying time and can deliver drugs in higher concentrations to the absorption site (i.e. upper part of the small intestine)\textsuperscript{[1]}. The precise mechanism by which Atomoxetine produces its therapeutic effects in Attention-Deficit/Hyperactivity Disorder (ADHD) is unknown, but is thought to be related to selective inhibition of the presynaptic norepinephrine transporter, as determined through in-vitro studies. Atomoxetine appears to have minimal affinity for other noradrenergic receptors or for other neurotransmitter transporters or receptors. Marketed available dosage is 10, 20, 30, 40 and 60 mg in India. Also available in 80 and 100 mg capsules in USA \textsuperscript{[2]}. To study the effect of nature of polymers individually & in combination on drug release. Study the In-vitro release of drug from the formulated floating microspheres; enhance the bioavailability of the drug. To evaluate the drug entrapment efficiency and other floating parameters

**MATERIALS AND METHODS**

Atomoxetine Hcl pure drug was obtained as a gift sample from Hetero Drugs Ltd, Hyderabad. Ethyl cellulose, Light liquid paraffin, Span 80, Dichloromethane, Sodium chloride was procured from Himedia laboratories. Ethanol, n-hexane was obtained from SD fine chemicals Ltd, Mumbai and Hydrochloric acid from Ranbaxy Fine chemicals Ltd, New Delhi.

**Preparation of microspheres**

Microspheres containing Atomoxetine Hydrochloride as a core material (Drug) were prepared by Non Aqueous solvent evaporation method. The drug and polymers in different proportions (1:1, 1:2, 1:3, 1:4) were weighed and co-dissolved at room temperature into a mixture of ethanol-dichloromethane mixture(1:1v/v) with vigorous agitation to form an uniform drug-polymer dispersion. This solution was slowly poured into the dispersion medium consisting of light liquid paraffin (200ml) containing 0.1% span60 which was previously melted. The system was stirred using an overhead propeller agitator at 500 rpm, at room temperature over a period of 2-3 hours, to ensure the complete evaporation of the solvent. The liquid paraffin was then decanted and the microspheres were separated by filtration, washed thrice with n-hexane and air dried for 24 hrs and stored in a desiccators.

**Yield of Floating microspheres**\textsuperscript{[3]}

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the
microspheres.

\[
\% \text{yield} = \frac{\text{actual weight of product}}{\text{total weight of excipients and drug}} \times 100
\]

**Particle size** \(^4\)\(^5\)

The particle size was measured by microscopic technique with the help of ocular and stage micrometer. A drop of suspension was mounted on a slide and observed under optical microscope about 100 particles were measured and their average particle size was determined.

**Micromeritic properties** \(^6\)\(^7\)\(^8\)

The floating microspheres were characterized by their micromeritic properties such as particle size, bulk density, tapped density, compressibility index, Hausner’s ratio and angle of repose.

a) **Tapped density**

The prepared floating microspheres were transferred to a measuring cylinder and tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density.

\[
\text{Tapped density} = \frac{\text{mass of microspheres (gm)}}{\text{volume of microspheres after tapping}}
\]

b) **Bulk density**

The prepared floating microspheres were transferred to a measuring cylinder and the volume occupied by the microspheres was noted. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

\[
\text{Bulk density} = \frac{\text{wt of microspheres (gm)}}{\text{bulk volume of microspheres (cm}^3\text{)}}
\]

c) **Carr’s compressibility index**

The compressibility index is a measure of flow of a powder to be compressed. It was determined from the bulk and tapped densities.

\[
\text{Carr’s compressibility index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100
\]
d) Hausner’s ratio

Tapped density and bulk density were measured and the Hausner’s ratio was calculated using the following formula:

\[
\text{Hausner’s ratio} = \frac{\text{tapped density}}{\text{bulk density}}
\]

Angle of repose

Angle of repose is defined as the maximum angle possible between the surface of the pile and the horizontal plane. The fixed funnel method was employed to measure the angle of repose.

A funnel was secured with its tip at a given height \( h \) above a graph paper that is placed on a flat horizontal surface. Accurately weighed microspheres were carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius \( r \) of the base of the conical pile was measured. The angle of repose \( \theta \) was calculated following formula.

\[
\tan \theta = \frac{h}{r}
\]

Where, \( \theta \) = angle of repose,
\( h \) = height in cm,
\( r \) = radius in cm.

Estimation of drug entrapment efficiency \(^9\)

Microspheres weighing 25 mg were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting the drug using SGF (pH 1.2)(10 ml). The extract was transferred to 100 ml volumetric flask and volume was made up by using SGF (pH 1.2). The solution was filtered and from the filtrate 10 ml was taken and further diluted to 100 ml and the absorbance was measured spectrophotometrically at 270nm against SGF (pH1.2) as blank.

\[
\% \text{ Drug entrapment efficiency} = \frac{\text{practical amount of drug present (mg)}}{\text{theoretical amount of drug taken (mg)}} \times 100
\]

In-vitro buoyancy studies \(^10\)

The microspheres weighing about 300 mg were spread over the surface of USP Paddle type dissolution apparatus(type II) which was filled with 900 ml of SGF (pH 1.2) containing 0.02% span 80. The medium was agitated with a paddle rotating at 100 rpm for 12 hours. The floating and settled portions of microspheres were recovered separately and were dried and weighed. Buoyancy percentage was calculated by using following formula.
\[
\text{% Buoyancy} = \frac{Q_f}{Q_f + Q_s}
\]

Where, \(Q_f\) = weight of floating microspheres
\(Q_s\) = weight of settled microsphere

**In-vitro drug release studies** [11]

Microspheres were weighed (weight equivalent to 50 mg of drug) and spread over the surface of USP paddle type dissolution apparatus which was filled with 900 ml of SGF (pH 1.2) containing 0.02% span 80. The dissolution medium was maintained at 37 ± 0.5°C and stirred at 100 rpm. The sample was withdrawn at a suitable interval from the dissolution vessel and assayed spectrophotometrically at 270 nm against suitable blank (SGF). The volume was replenished with the same amount of fresh dissolution medium each time to maintain sink condition.

**RESULTS AND DISCUSSION**

**Preformulation studies**

The Atomoxetine HCl sample is found to be soluble in water, methanol and ethanol. It is insoluble in chloroform. The melting point of obtained drug sample was found to be 169.8 ± 1.2°C, which is in the specified range (168-172°C), indicating purity of the drug sample. The IR spectrum of pure drug was found to be similar to the standard spectrum of Atomoxetine HCl. The IR spectrum of pure drug was shown in Fig.1. In IR spectrum of Atomoxetine Hcl, the presence of peaks at 3442.98 cm\(^{-1}\) (Ar-H), 2824.87 cm\(^{-1}\) (R-CH\(_2\)), 1016.53 cm\(^{-1}\) (R-NH), 1243.18 cm\(^{-1}\) (Ar-o-R), 771.56 cm\(^{-1}\) (o-disubstitution), 703.08 cm\(^{-1}\) (Mono substitution) were characteristic to that of the pure drug and all of them remained unaltered in the IR spectra of physical mixtures containing drug and polymer in Figure:1

![Figure 1: A) FT-IR spectrum of Atomoxetine Hcl B) FT-IR spectrum of drug and polymer.](image-url)
Calibration curve of Atomoxetine Hcl

The calibration curve of Atomoxetine Hcl was linear over the Beer’s range (10 -90 µg/ml) with R² value 0.999. The calibration curve of Atomoxetine Hcl is shown in Fig.2

![Calibration curve of Atomoxetine Hcl](image)

**Figure 2: Calibration curve of Atomoxetine Hcl**

Micromeritic properties

The micromeritic properties of all the formulations were within the acceptable limit the results shown in Table.1

<table>
<thead>
<tr>
<th><strong>Table 1.</strong> Micromeritic parameters of floating microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>F1</td>
</tr>
<tr>
<td>F2</td>
</tr>
<tr>
<td>F3</td>
</tr>
<tr>
<td>F4</td>
</tr>
</tbody>
</table>

* All the values are an average of three determinations.

Particle size analysis

With increase in polymer concentration in microspheres, the particle size of microspheres increases. This may be because of viscosity of the polymer solution which increases as the polymer concentration increases which in turn decreases the stirring efficiency. Table.2 shows the average particle size of the floating microspheres.

Percentage yield

The percentage yield of different formulations F1 to F4 were calculated and tabulated in Table.2. The loss of material during the preparation of microspheres may be due to process parameters as well as during filtration of microspheres.
Drug entrapment efficiency  
The values of entrapment efficiency are shown in Table 2. From the results it was seen that as the polymer concentration increased, viscosity of the dispersed phase increased, encapsulation efficiency increased.

In-vitro buoyancy studies  
In-vitro buoyancy studies of the prepare microspheres were evaluated in SGF pH 1.2. The microspheres having higher polymer concentrations were less buoyant than those with lower polymer concentrations. The porosity was found to be lesser at higher polymer concentrations probably due to the slowing down of solvent evaporation due to increase in viscosity of the polymer solution the results tabulated in Table 2.

Table 2. Particle size (µm), Percentage, Entrapment efficiency yield, In-vitro buoyancy studies of all formulations.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Formulation code</th>
<th>*Mean particle size(µm)</th>
<th>Percentage yield (%)</th>
<th>Entrapment efficiency (%)</th>
<th>In-vitro buoyancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>95.03</td>
<td>89.31</td>
<td>78±0.35</td>
<td>94.18±4.4</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>117.21</td>
<td>86.48</td>
<td>84±0.65</td>
<td>91.64±3.7</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>135.17</td>
<td>79.29</td>
<td>89±0.38</td>
<td>87.73±2.2</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>151.48</td>
<td>71.87</td>
<td>93±0.82</td>
<td>82.25±1.6</td>
</tr>
</tbody>
</table>

In-vitro drug release studies:
In-vitro drug release studies showed that the increase in polymer concentration decreases the drug release. Among all the formulations F1 showed that release from microspheres got successfully retarded in 12 hours the drug release studies were shown in table 3.

Table 3: In-vitro drug release studies of different formulations

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Time(hr)</th>
<th>F1(%CDR)</th>
<th>F2(%CDR)</th>
<th>F3(%CDR)</th>
<th>F4(%CDR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>25.30±0.01</td>
<td>21.20±0.69</td>
<td>18.43±0.20</td>
<td>16.38±0.06</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>32.71±0.61</td>
<td>27.98±0.06</td>
<td>25.93±0.83</td>
<td>23.41±0.64</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>40.01±0.58</td>
<td>34.86±0.76</td>
<td>31.93±0.47</td>
<td>28.41±0.77</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>46.36±0.65</td>
<td>41.10±0.09</td>
<td>37.44±0.57</td>
<td>34.82±0.18</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>52.65±0.80</td>
<td>46.08±0.73</td>
<td>42.77±0.09</td>
<td>41.89±0.25</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>59.20±0.36</td>
<td>53.54±0.89</td>
<td>49.53±0.91</td>
<td>46.68±0.05</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>62.21±0.29</td>
<td>66.01±0.22</td>
<td>62.47±0.48</td>
<td>61.52±0.78</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>72.28±0.63</td>
<td>78.25±0.01</td>
<td>73.97±0.55</td>
<td>70.28±0.04</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>95.41±0.87</td>
<td>90.35±0.16</td>
<td>87.34±0.75</td>
<td>84.77±0.54</td>
</tr>
</tbody>
</table>
Figure 2: A) Zero order kinetics B) First order kinetics C) Higuchi matrix D) Peppas

Data analysis
The curve fitting results of the release rate profile of the designed formulation shown in Figure 2 gave an idea on the release profile. The regression coefficient for different drug release kinetic models are shown in Table 4. Models with the highest regression coefficient were judged to be the most appropriate model for the dissolution data.

Table 4: Results of model fitting for Atomoxetine Hcl floating microspheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order $r^2$ value</th>
<th>First order $r^2$ value</th>
<th>Higuchi matrix $r^2$ value</th>
<th>Peppas $r^2$ value</th>
<th>n value</th>
<th>Best fit model</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.9658</td>
<td>0.8849</td>
<td>0.9849</td>
<td>0.9859</td>
<td>0.5501</td>
<td>Peppas model</td>
</tr>
<tr>
<td>F2</td>
<td>0.9797</td>
<td>0.9238</td>
<td>0.9771</td>
<td>0.9832</td>
<td>0.602</td>
<td>Peppas model</td>
</tr>
<tr>
<td>F3</td>
<td>0.9847</td>
<td>0.9315</td>
<td>0.9747</td>
<td>0.9855</td>
<td>0.6365</td>
<td>Peppas model</td>
</tr>
<tr>
<td>F4</td>
<td>0.9873</td>
<td>0.9458</td>
<td>0.9874</td>
<td>0.9707</td>
<td>0.675</td>
<td>Higuchi matrix</td>
</tr>
</tbody>
</table>

CONCLUSION
The present study has been a satisfactory attempt to formulate floating microspheres of Atomoxetine Hcl with a view of improving its oral bioavailability and also to prolong the
gastric residence of the drug. From the results it can be concluded that biocompatible like ethyl cellulose can be used to formulate an efficient floating micro particulate system with good percentage entrapment efficiency and practical yield. The particle size analysis revealed that the prepared microspheres were within acceptable range, hence showed good flow properties. The prepared microspheres were porous and had low densities, thus exhibiting excellent buoyancies in simulated gastric fluid. In-vitro drug release studies showed that release from microsphere get successfully retarded for 12 h. It was concluded that these floating microspheres can play an important role in increasing therapeutic efficiency by providing a sustained effect of the drug by reducing dosing frequency.

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