Introduction

Development in the field of novel drug delivery is getting more attention, but still the oral route of administration is the most preferred route of administration as the physiology of the gastrointestinal tract offer more opportunities for the development of the dosage form. Thus the focus of the researcher is on the study of extended release of the drug with well controlled release profile (Dubey et al., 2013). Retention of the dosage form in the gastric fluid increases the gastric transit time which ultimately increases the bioavailability of poorly soluble drugs (Patel et al., 2011). These systems are suitable for the drugs that are absorbed from the stomach (Rao et al., 2016). There are different approaches based on their mechanism of release such floating, expansion/plug time, high density or adhesion to mucosa, low density (Singh et al., 2013). Recently floating system has gained more attention because of increased gastric residence time. Immediate floating can be achieved either by the entrapment of the air (Krogel et al., 1999) or by the use of low density materials such as oils (Desai et al., 1993) or foam powders (Streuble et al., 2002).

The floating wax microspheres were prepared by the ionotropic gelation method. The formation of microspheres occurs by the cross-linking of the calcium ions with pectin to form calcium pectinate. The morphology, floating properties, swelling studies, drug content, drug entrapment efficiency and drug release study was carried out. The purpose of the present study was to investigate the influence of incorporated wax, which prepared by the ionotropic gelation technique on the drug release profile.

Materials and methods

Materials

Tizanidine hydrochloride was received as generous gift from Blue Cross Laboratories Pvt. Ltd. Nasik. Pectin, olive oil, carnauba wax, Calcium chloride were of laboratory grade and available at institute.

Preparation of floating wax microspheres

The emulsion of pectin, olive oil and Tizanidine hydrochloride was prepared in distilled water using a high speed homogenizer (IKA T25) at 3000 rpm. The weighed amount of the wax was melted on the water bath at the temperature of more than 5°C of melting point of the wax.

Abstract

Objective: The purpose of this study was to formulate and evaluate the floating wax beads by the ionotropic gelation method. Materials and methods: The wax in the pectin-oil emulsion was hot melted, homogenized and extruded into calcium chloride solution i.e. by ionotropic gelation method. The formulated microspheres were allowed to dry for 24 hrs. Further the formulated microspheres were evaluated for the parameters of micromeritic properties, floating lag time, floating time, surface characterization, in-vitro drug release etc. Results: The effect of different amount of the wax and oil on the floating time and drug release was studied. The drug loaded microspheres were found to be float on the 0.1N hydrochloric acid solution. Addition of the wax into the microspheres affected the drug release. Conclusion: The results suggest that the increase in the concentration of the wax had significantly retarded the drug release and the wax microspheres could be used as the potential drug carrier for the sustained drug delivery system. Keywords: Wax, microspheres, floating, pectin, drug carrier, sustained drug delivery

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The molten wax was dispersed in the previously heated homogenized emulsion of pectin, olive oil and Tizanidine hydrochloride and mixed until the homogeneous mixture was obtained. The hot melted mixture was extruded in the 2% w/v Calcium chloride solution through 22G syringe. The microspheres formed were allowed to remain in the calcium chloride solution for 10 – 20 mins for the hardening of the microspheres. The microspheres formed were then filtered and washed thoroughly with water to remove the excess of calcium from the surface of the microspheres (Pornsak et al., 2008).

**Micromeritic properties**

All the prepared formulations of floating microspheres were evaluated for bulk density, tapped density, Carr's index and Hausner's ratio (Patel et al., 2009; USP-2008).

\[
\text{Bulk density} = \frac{\text{Total mass of formulation}}{\text{Total volume of formulation}}
\]

\[
\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume of powder}}
\]

\[
\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}
\]

\[
\text{Carr's compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100
\]

**Percentage yield**

All the prepared formulations of floating microspheres were evaluated for the percentage yield by using following formula (Patel et al.,2009; Tekde et al.,2009):

\[
\% \text{ yield} = \frac{\text{Total mass of formulation}}{\text{Total mass of raw material}} \times 100
\]

**Determination of drug content and drug entrapment efficiency**

The 50mg of floating microspheres was dissolved in 0.1 N Hydrochloric acid under sonication and filtered. The drug content was assayed using UV-spectrophotometer (V – 630, Shimadzu Co Ltd., Japan) at 228 nm after suitable dilution with 0.1 N Hydrochloric acid. Percent drug content and entrapment efficiency was determined using formula (Yadav et al., 2014):

\[
\% \text{ Drug content} = \frac{\text{Actual drug content}}{\text{Total drug amount taken}} \times 100
\]

\[
\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theroretical drug content}} \times 100
\]

**Floating lag time and floating time**

The formulated bead sample (n=20) were placed in a beaker filled with 0.1N HCl (pH 1.2) solution. Temperature was maintained at 37°c. The floating time of microspheres was observed for 12 hrs. The preparation was considered to have buoyancy in the test solution only when all the gel microspheres floated in it. The time the formulation took to emerge on the surface of the medium (floating lag time) and the time for which the formulation remains float on the surface of the medium (floating time) were noted (Jaiswal et al., 2009).

**Swelling studies**

Microspheres were studied for swelling characteristics. Only those batches were selected which have good drug content and entrapment efficiency more than 50%. Sample from drug loaded microspheres were taken, weighed and placed in wire basket of USP dissolution apparatus II. The basket containing microspheres put in a beaker containing 100 ml of 0.1N HCl (pH1.2) maintained at 37°c. The microspheres were periodically removed at predetermined intervals and weighed. Then swelling ratio was calculated as per the following formula (Gareeb et al., 2014):

\[
\text{Swelling index} = \frac{W_s - W_o}{W_o} \times 100
\]

Where, \(W_s\) = weight of swollen microspheres,

\(W_o\) = weight of dried microspheres

**Particle size determination**

The particle size of microspheres was determined by the dry state using optical microscopy method. The stage micrometer and eyepiece micrometer were used for the measurement of the particle size. The size of the microspheres present in the 1cm² area of the slide was counted (Pursale et al., 2009).

**Surface characterization**

Surface characterization of microspheres was examined with a scanning Electron Microscopy (SEM Sophisticated Test and Instrumentation centre, Cochin). Microspheres were mounted on metal grids using double-sided tape and coated with gold under vacuum (Khan et al., 2011).

**Differential Scanning Calorimetry (DSC)**

The DSC measurements were performed on a DSC 60, Shimadzu, Japan differential scanning calorimeter with thermal analyzer. All accurately weighed samples were deposited in a sealed aluminum pans, before heating under nitrogen flow (10 ml/min) at a scanning rate of 10 °C per min from 25 to 300 °C. An empty aluminum pan was used as
Fourier Transform Infrared Spectroscopy (FTIR)

The compatibility study was carried out using Fourier transform infrared spectrophotometer (BRUKER – ECO ATR). FTIR study was carried out on pure drug and physical mixture of drug and polymer. Physical mixtures were prepared and samples were kept for 1 month at room temperature. Infrared absorption spectrum of Tizanidine hydrochloride and physical mixture was recorded over the wave number 4000 to 400 cm\(^{-1}\) using Fourier Transform spectrophotometer (Bruker, ECO-ATR)(IP-2014; Srivastav 2012; Pavia et al., 2007).

In-vitro drug release study

The release of Tizanidine hydrochloride from sustained release floating wax microspheres was determined using USP dissolution apparatus II at 50 rpm. The dissolution medium used was 900 ml of 0.1N HCl (pH1.2) and temperature was maintained at 37°C. A sample (5ml) was withdrawn from the dissolution apparatus at 0 min., 1hr, 2hr, 4hr, 6hr, 8hr, 10hr, 12hr. The samples were filtered through whatman filter paper and analysed using UV method. Cumulative % drug release was calculated and observed. The dissolution of the formulation was compared with the 250mg of the capsule containing 4mg of the drug (Pornsak et al., 2008).

Best fit kinetic model for optimized formulation

The data obtained from study of diffusion kinetics of the optimized formulation was studied to obtain the best fit model. The best fitted model is the one which gives the highest R\(^2\) value and least slope value.

Stability study

Stability study of the formulation which gave maximum dissolution rate was carried out to point out any visual physical or chemical change made in the formulation after storing it at elevated temperature and humidity conditions. The optimized formulation was store in ambient colour bottle and stored at 40°C ± 2°C and 75% ± 5% Relative humidity for three months. Floating wax microspheres was analysed for the drug content (ICH Q1A(R2), 2003).

Statistical Analysis

Results of ex-vivo experiments are reported as SEM analysis. The classical zero order release curve was found to be linear. The curves plotted according to first order and Higuchi model were also found to be linear. For the Korsemeyer-Peppas release curves R\(^2\) was found to be ≥ 0.886 for all 4 formulations. The drug release occurs probably by diffusion and erosion and dissolution. From the above tables it was seen that the best fit model for formulation was Zero order kinetic, such type of model was applicable when sustained release dissolution mechanism are seen.

Results and discussion

Microemeric properties

From the study of the micromeritics properties of the formulation it was found that the bulk density of the formulation lies within range of 0.3614 – 0.4734 g/cm\(^3\), tapped density within range of 0.3849- 0.5036. The Carr's index lies within range of 5.99 – 8.22 and Hausner's ratio within range of 1.0270 – 1.0834 which indicates that the prepared formulation have excellent flow property (Table 1).

Percentage yield

All formulations F1 – F4 showed percentage yield 97.79 – 99.26% which lied in the normal range (Table 2).

Drug content and drug entrapment efficiency

The percentage drug content of all prepared formulations was found to be in the range of 92.56 – 98.77%. Therefore uniformity of drug content was maintained in all formulations (Table 2).

The percentage drug entrapment efficiency of all prepared formulations was found to be in the range of 90.28% - 92.62%. Therefore entrapment efficiency was found to be less due to the diffusion of the drug into the calcium chloride solution during the formation of the microspheres (Table 2).

Floating lag time and floating time

Floating lag time in the range of 1.18 – 3.28 min. and floating time >12hr for all formulations F1-F4. This is due the increase in the concentration of the carnauba wax (Table 3).

Swelling studies

For all prepared batches (F1-F4), percent swelling ratio was found to be in the range of 8.92 – 19.04 % from table 6. The

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Bulk density (gm/ml) ± SD</th>
<th>Tapped density (gm/ml) ± SD</th>
<th>Carr’s index ±SD</th>
<th>Hausner’s ratio ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.3953 ±0.0033</td>
<td>0.4283 ± 0.0052</td>
<td>7.70 ± 0.0578</td>
<td>1.0834± 0.0032</td>
</tr>
<tr>
<td>F2</td>
<td>0.4734 ±0.0066</td>
<td>0.5036± 0.0079</td>
<td>5.99 ± 0.0357</td>
<td>1.0637± 0.0041</td>
</tr>
<tr>
<td>F3</td>
<td>0.4462 ±0.0050</td>
<td>0.4862± 0.0061</td>
<td>8.22 ± 0.0441</td>
<td>1.0270± 0.0048</td>
</tr>
<tr>
<td>F4</td>
<td>0.3614 ±0.0031</td>
<td>0.3849± 0.0036</td>
<td>6.10 ± 0.0482</td>
<td>1.0650±0.0053</td>
</tr>
</tbody>
</table>
F1 batch showed the maximum swelling index. This is because of the lipophilic nature of the carnauba wax which affected the swelling of the microspheres (Table 4).

**Particle size determination**

For F1-F4 batches average particle size was found to be in the range of 1.21 – 1.52 mm (Table 4).

**Surface characterization**

The SEM result showed that the particle size of formulation was found to have regular and spherical shape with rough and uneven surface (Figure 1).

**Table 2. Percentage yield, Drug content and Drug entrapment efficiency of the formulations**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Percentage yield (%)</th>
<th>Contents(%)±SD</th>
<th>% DEE± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>97.79</td>
<td>97.17 ± 0.1021</td>
<td>90.28 ±0.5802</td>
</tr>
<tr>
<td>F2</td>
<td>98.28</td>
<td>97.85 ± 0.2313</td>
<td>90.7 ± 0.6818</td>
</tr>
<tr>
<td>F3</td>
<td>98.77</td>
<td>98.77 ± 0.09</td>
<td>91.77 ±0.6265</td>
</tr>
<tr>
<td>F4</td>
<td>99.26</td>
<td>92.56± 0.0869</td>
<td>92.62 ± 0.3693</td>
</tr>
</tbody>
</table>

**Table 3. Floating lag time and floating time of the formulations**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Batch</th>
<th>Floating lag time (min.)</th>
<th>Floating time ( hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>3.28 ± 0.04330</td>
<td>&gt; 10.25</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>1.44 ± 0.0391</td>
<td>&gt; 12</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>1.20 ± 0.00707</td>
<td>&gt; 12</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>1.18 ± 0.02121</td>
<td>&gt; 12</td>
</tr>
</tbody>
</table>

**Table 4. Swelling studies and Particle size of the formulations**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Swelling index</th>
<th>Particle size (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>19.04 ± 0.05612</td>
<td>1.21 ±0.01204</td>
</tr>
<tr>
<td>F2</td>
<td>16.66 ± 0.04716</td>
<td>1.33 ± 0.01767</td>
</tr>
<tr>
<td>F3</td>
<td>13.79 ± 0.02549</td>
<td>1.43 ± 0.02263</td>
</tr>
<tr>
<td>F4</td>
<td>8.92 ± 0.02179</td>
<td>1.52 ± 0.01118</td>
</tr>
</tbody>
</table>

Differential scanning calorimetric studies

Tizanidine Hydrochloride was compatible with polymer. There is slightly peak broadening in physical mixture of polymer to pure Tizanidine Hydrochloride (Figure 2).

Fourier transform infrared spectroscopy

FTIR spectrum of the physical mixture shows that there is no interaction between drug and polymer (Figure 3).
**In vitro drug release**

Maximum drug release 94.60% was shown by F2 batch. The data also suggested that floating microspheres formulation were capable to produce linear drug release for longer period of time. Drug release profile of formulation F1 to F4 and dissolution profile F1 to F4 signified sustained drug release. Out of four formulations maximum release after 12 hr was found for F2 formulation (Table 5 and Figure 4).

From the comparative study of the formulation with capsule containing the dose of 4mg of Tizanidine hydrochloride, it was found that the capsule containing drug showed the 98.49% drug release within 6 hrs while the prepared formulation (F2 Batch) showed maximum drug release up to 94.60% within 12 hrs (Table 6 and Figure 5).

**Table 5. In-vitro drug release of the formulations**

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 hr</td>
<td>8.91 ± 0.031</td>
<td>10.46 ± 0.019</td>
<td>6.58±0.050</td>
<td>5.34 ± 0.031</td>
</tr>
<tr>
<td>2 hr</td>
<td>19.00 ± 0.026</td>
<td>19.78 ±0.024</td>
<td>12.83 ±0.066</td>
<td>10.90 ± 0.024</td>
</tr>
<tr>
<td>4 hr</td>
<td>44.63± 0.037</td>
<td>39.21 ±0.022</td>
<td>20.97 ±0.077</td>
<td>18.26 ± 0.068</td>
</tr>
<tr>
<td>6 hr</td>
<td>61.77 ±0.042</td>
<td>54.42 ±0.018</td>
<td>23.73 ±0.070</td>
<td>27.23 ± 0.048</td>
</tr>
<tr>
<td>8 hr</td>
<td>83.56±0.030</td>
<td>66.50 ±0.021</td>
<td>28.41 ±0.045</td>
<td>29.20 ± 0.037</td>
</tr>
<tr>
<td>10 hr</td>
<td>97.97±0.033</td>
<td>83.50±0.023</td>
<td>32.71±0.104</td>
<td>40.49 ± 0.046</td>
</tr>
<tr>
<td>12hr</td>
<td>98.12±0.037</td>
<td>94.60±0.016</td>
<td>53.31±0.060</td>
<td>45.57 ± 0.029</td>
</tr>
</tbody>
</table>

**Kinetic model for F2 batch**

In order to investigate the mode of release from floating microspheres data were analysed with following mathematical model.

A. Zero order kinetic

B. First order kinetic

C. Higuchi equation

D. Korsemeyer-peppas equation

The classical zero order release curve was found to be linear. The curves plotted according to first order and Higuchi model were also found to be linear. For the Korsemeyer-Peppas release curves R² was found to be ≥ 0.886 for all 4 formulations. The drug release occurs probably by diffusion and erosion and dissolution. From the table it was seen that the best fit model for formulation was Zero order kinetic, such type of model was applicable when sustained release dissolution mechanism are seen (Table 7 and Figure 6: (A),(B),(C) and (D)).

**Table 6. Comparative dissolution profile of the formulation with marketed formulation**

<table>
<thead>
<tr>
<th>Marketed formulation</th>
<th>F2 Batch formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hrs.)</td>
<td>% drug release</td>
</tr>
<tr>
<td>1</td>
<td>35.68</td>
</tr>
<tr>
<td>2</td>
<td>56.66</td>
</tr>
<tr>
<td>3</td>
<td>61.77</td>
</tr>
<tr>
<td>4</td>
<td>79.29</td>
</tr>
<tr>
<td>5</td>
<td>91.02</td>
</tr>
<tr>
<td>6</td>
<td>98.49</td>
</tr>
<tr>
<td>12</td>
<td>94.60</td>
</tr>
</tbody>
</table>

**Stability study**

The sample were withdrawn after 1, 2 and 3 months and subjected to following tests a shown in. The accelerated stability studies (carried for 3 months), at temperature of 40°C ± 2°C and % RH 75% ± 5% RH indicated that the developed floating pectinate microspheres were unaffected after 03 months storage under accelerated condition as no change was observed in the appearance and colour of the formulation. On the basis of these results, it may be concluded that the F2 formulation developed is stable under accelerated condition of 03 months (Table 8).
Conclusion

From the above study it may be concluded the use of hydrophobic carriers like waxes can be done for achieving the sustain release action. The low density materials like oils were used to attend the floating of the formulation. The study also suggested that the floating wax microspheres can be implemented as a suitable drug carrier for sustaining the release of the drugs with short biological half life.

Acknowledgement

The authors are thankful to the Blue Cross Laboratories for providing the gift sample of the drug and Principal of KCT’s, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik for permitting the use of the college facilities.

Conflict of interest

Authors declare that they do not have any conflict of interest.

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