**ABSTRACT**

The gastroretentive sustained release system of cefixime was formulated to increase the gastric residence time and modulate its release behaviour. Olive oil entrapped beads containing cefixime capable of floating in the gastric condition were formulated and evaluated. Cefixime is a third generation cephalosporin antibacterial agent which results from inhibition of mucopeptide synthesis in the bacterial cell wall. Cefixime is considered as a suitable drug candidate for floating controlled drug delivery system. Hence, cefixime beads were prepared by emulsion gelation method by employing pectin and sodium alginate as sustained release polymers in three different ratios and olive oil was used to enable floating property to the beads. The effect of validation in polymer and their concentration was investigated. The beads were evaluated for production yield, particle size, swelling index, density measurement, in-vitro buoyancy, drug content, drug entrapment efficiency, in-vitro release characteristics, and in-vitro release kinetic study. Based on drug entrapment efficiency, buoyancy, swelling and in-vitro release, F9 was selected as the optimized formulation. F9 was further subjected to surface morphology by SEM. In-vivo floating study in rabbits was performed and revealed that the beads were floating in the rabbit stomach up to 10h. In-vitro release follows first order and fitted in Higuchi model with a Fickian diffusion mechanism according to Korsemeyer- Peppas(n=0.389).
Therefore, the rate of drug release is due to drug diffusion from a polymer system. Thus, it was concluded that the sustained release formulation containing cefixime was found to improve patient compliance, minimize side effects, and decrease the frequency of administration.

**Keywords**: Cefixime, Floating drug delivery system, Sodium alginate, olive oil, oil entrapped floating bead.

**INTRODUCTION**

Gastric residence time is an important parameter for different dosage forms and the prolongation and control of this time, especially for the dosage forms which remain in the stomach for a longer period of time result in better absorption and enhanced bioavailability.

Gastro retentive drug delivery systems are systems that can reside in the gastric region for several hours and can significantly prolong and control the gastric residence time of drugs. Prolonged gastric retention enhances bioavailability and improves solubility for drugs that are less soluble high pH environment\[1, 2\]. Floating drug delivery systems (FDDS) have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time without affecting the gastric emptying time. When the system is floating on the gastric juice, the drug is released slowly and this in an increased gastric residence time and better control of the variability in plasma drug concentration. Alginate beads have been designed in the present study as vehicle for drug delivery systems alginate is a hydrophilic polymer, stable in acidic media and easily depredated in alkaline media. These properties have enabled wide spread use of alginate beads in GRDFs for sustained release of drugs \[3\].

Floating dosage forms can be made by a gelling process of hydrocolloid materials or by incorporating a vacuum or gas filled chambers. Floating properties of dosage forms can also be fabricated using oils, here we incorporated olive oil \[4\]. Alginate is a polysaccharide which contains varying amounts of 1,4-linked beta-d-mannuronic acid, alpha-L-glucuronic acid residues. As biocompatible and biodegradable biopolymer, it forms a bio adhesive and stable gel with divalent cations such as Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\) \[5,6\]. These preparations have widespread use for sustained release of drugs \[7,8\]. They can also function as carriers of Bifidobacteria \[9\]. Floating alginates beads are particularly effective for site specific controlled release antibacterial agents effective against harmful stomach bacteria such as H.pylori \[10,11\].
Cefixime was taken as a model drug. It is an orally active third generation semisynthetic cephalosporin type of beta lactam antibiotic. The antibacterial effect of cefixime results from inhibition of mupopeptide synthesis in the bacterial cell wall. Like all beta-lactam antibiotics, cefixime binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefixime interferes with an autolysin inhibitor.

The aim of present work is to prepare cefixime floating alginate beads to control the drug release using olive oil as a floating agent. The influence of pectin as hydrophilic copolymer and olive oil on in-vitro drug release was studied.

**MATERIALS AND METHODS**

**Materials**

Cefixime was the generous gift from Aurobindo, Pvt.ltd. Hyderabad,(India), Pectin was received from Krishna pectin’s (India), Sodium alginate(SA) from S.D fine chemicals (India).Other materials used in the study were Calcium chloride from Lobachem Pvt ltd(India),Olive oil from Hi-media laboratories(India). All chemical reagents used were of analytical grade.

**Methods**

**Formulation of floating beads of Cefixime**

Floating alginate beads were prepared by ionotropic gelation method. Drug, pectin, sodium alginate were passed through sieve 80, separately. Drug was dissolved in different proportion of olive oil (2, 5, 10) ml. Pectin (3%) and S.A in 3 different concentrations (2%, 3%, 4%) were dissolved in water. The prepared drug solution was added to the above polymer solution and was stirred to form a homogenous emulsion. This drug loaded emulsion was extruded through a 23G syring needle into (5% w/v) cacO₂ solution maintained under gentle agitation. The beads were allowed to remain in the same solution for 30min to improve their mechanical strength. This solution was filtered and the formed beads were allowed to dry at room temperature for 48hrs. Nine formulations were prepared using different concentrations of polymer and oil.
CHARACTERIZATION OF CEFEXIME FLOATING BEADS

Determination of percentage yield, drug loading (DL) and encapsulation efficiency of the prepared beads

Percentage yield for the formulation beads was calculated from the following equation.\textsuperscript{[12]}

\[
\text{Yield\%} = \frac{\text{weight of the beads}}{\text{weight of drug+ polymer weight}} \times 100
\]

Determination of Drug Loading

100mg of Cefexime prepared floating beads were weighed and were dissolved in few ml of methanol and the volume was made up to 100ml using buffer pH 1.2 centrifuged, filtered and then the filtrate was analyzed at 288nm using a UV visible spectrophotometer. The drug loading was calculated from the following equation\textsuperscript{[12]}

\[
\text{Drug loading}= \frac{\text{Weight of drug in beads}}{\text{Total weight of beads}} \times 100
\]

The % drug entrapment efficiency of each bead formulation was calculated using the following equation

\[
\text{Actual amount of drug (AQ)}/ \text{Theoretical amount of drug (TQ)} \times 100
\]

Scanning Electron Microscopy: The prepared floated beads were examined for its surface morphology by SEM (JEOL JSM T-330, Japan). The samples were fixed in individual stubs and coated uniformly with gold

Determination of Particle Size: Average particle size of beads was determined using sieve analysis. Twenty gram beads were weighed carefully and placed on the first screen and shaken for certain time. Each fraction then taken and weighed. The particle size was determined as follows.

\[
D_{\text{ave}} = \frac{\sum nd}{\sum n}
\]

where, \(D_{\text{ave}}\) is the average diameter of beads, \(n\) is percent of each fraction retained on each sieve and \(d\) is the arithmetic mean size of sieve opening.\textsuperscript{[13]} The experiment was performed in triplicate.

Determination of Swelling Properties:

Known weight (100mg) of floated beads were taken in a dissolution basket and weighed, the basket along with the beads was immersed in SGF (pH 1.2). The weight of the basket along
with the beads was determined after a time period of 12hrs. The swelling index (S.I) was calculated using the following equation \[^{[14]}\].

\[
\% \text{ S.I} = \frac{W_2 - W_1}{W_1} \times 100
\]

Where, \( W_1 \) is weight of drug beads and basket
\( W_2 \) is weight of the swollen beads and basket

**Determination of Lag and buoyant time:** Specified weight (100mg) of floated beads was placed in a beaker containing 100ml of buffer 1.2 pH and shaked at 50rpm in a water bath 37±0.5°C. The time taken by beads to float on the surface was removed and the layer at the bottom was separated by filtration. The upper and lower layer were dried at 40°C until constant weight, buoyant time was calculated by the following equation % Floating = weight of floated beads (\( W_f \)) / weight of floated beads (\( W_f \)) + weight of settled beads (\( W_s \)) \[^{[15]}\].

**In-vitro release study:** The dissolution rate of the prepared beads was studied using USP rotating paddle dissolution apparatus II (Labindia). Known weight of beads containing equivalent amount of 100mg of cefixime was placed in the dissolution medium containing 900ml of pH 1.2 for 2 hrs followed by pH 6.8 for the remaining time. The temperature was maintained at 37±0.5°C with an rpm of 50. 5ml of sample was withdrawn at specified time intervals and replaced with fresh dissolution medium. The samples were filtered with 0.45µm Whatmann membrane filter and the quantity of the drug released was determined using UV spectrophotometer at 388nm.

**Kinetic study:** The release date were analyzed using different kinetic equations which include Zero-order, First order kinetics, Higuchi diffusion and Korsemayer –peppas models \[^{[16]}\].

**2.3.8 Density measurements:** The mean weight and diameter of the beads were measured and used to calculate the densities of these spherical olive oil entrapped calcium alginate beads containing cefixime using the following equation \[^{[17]}\].

\[
D = \frac{M}{V} \quad \text{and} \quad V = \frac{4}{3}\pi r^3
\]

Where \( D \) -is the density of the beads
\( M \) -is the weight of the beads
\( V \) -volume of the bead
\( R \) - is the radius of the beads
Drug –excipient compatibility studies: The pure drug and the formulations mixed with polymers were subjected to IR studies. The pure drug and formulations mixed with polymers were separately mixed with IR grade KBr and pellets were prepared by applying 10 metric ton of pressure in hydraulic press. The pellets were then scanned over range of 4000-400cm\(^{-1}\) n FTIR instrument.

RESULTS AND DISCUSSIONS
The characteristic peaks of standard Cefixime were reported in the IR spectrum which indicated that the obtained sample was of Cefixime and was pure and when compared indicated there was no interaction between the drug and polymers as the characteristic peaks of drug were reported in the physical mixture. This confirms the compatibility of the drug with the polymer used for the formulation of oil entrapped gastro retentive beads as depicted in fig 1 & 2.

Drug – Polymer interaction study
The FT-IR spectra study showed no change in the finger print of pure drug spectra, thus confirming absence of drug and polymer interaction. The result shown in fig1 and 2.

![Fig. 1. IR Spectrum of pure Cefixime](image-url)
Formulation of Beads
Gastroretentive floating beads of cefixime (F1-F9) were prepared using pectin, sodium alginate each in different ratios alone and in combination, containing 100mg of cefixime using olive oil. It is well known that the gelation and cross linking of alginate and pectin molecules are due to the stacking of the glucuronate blocks in the alginate and pectin chains with the formation of the ‘egg-box junction’ upon adding chelating divalent cations such as calcium ion(Ca²⁺).

Characterization of Beads
Production yield
The Production yield was maximum for formulation F9 containing high drug: polymer ratio (Pectin: sodium alginate). The practical yield was increased with increasing in concentration of polymer. The practical yields of different formulations are given in table 1.

Particle size
The prepared beads were almost spherical. The mean surface diameter of all 9 formulations was between 1.251mm and 1.628mm tabulated in table 1. The size of the bead was found to be increased as the concentration of polymer and oil was increased.

Swelling index
The results showed that the swelling was related to the polymer concentration with swelling being more significant for beads containing high polymer content. The swelling index was found to be in range between 0.64% to 1.15% as shown in the table 1.
Density measurements: Density measurement showed that the calculated densities of all the prepared beads were less than the density of SGF (i.e. 1.004 g/cm$^3$) imparting floating behaviour to the beads. Their values ranged from 0.75 g/cm$^3$ to 0.95 g/cm$^3$ as shown in Table 1.

Table no. 1 Characterization of floating beads

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Practical yield (%)</th>
<th>Particlesize (mm) Mean±SD</th>
<th>Swelling index (%) Mean ±SD</th>
<th>Density (g/cm$^3$) Mean ±SD</th>
<th>Actual drug content (%) Mean ±SD</th>
<th>Entrapment efficiency (%) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>66.53</td>
<td>1.251±0.014</td>
<td>0.64±0.23</td>
<td>0.752±0.28</td>
<td>56.9±0.195</td>
<td>40.45±0.28</td>
</tr>
<tr>
<td>F2</td>
<td>70.63</td>
<td>1.290±0.024</td>
<td>0.85±0.20</td>
<td>0.880±0.72</td>
<td>73.2±0.217</td>
<td>53.51±0.16</td>
</tr>
<tr>
<td>F3</td>
<td>75.86</td>
<td>1.342±0.023</td>
<td>1.01±0.15</td>
<td>0.945±0.54</td>
<td>86.1±0.092</td>
<td>61.85±0.67</td>
</tr>
<tr>
<td>F4</td>
<td>80.16</td>
<td>1.477±0.017</td>
<td>0.68±0.13</td>
<td>0.770±0.86</td>
<td>78.5±0.650</td>
<td>71.64±0.58</td>
</tr>
<tr>
<td>F5</td>
<td>81.94</td>
<td>1.491±0.018</td>
<td>0.70±0.11</td>
<td>0.890±0.38</td>
<td>82.3±0.064</td>
<td>75.43±0.71</td>
</tr>
<tr>
<td>F6</td>
<td>82.45</td>
<td>1.532±0.017</td>
<td>0.87±0.03</td>
<td>0.952±0.53</td>
<td>88.5±0.092</td>
<td>80.64±0.22</td>
</tr>
<tr>
<td>F7</td>
<td>84.32</td>
<td>1.615±0.068</td>
<td>0.72±0.26</td>
<td>0.900±0.25</td>
<td>73.1±0.084</td>
<td>81.85±1.19</td>
</tr>
<tr>
<td>F8</td>
<td>89.26</td>
<td>1.620±0.052</td>
<td>1.05±0.14</td>
<td>0.935±0.43</td>
<td>74.6±0.049</td>
<td>82.65±0.42</td>
</tr>
<tr>
<td>F9</td>
<td>105.02</td>
<td>1.628±0.026</td>
<td>1.15±0.04</td>
<td>0.950±0.14</td>
<td>90.0±0.028</td>
<td>88.72±0.63</td>
</tr>
</tbody>
</table>

In-vitro buoyancy studies: All the Formulations showed good buoyancy, formulations F1, F2, F4, F5, F7 were floating for more than 12hr and F3, F6, F8, F9 for 24hr. In-vitro buoyancy studies of beads are shown in Table 2. This may be attributed due to proper entrapment of oil into the beads considering the polymer ratio. Hence, as the polymer concentration increases, oil is found to be better entrapped into the beads. Therefore, lag time increases as the polymer ratio increases hence exhibit better buoyancy property.

Table . no. 2 In-vitro buoyancy studies

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>FLOATING LAG TIME(min)</th>
<th>BUOYANCY DURATION(h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>10</td>
<td>&gt;12</td>
</tr>
<tr>
<td>F2</td>
<td>11</td>
<td>&gt;12</td>
</tr>
<tr>
<td>F3</td>
<td>13</td>
<td>&gt;24</td>
</tr>
<tr>
<td>F4</td>
<td>9</td>
<td>&gt;12</td>
</tr>
<tr>
<td>F5</td>
<td>8</td>
<td>&gt;12</td>
</tr>
<tr>
<td>F6</td>
<td>9</td>
<td>&gt;24</td>
</tr>
<tr>
<td>F7</td>
<td>4</td>
<td>&gt;12</td>
</tr>
<tr>
<td>F8</td>
<td>5</td>
<td>&gt;24</td>
</tr>
<tr>
<td>F9</td>
<td>7</td>
<td>&gt;24</td>
</tr>
</tbody>
</table>
**Drug content & Drug entrapment Efficiency**

All beads were evaluated for actual drug content and entrapment efficiency. Results revealed that entrapment efficiency was found to be minimum for F1 (40.45%) and maximum for F9(88.72%). Drug entrapment efficiency were found to be increasing with respect to increase in concentration of polymer. % entrapment efficiency of all the formulations is given in table1.

Results revealed that drug content was found to be minimum for F1 (56.9%) and maximum for F9 (90.0%). This may be because of increase in viscosity of polymeric solution which in turn increases the cross linking of polymer and prevent drug from diffusing out of the system.

**Scanning electron microscopy**

Surface morphology of the prepared floating beads was examined by SEM. The SEM images was taken for the optimized formulation F9. The beads were spherical with a uniform texture and smooth surface. The external surface was slightly rough surface/shrinkage which could be due to drying.

![SEM Image](image.png)

**Fig. 3.** SEM of optimized formulation F9 (a) Spherical shape of beads.
In-vitro drug release profiles

The in-vitro release study of cefixime floating beads was carried out. The drug release profiles were presented by plotting the amount of cefixime released against time. The cumulative drug release of formulations F1 to F9 was calculated. The beads exhibited a sustained release initially which may be by swelling and then diffusion. Formulation F1 which contained least concentration of polymer couldn’t sustain the cefixime release upto 10h and released the drug completely at 9h. Formulation F6 and F9 containing high concentration of polymer and oil showed a sustained release profile at the end of 10h as shown in fig 5, 6, & 7.

![SEM of optimized formulation F9 (b) slightly rough surface](image-url)

Fig. 4. SEM of optimized formulation F9 (b) slightly rough surface

![Comparative In-vitro release profile of Cefixime floating beads for formulation F1, F2 and F3 in 0.1N Hcl](image-url)

Fig. 5. Comparative In-vitro release profile of Cefixime floating beads for formulation F1, F2 and F3 in 0.1N Hcl
Analysis of in-vitro drug release kinetics and mechanism:

In order to determine the release mechanism that provides the best description to the pattern of drug release, data of the in-vitro release of optimized formulation (F9) were fitted to different kinetic models. When the data were plotted according to Zero-order equation, the formulation showed correlation coefficient value of 0.793. But when the data were plotted according to the first order equation, the formulation showed significantly higher correlation coefficient value than the Zero order plot i.e 0.957. Hence, the result revealed that the drug
release kinetics of formulation (F9) is by Zero-order kinetics. Higuchi model was applied to the in-vitro release data, linearity was obtained with high ‘r’ value (0.966) indicating that the drug release from the controlled-release beads through diffusion. The value of ‘n’ obtained for the optimized formulation is 0.389 suggesting probable release by Fickian diffusion.

CONCLUSION
A new sustained release system of oil entrapped beads were designed and prepared and the conclusions were drawn from the study. Preformulation studies were carried out and the results were found to be as given in the literature within the prescribed limits. Nine formulations containing cefixime beads were efficiently prepared and characterized were efficiently prepared and characterized by emulsion gelation method using pectin and sodium alginate. The practical yield, particle size and swelling was directly proportional to the increase in the concentration of the polymer. The calculated densities of all the prepared beads were less than i.e.1.004gcm⁻³ imparting floating behaviour to the beads, some for more than 12h and others for 24 h. Percentage entrapment efficiency increases as the polymer concentration increases. Formulation F9 was found to have maximum release and sustained release profile. Higher r² value was obtained for first order. Higuchi(Fickian release) was found to be the best fit kinetic model. The beads were spherical and their external surface was slightly rough. From the studies performed it was concluded that the oil entrapped gastroretentive floating beads of cefixime showed excellent buoyancy and sustained drug release upto 12h and thus enhanced the bioavailability.

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