Formulation and evaluation of floating alginate: Chitosan microspheres of cefixime

PG Sindhumol, Dr. CR Sudhakaran Nair and Dr. Jyoti Harindran

Abstract

The aim of the present study is to formulate floating microspheres of cefixime trihydrate using sodium alginate and chitosan to prolong gastric residence time and increase drug bioavailability with decreased gastro intestinal side effects. The microspheres were prepared by the ionotropic gelation method with calcium carbonate (CaCO₃) being used as gas forming agent. The floating microspheres were prepared by varying concentrations of the polymers, CaCO₃, CaCl₂ and varying the stirring rate. The prepared microparticles were evaluated for percentage yield, drug entrapment efficiency, particle size, drug excipient compatibility studies, buoyancy and in vitro drug release studies. The entrapment efficiency was found to increase with increase in polymer concentration. The beads formulated without gas forming agents showed maximum entrapment efficiency. The stirring speed influenced the particle size and entrapment efficiency of the formulation. The drug-excipient compatibility studies were done by Fourier Transform – Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). The results of the floating studies were shown that the microsphere formulated with CaCO₃ showed excellent floating behavior compared with the microspheres without CaCO₃. Drug release studies showed that sustained drug release pattern was observed for a period of 24 hrs. From the study an optimum concentration of alginate at 3%, chitosan 1.5%, the CaCO₃: Alg. ratio of 0.75:1, CaCl₂ at a minimum concentration of 0.5% and the stirring speed of 600 rpm was selected for the formulation of efficient floating microspheres.

Keywords: cefixime trihydrate, chitosan, floating microspheres, ionotropic gelation, sodium alginate

Introduction

Oral controlled release drug administration offers a number of advantages in therapeutics. Prolonged and efficient delivery of drugs, patient compliance, and localization of therapy and minimization of undesirable local action within the GIT are the important advantages of the system [1]. Although studies revealed that this route is subjected to two physiological influences of short gastric residence time (GRT) and variable gastric emptying time (GET), which may lead to unpredictable bioavailability and times to achieve the peak plasma levels. Furthermore, the brief GET in humans, which normally averages 2-3 hour through the major absorption zone (stomach and upper part of intestine), can result in incomplete drug release from the drug delivery system leading to diminished efficacy of administered dose [2]. Thus control of placement of drug delivery system in a specific region of the gastro intestinal tract (GI) offers numerous advantages like improved bioavailability and therapeutic efficacy, local delivery of drugs and possible reduction of dose size. All these features have lead to the development of drugs of oral controlled release (CR) dosage forms possessing gastric retention capabilities.

Cefixime is an orally active, broad spectrum antibiotic. It is active against the gram-positive organisms like, Streptococcus pneumoniae, Streptococcus pyogenes and Gram-negative organisms like, E. coli, H. influenzae, Moraxella catarrhalis, Proteus mirabilis and N. Gonorrhoeae. It is indicated for the treatment of upper respiratory tract infections like pharyngitis and tonsillitis, otitis media, sinusitis, acute bronchitis, urinary tract infections, typhoid fever etc. Cefixime is primarily absorbed from the stomach and upper part of intestine. After its oral administration, it is slowly and incompletely absorbed from the gastrointestinal tract, which resulting into the poor bioavailability of 40-50% [3-4]. Considering the wide range of activity of cefixime, if the gastric residence time of cefixime containing formulation is prolonged and allowed to float in the stomach for a long period, the oral bioavailability might be increased. So formulating in to a floating dosage form will increase the gastric residence time and sustain the drug release [5].
Ragavendra Rao et al. [6] formulated the gastric oral floating tablets of cefixime to improve the therapeutic effect of the drug by increasing its oral bioavailability.

It has been found that products based on a multiple unit system as polymer microspheres comprising many small units have advantages over single unit preparations such as matrix tablets and capsules. The multiple unit floating systems have the potential to distribute widely over a large area in the stomach and intestine provides a longer lasting and more predictable drug release by suppressing the effects of many variables in the gastro intestinal environment. The gastric emptying of multiple unit dosage forms occur gradually, in a more consistent manner with less individual variation. As multiple unit dosage forms consists of many small units, less risk of dose dumping is expected. Another advantage over single unit dosage forms demonstrated as flexibility during formulation development and therapeutic benefits for the patient [7]. Floating calcium alginate beads of riboflavin was prepared by Stops et al. [8] to prolong gastric retention and improve the bioavailability.

So the aim is to investigate the possibility of obtaining controlled and relatively constant therapeutic level of the drug from the microspheres floating on the gastro intestinal fluid. The floating nature of the microspheres increases the gastro intestinal residence time of the dosage form and improves the bioavailability of antibacterial agents as reported by Sahastian et al. [9].

Alginate, a pH sensitive, biocompatible and non-toxic natural polymer is selected as the matrix material for drug encapsulation. Chitosan, another non-toxic biocompatible natural polymer, is also incorporated in the formulation of microspheres. In this study the floating drug delivery system employs calcium carbonate (CaCO₃) as gas forming agent dispersed in an alginate matrix.

Materials and Methods

Materials and Equipment’s
Cefixime was gifted from Sance Pharmaceuticals Pala, Kerala, India. Sodium alginate was purchased from Loba Chemie Pvt.Ltd. Mumbai. Chitosan was gifted by India Sea Foods, Kochi, Kerala. Calcium carbonate was purchased from S.D fine laboratories. All other chemicals were analytical grade. UV- Visible Spectrophotometer-1800, Shimadzu, Kyoto, Japan. Fourier Transform Infrared Spectrophotometer, Affinity-1, FTIR-8400S, Shimadzu, Japan. Differential Scanning Calorimeter, DSC-60, Shimadzu, Japan.

Methods

Drug excipient compatibility studies

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Cefixime (mg)</th>
<th>Concentration of Sodium alginate (%)</th>
<th>Concentration of Chitosan (%)</th>
<th>CaCO₃: Alginate CaCl₂ (%/w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₀</td>
<td>100</td>
<td>0</td>
<td>1.5</td>
<td>0.75:1</td>
</tr>
<tr>
<td>A₁</td>
<td>100</td>
<td>1</td>
<td>1.5</td>
<td>0.75:1</td>
</tr>
<tr>
<td>A₂</td>
<td>100</td>
<td>2</td>
<td>1.5</td>
<td>0.75:1</td>
</tr>
<tr>
<td>A₃</td>
<td>100</td>
<td>3</td>
<td>1.5</td>
<td>0.75:1</td>
</tr>
<tr>
<td>A₄</td>
<td>100</td>
<td>4</td>
<td>1.5</td>
<td>0.75:1</td>
</tr>
</tbody>
</table>

Effect of chitosan concentration

Chitosan was used at different concentrations from 0% to 2%. Fourier Transform Infrared (FTIR) Spectral Studies

The drug- excipients compatibility or interactions can be understood by the FTIR studies [10]. FTIR spectra were recorded for pure drug, individual polymers and alginate-chitosan physical mixture with the drug. About 1mg of the samples was triturated with approximately 300mg of dry finely powdered potassium bromide 1R. The mixture was grinded thoroughly and was spread uniformly in a suitable die and compressed under vacuum at a pressure of about 800 Mpa. The resultant disc was mounted in a suitable holder in the spectrophotometer. Infrared spectra were measured in the scanning range of 400 to 4000 cm⁻¹. (Fourier Transform Infrared Spectrophotometer, Affinity-1, ftir-8400s, Shimadzu, Japan.)

Differential Scanning Calorimetry

DSC thermogram of cefixime, individual polymers (chitosan and sodium alginate) and the physical mixture of both drug and polymers were recorded using Differential Scanning Calorimeter (DSC-60, Shimadzu, Japan). The physical mixtures of pure drug, polymers and drug-polymer were sealed in DSC aluminium pan and scanned between 40°C to 340°C with heating rate of 10°C/minute under nitrogen atmosphere. An empty aluminium pan served as reference. Thermogram obtained was observed for any interaction [11].

Preparation of floating alginate-chitosan microspheres

Alginate was dissolved in 100ml distilled water (at a concentration of 0%(w/v), 1%(w/v), 2%(w/v), 3%(w/v) and 4%(w/v)), 100 mg of the drug cefixime and CaCO₃ was added to the solution with levels from 0:1 to 1:1 (gas forming agent: alginate, w/w). The solution was stirred thoroughly. The gelation medium was prepared by dissolving calcium chloride (CaCl₂) of different concentrations (0.5, 1, 2, and 3% w/v) in 2 % glacial acetic acid. To 100 ml of the gelation medium chitosan was added at different concentrations (0.5, 1, 1.5 and 2% w/v). The homogenous alginate solution was extruded using a 21G syringe needle into the gelation medium. The dropping rate was 30 drops/minute and the falling distance was 5cm. The solution containing the suspended micro carriers were stirred with a magnetic stir bar for 10 minutes to improve the mechanical strength of the microspheres and allowed to complete the reaction to produce gas. The microspheres were collected, washed twice with distilled water and subsequently air dried [12, 13].

Effect of Sodium alginate concentration

Floating microspheres were prepared by varying the concentration of sodium alginate and the formulation details are shown in table 1.
Effect of Gas forming agent
To study the floating levels of the microspheres CaCO₃ was selected as the gas forming agent and added at levels from 0.1 to 1:1 (CaCO₃/alginate, w/w). The formulation details are shown in table 3.

Table 3: Formulation details of alginate–chitosan floating microspheres prepared with different concentrations of CaCO₃.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Cefixime (mg)</th>
<th>Concentration of Sodium alginate (%)</th>
<th>CaCO₃: Alginate</th>
<th>CaCl₂ (%w/v)</th>
<th>Concentration of Chitosan (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>100</td>
<td>3</td>
<td>0:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>C₁</td>
<td>100</td>
<td>3</td>
<td>0.25:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>C₂</td>
<td>100</td>
<td>3</td>
<td>0.5:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>C₃</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>C₄</td>
<td>100</td>
<td>3</td>
<td>1:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Effect of CaCl₂ concentration
In order to study the effect of CaCl₂ in the formulation, the floating microspheres were prepared using different concentrations of CaCl₂ from 0.5% to 3%. The details are given in table 4.

Table 4: Formulation details of alginate–chitosan floating microspheres prepared with different concentrations of CaCl₂ solution.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Cefixime (mg)</th>
<th>Concentration of Sodium alginate (%)</th>
<th>CaCO₃: Alginate</th>
<th>CaCl₂ (%w/v)</th>
<th>Concentration of Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₁</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>D₂</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>D₃</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>D₄</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Effect of Stirring Rate
The floating microspheres were prepared by setting different speed of the mechanical stirrer from 200 to 1000 rpm. Results showed that increased speed of mechanical stirrer decrease particle size and the drug entrapment efficiency. At 300 rpm microspheres were irregular in shape and at 1000 rpm spherical shape of the microspheres were observed but particles were coalesced to beaker wall [14, 15]. Therefore, 400,600, 800 rpm was used for further study (table 5).

Table 5: Formulation details of alginate–chitosan floating microspheres prepared at different stirring speeds.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Cefixime (mg)</th>
<th>Concentration of Sodium alginate (%)</th>
<th>CaCO₃: Alginate</th>
<th>CaCl₂ (%w/v)</th>
<th>Concentration of Chitosan</th>
<th>Stirring speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>0.5</td>
<td>1.5</td>
<td>400</td>
</tr>
<tr>
<td>R₂</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>0.5</td>
<td>1.5</td>
<td>600</td>
</tr>
<tr>
<td>R₃</td>
<td>100</td>
<td>3</td>
<td>0.75:1</td>
<td>0.5</td>
<td>1.5</td>
<td>800</td>
</tr>
</tbody>
</table>

Evaluation of Floating Alginate-Chitosan Microspheres
Determination of percentage yield
The prepared batches of all the microspheres were accurately weighed. The weighed quantity of prepared microspheres was divided by the total amount of the drug and all the polymers used in the formulation of microspheres, which gave the total % yield of all the microspheres [16, 17]. The procedure was done in triplicate and the mean value of % yield was calculated using the formula:

\[
\text{Percentage Yield} = \frac{\text{Weight of the microspheres prepared}}{\text{Total weight of excipients and drug}} \times 100
\]

Determination of drug entrapment efficiency
The drug content in the microspheres was determined by pulverizing the drug loaded microspheres followed by immersing them in 1000 ml simulated gastric fluid (pH 1.2 buffers) with agitating at room temperature for 24 hour. After filtration through Whatmann No.1 filter paper, the drug concentration was determined spectrophotometrically at wave length 284nm using a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). The filtered solution from the empty microspheres was taken as blank. All samples were analyzed in triplicate [13].

Encapsulation efficiency (EE %) = \( W_A/W_T \times 100 \)

Where
EE: Encapsulation efficiency;
\( W_A \): Actual drug content;
\( W_T \): Theoretical drug content.

Study of particle size and morphology of microspheres
Particle size analysis plays an important role in determining the release characteristics and floating property. The size of the microspheres was determined using an optical microscope fitted with an ocular micrometer and a stage micrometer.
Randomly measured the particle diameters of about 100 microspheres and the average particle size were determined using the Edmondson’s equation:
\[ d_{\text{mean}} = \frac{\sum n d}{\sum n} \]
Where “n” stands for the number of counted microspheres, and “d” for the mean size range

_In vitro_ evaluation of floating ability (buoyancy) of microspheres

The time between the introduction of the Floating Drug Delivery Systems (FDDS) into the medium and its buoyancy to the upper one third of the dissolution vessel is called floating lag time (FLT) or buoyancy lag time (BLT) and duration of time the dosage form constantly floated on the surface of the medium (floating duration) is called total floating time (TFT).

The floating properties of the microspheres were evaluated in a dissolution vessel filled with simulated gastric fluid (pH 1.2) containing 0.02% of Tween 80. Paddle rotation speed was at 100 rpm, temperature was maintained at 37±0.5°C. For each sample of microspheres, 50 individual microspheres were placed in the dissolution vessel. Both the number of microspheres _N_F_ (observed visually) and the floating duration _F_T_ (which is the time during which the microspheres remain buoyant on test solution) were then determined at fixed time intervals during a 24 hour period. Experiments were performed in triplicate and the percentage of floating microspheres was calculated according to the equation [10],

\[ F\% = \left( \frac{N_F}{N_T} \right) \times 100 \]

Where, _NF_ = Number of floating Microspheres

_N_T_ = Total number of the microspheres.

_In vitro_ drug release studies

The dissolution studies of beads equivalent to 100mg of cefixime were performed using USP dissolution type apparatus II (paddle type). The drug release study was carried out using 900ml of pH 1.2 buffer, maintained at 37± 0.5°C. The speed of stirrer was maintained at 100 rpm. An aliquot of 5ml of the solution was withdrawn at pre determined time intervals and perfect sink conditions was established during the dissolution study period by replacing an equivalent volume of fresh dissolution medium. The sample solution was filtered through Whatman No.1 filter paper and analyzed for the concentration of cefixime using a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) at wavelength of 284 nm. The amount of cefixime released was calculated from the calibration curve of the same dissolution medium. All experiments were performed in triplicate [13].

Results and Discussion

Drug excipient compatibility studies

Fourier Transform Infrared (FTIR) Spectral Studies

The spectrum of pure drug showed characteristic peak at 1780-1710 cm⁻¹ (C==O stretching of lactam), 1690-1630 cm⁻¹ (C=O stretching of amide), 1565-1700 cm⁻¹ (C=N stretching of oxime), 1540-1380 cm⁻¹ (N=O stretching) and 1340-1300 cm⁻¹ (-NH₂ of carbamate) (fig. 1). Similarly all the characteristic peaks were observed in individual polymers and drug-polymers mixture as shown in fig. 2.

![Fig 1: FTIR spectrum of pure drug cefixime](image)
**Fig 2:** FTIR spectra of the pure drug cefixime and the physical mixture of drug and the polymers

**Differential Scanning Calorimetry**

The DSC thermogram of pure drug cefixime, pure polymers chitosan and sodium alginate and physical mixture of drug and polymers are shown in figures 3 to 6. The characteristic endothermic peak value of pure drug, polymers and drug-polymer mixture is shown in the figures. The characteristic peak of cefixime at 250.10°C (fig. 3) provided the endothermic melting value of the pure drug. The characteristic peaks were appeared at the melting point of the polymers (fig. 4 & 5). The physical mixture of the pure drug and the polymers were used to determine the drug polymer compatibility study. The mixture showed an endothermic peak at 282.08°C (fig. 6). The minor changes observed in peak values of drugs are not indication of any potential incompatibility. The minor changes in the peak values revealed no interaction.
Fig 4: DSC thermogram of chitosan

Fig 5: DSC thermogram of Sodium alginate.
Evaluation of floating Alginate-Chitosan microspheres

Percentage yield of the microspheres

The percentage yield of the microspheres was found in the range between 30.53±2.17% to 84.13±1.98% as given in table 6. In formulation F₀, the percentage yield is 48.64±1.23% in which the chitosan concentration was 0%. As the concentration of chitosan is increased from 0.5% to 1.5%, the percentage yield is also increased from 56.42±3.46% to 74.12±3.42%. Also in formulation A₀ which contain 0% alginate the percentage yield is 30.53±2.17%. When the concentration of alginate is increased from 1% to 4% in formulations A₁ to A₄, the percentage yield is also found to be increased from 44.56±2.64% to 78.96±3.65%. The formulation C₀, without gas forming agent showed high production yield as 84.13±1.98% compared to other formulations. In the case of formulations C₁, C₂, C₃ and C₄ the percentage yield are 78.95±2.43%, 76.36±3.06%, 74.12±4.03% and 76.42±1.06 respectively. In the case of formulations with varying concentrations of CaCl₂ as 0.5% (D₁), 1% (D₂), 2% (D₃), 3% (D₄) the percentage yield is 74.09±1.05%, 72.31±2.13%, 70.19±3.74% and 74.96±2.45% as shown in table 6. With increased speed of the mechanical stirrer at 400 rpm, the percentage yield is 69.13±3.86% in formulation R₁, at 600 rpm 76.75±2.87% in formulation R₂ and 73.28±1.96% at 800 rpm in the case of formulation R₃.

Table 6: Percentage yield and entrapment efficiency of the floating alginate-chitosan microspheres

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Percentage yield</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀</td>
<td>48.64±1.23</td>
<td>30.36±2.25</td>
</tr>
<tr>
<td>F₁</td>
<td>56.42±3.46</td>
<td>76.93±2.64</td>
</tr>
<tr>
<td>F₂</td>
<td>62.38±2.94</td>
<td>82.87±0.54</td>
</tr>
<tr>
<td>F₃</td>
<td>72.24±1.86</td>
<td>84.93±0.89</td>
</tr>
<tr>
<td>F₄</td>
<td>74.12±3.42</td>
<td>83.49±0.06</td>
</tr>
<tr>
<td>A₀</td>
<td>30.53±2.17</td>
<td>32.12±2.32</td>
</tr>
<tr>
<td>A₁</td>
<td>44.56±2.64</td>
<td>64.93±2.64</td>
</tr>
<tr>
<td>A₂</td>
<td>52.24±3.06</td>
<td>72.87±0.54</td>
</tr>
<tr>
<td>A₃</td>
<td>70.69±2.84</td>
<td>84.93±1.05</td>
</tr>
<tr>
<td>A₄</td>
<td>78.96±3.65</td>
<td>85.49±2.30</td>
</tr>
<tr>
<td>C₀</td>
<td>84.13±1.98</td>
<td>88.12±2.32</td>
</tr>
<tr>
<td>C₁</td>
<td>78.95±2.43</td>
<td>84.93±2.64</td>
</tr>
<tr>
<td>C₂</td>
<td>76.36±3.06</td>
<td>83.87±0.54</td>
</tr>
<tr>
<td>C₃</td>
<td>74.12±4.03</td>
<td>82.93±0.86</td>
</tr>
<tr>
<td>C₄</td>
<td>76.42±1.06</td>
<td>79.49±0.06</td>
</tr>
<tr>
<td>D₁</td>
<td>74.09±1.05</td>
<td>84.19±0.74</td>
</tr>
<tr>
<td>D₂</td>
<td>72.31±2.13</td>
<td>83.87±0.43</td>
</tr>
</tbody>
</table>
Drug entrapment efficiency
Effect of chitosan concentration
The formulation F0 which contain no chitosan the entrapment efficiency was only 30.36±2.25%. Incorporation of chitosan in the formulation resulted in increase in drug entrapment efficiency. It is found that the drug loading increased from 76.93±2.64% to 84.93±0.89% as the concentration of chitosan increased from 0.5 to 1.5%, while the value decreased to 83.49±0.06% when the concentration of chitosan was 2% as shown in table 6. So in later studies chitosan concentration was selected as 1.5% (w/v).

Effect of sodium alginate concentration
It was found that the incorporation efficiency increased progressively with increasing sodium alginate concentration as given in table 6. In the formulation A0, in which the concentration of alginate is 0% the entrapment efficiency was less 32.12±2.32%. As the concentration of alginate increased from 1% to 4% the entrapment efficiency also increased from 64.93±2.64% to 85.49±2.30%. As the concentration increased to 4% the dispersion was somewhat thick and difficult to extrude through the syringe needle. So alginate solution at 3% concentration was selected for further formulation [19].

Effect of Gas Forming agent on Drug Entrapment
CaCO3 was selected as the gas forming agent in the formulation. The formulations without gas forming agent showed high entrapment efficiency as compared with gas forming agent as shown in table 6. The batch prepared with CaCO3: alginate in the ratio 1:1 produced microspheres of poor mechanical strength with no spherical shape due to excessive liberation of gas, which made alginate matrix too weak to sustain the shape after drying. So this type of microspheres with CaCO3: Alg. 1:1 ratio is avoided in the subsequent formulations.

The batches prepared without CaCO3 showed high drug entrapment of 88.12±2.32%. In other batches drug entrapment decreased with increase in the amount of CaCO3 i.e from 84.93±2.64% to 79.49±0.06%. The floating microspheres prepared with CaCO3: alginate in the ratio 0.75:1 showed good entrapment efficiency, buoyancy and sustaining effect. So that concentration is preferred in the later studies [13].

Effect of CaCl2 concentration
Variation in the concentration of CaCl2 had little effect on drug entrapment efficiency. It was observed that with different concentrations of CaCl2 as 0.5%, 1%, 2% and 3% the entrapment efficiency was 84.19±0.74%, 83.87±0.43%, 81.93±0.89% and 80.49±0.06% respectively as shown in table 6. These results are more clearly observed in the release profiles (fig. 10). Thus 0.5% (w/v) CaCl2 was chosen in the optimization studies.

Effect of stirring rate on particle size and drug entrapment
The floating microspheres were prepared by setting the speed of the mechanical stirrer between 200 to 1000 rpm. Results showed that increased speed of mechanical stirrer decreased particle size and the drug entrapment efficiency. At 300 rpm microspheres were irregular in shape and at 1000 rpm spherical shape of the microspheres were observed but particles were coalesced to beaker wall. Therefore, 400,600, 800 rpm was used for further study. Results are shown in table7. At 400 rpm the particle size was found to be 936±6.9µm and entrapment efficiency as 84.2±6.4%. While at 600 rpm particle size and drug entrapment efficiency were 542±2.05 µm, 83.87±0.54% and at 800 rpm the values were 360±6.3 µm, 78.93±0.89% respectively. So the stirring rate was set at 600 rpm for further studies.

Table 7: Drug entrapment efficiency and particle size of floating alginate-chitosan microspheres prepared at different stirring speeds.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Entrapment efficiency (%)</th>
<th>Particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>84.12±2.64</td>
<td>936±6.9</td>
</tr>
<tr>
<td>R2</td>
<td>83.87±0.54</td>
<td>542±2.05</td>
</tr>
<tr>
<td>R3</td>
<td>78.93±0.89</td>
<td>360±6.3</td>
</tr>
</tbody>
</table>

In vitro evaluation of floating ability (buoyancy) of microspheres
The formulations specified in table 3 (C0 to C4) was evaluated for the floating ability. The results of the study are given in the table 8. By the visual observation it was found that the microspheres without CaCO3 (C0) sank in the simulated gastric fluid. In contrast, the microsphere with CaCO3: alginate ratio of 0.25:1(C1) and CaCO3: alginate ratio of 0.5:1 (C2) was floating for 12 hours and 14 hours respectively. More excellent floating ability was observed with microspheres, of CaCO3: alginate ratio 0.75:1 (C3) and 1:1 (C4) for 18 hours. They exhibited buoyancy of 78.12±2.07% and 77.74±2.45% respectively. This shows that the floating ability is found to be directly related to the gas content of the polymer matrix [20] which might have imparted buoyancy to the formulations for sufficient time period. But in the case of formulation C4, microspheres had no spherical shape due to excessive liberation of gas, which made alginate matrix too weak to sustain the shape after drying. So these concentrations were avoided in the subsequent studies.

Table 8: In vitro floating characteristics of the alginate–chitosan floating microspheres with different concentrations of CaCO3s.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Floating lag time (sec)</th>
<th>Total floating time (hours)</th>
<th>Buoyancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C1</td>
<td>24±2</td>
<td>12</td>
<td>74.52±2.13</td>
</tr>
<tr>
<td>C2</td>
<td>24±3</td>
<td>14</td>
<td>75.12±1.08</td>
</tr>
<tr>
<td>C3</td>
<td>22±3</td>
<td>18</td>
<td>78.12±2.07</td>
</tr>
<tr>
<td>C4</td>
<td>26±2</td>
<td>18</td>
<td>77.74±2.45</td>
</tr>
</tbody>
</table>

In vitro drug release studies
In order to prolong the gastric retention time (GRT) and for sustaining the drug release chitosan was added to the gelation medium. As previously described, it increased the drug loading and encapsulation efficiency. On the addition of chitosan a controlled release profile was obtained with prolonged duration for 24 hours.

In the case of formulation F0 (0% chitosan) the drug release

![Table 7: Drug entrapment efficiency and particle size of floating alginate-chitosan microspheres prepared at different stirring speeds.](image-url)
The Pharma Innovation Journal

was only up to 4 hours and the microspheres were disintegrated completely in the pH 1.2 buffer. On the addition of chitosan into the gelation medium a substantial control and prolonged release pattern was observed in the release profile as shown in fig. 7. In the case of formulation $F_1$ (0.5% chitosan), initial burst release was observed and 49.51±3.05% of the drug was released within 6 hour, thereafter the release rate was slow and 90.12±3.18% of the drug was released within 24 hours. As the concentration of the chitosan was increased from 1 to 2% a sustained effect was observed in the release pattern. In the case of formulation $F_2$ 78.32±3.62% of the drug was released after 24 hours and for the formulation $F_3$ the drug released was 74.73±1.04%. As the concentration of chitosan exceeds 1.5%, as in the case of formulation $F_4$, the drug release rate was very slow and 58.73±2.43% of the drug was released after 24 hours.

**Fig 7:** Profiles of cefixime release from Cs-Alg floating microspheres prepared with varying concentrations of chitosan.

In vitro release profiles of floating alginate- chitosan microspheres prepared with varying concentration of alginate is shown in fig. 8. The results indicated more sustained effect with increase in the concentration of sodium alginate. In formulation $A_0$ (0% alginate), 92.42±1.46% of the drug was released up to 10 hours. In this case the drug release was not sustained effectively. As the concentration of alginate was increased from 1 to 4%, a prolonged release profile was observed. In the formulation $A_1$ (1% alginate) 94.74±0.97% of the drug was released at 24 hours. In the case of formulations $A_2$ (2% alginate), $A_3$ (3% alginate) and $A_4$ (4% alginate), the most sustained effect was observed with increase in the alginate concentration with release of 92.53±2.74%, 84.32±2.62% and 78.73±1.04% respectively in 24 hours.

**Fig 8:** Profiles of cefixime release from Cs-Alg floating microspheres prepared with varying concentrations of alginate.

The alginate- chitosan microspheres prepared with CaCO$_3$: alginate ratio of 0.75:1, ($C_3$) showed good entrapment efficiency and sustaining effect as given in release profile (fig. 9). A decrease in cumulative percentage drug release with increased CaCO$_3$ concentration was observed. This may be due to the internal ionotropic gelation effect of CaCO$_3$. In the absence of CaCO$_3$ ($C_0$), the release rate was very slow and only 68.73±1.99% of the drug was released up to 24 hours but the microspheres were non buoyant. The microspheres prepared with CaCO$_3$: alginate 1:1 ratio ($C_4$) was not spherical, because the evolved carbon dioxide caused the bursting of the beads before their wall was sufficiently hardened. So the dissolution study was not conducted for the formulation ($C_4$). The formulation $C_3$ was observed to be optimum with respect to floating ability and extended release and hence was used in further studies.

**Fig 9:** Profiles of cefixime release from Cs-Alg floating microspheres prepared with varying concentrations CaCO$_3$.

Variation in the concentration of CaCl$_2$ had little effect on drug entrapment efficiency and drug release as shown in fig. 10. The release pattern was almost same. So the minimum concentration of CaCl$_2$ was sufficient for the formulation of the beads. Similar result was observed [13] in the evaluation of sustained release floating microspheres of diltiazem hydrochloride.

**Fig 10:** Profiles of cefixime release from Cs-Alg floating microspheres prepared with varying concentrations CaCl$_2$.

From the drug release studies shown fig. 11 it was found that as the particle size decreased the drug release rate was more ($R_1$), and 96.74±0.97% of the drug was released at 24 hours as compared to the larger sized microspheres ($R_2$) where the drug release % was 80.32±2.62 only. In the formulation $R_2$, 86.32±2.62% of the drug was released up to 24 hours. This was due to the increased surface area of the smaller microspheres compared to the larger ones. As the particle size
decreased, the surface area increased. So the stirring rate was kept at the optimum level of 600 rpm to control the particle size and drug release in further studies.

Fig 11: Profiles of cefixime release from Cs-Alg floating microspheres prepared at different RPM.

Conclusion
In this study, alginate–chitosan microspheres were successfully formulated as floating drug delivery systems by ionotropic gelation method. The method is simple and reproducible. It was found that increasing the polymer concentration increased the drug entrapment efficiency and sustaining the drug release for a period of 24 hrs. The in vitro buoyancy studies revealed that the floating behavior of the formulation increased with increasing weight ratios of CaCO3. The drug polymer compatibility studies revealed that there is no incompatibility between the polymers and drug. From this study it was found that alginate at a concentration of 3%, chitosan at 1.5% concentration, the CaCO3: alginate ratio of 0.75:1, CaCl2 at a minimum concentration of 0.5% and the stirring speed of 600 rpm can be selected as the optimum for the formulation of the efficient floating microspheres. It is hoped that further studies with animal models are needed to understand the in vivo floating characteristics of the formulation.

Acknowledgement
Sincerely express our gratitude to Sance Pharmaceuticals Pala, Kerala, for providing cefixime trihydrate as gift sample. Thankfully remember India Sea Foods, Kochi, Kerala for providing the sample of chitosan.

References