ABSTRACT

Objective: The objective of this research was to formulate and evaluate the different grades of rizatriptan benzoate loaded polysaccharide based microspheres for the nasal drug delivery system.

Methods: The polysaccharide was extracted from the seed of *Trigonella foenum-graecum* and microspheres were prepared by emulsification, followed by crosslinking using epichlorohydrin. A 3² full factorial design was employed in formulating the microspheres with polymer concentration (X₁), and stirring rate (X₂) as independent variables and particle size (Y₁) and entrapment efficiency (Y₂) were dependent variables.

Results: The microspheres were discrete and free-flowing. The mean particle size (Y₁) of microspheres ranged from 40.82±12 µm to 62.48±0.41 µm and the encapsulation efficiency (Y₂) was found to be increased from 60.7±0.2% to 79.22±0.2% as the drug polysaccharide ratio increased. A 3² full factorial design confirmed that the X₁ and X₂ both effect on particle size whereas X₁ alone effect on entrapment efficiency. SEM revealed the smooth spherical surface of microspheres whereas kinetic model revealed that drug release followed the case II transport. FTIR indicated good compatibility of the excipients with rizatriptan benzoate. Stability studies were carried out for formulation F7 at 4°C ambient, 25±2 °C/60±5%, 40±2°C/75±5% relative humidity revealed that the physical drug appearance, entrapment efficiency were within the permissible limits.

Conclusion: The result obtained in this research work indicate a promising potential of control release rizatriptan benzoate loaded microspheres whereas the *Trigonella foenum-graecum* polysaccharide used as rate controlling polymer for the effective treatment of migraine patients.

Keywords: Rizatriptan benzoate, Polysaccharide, Microspheres, Nasal drug delivery.
solutions containing different drug and polysaccharide ratios (1:1 to 1:3) were prepared by dissolving required amount of rizatriptan benzoate and polysaccharide in distilled water. The final volume was adjusted in such a way such a way that the concentration of the polysaccharide in the final solution was 2% w/v. The mixture was kept for 4 h under magnetic stirring at 100 rpm (round per minute) for completed hydration of polysaccharide. An aqueous phase was emulsified into castor oil using span 80 (1% v/v) as an emulsifying agent. The phase: volume ratio of the oil and aqueous phase was maintained at 1:1:1. The emulsion was homogenized for 5 min. with the addition of 0.2 ml H 2 SO 4 using high speed a mechanical stirrer (Yamato, LT400, Tokyo, Japan) at different rotation rate (2000 to 3000 rpm). Epichlorohydrin (4% v/v) was added for covalent crosslinking of droplets. Stirring was continued for 18 h at 40°C. The formed microspheres were separated by sedimentation and centrifugation at 1500 rpm for 5 min. Microspheres were washed thrice using isopropyl a alcohol [11].

**Experimental design**

The design of the experiment is an approach for effectively and efficiently exploring the cause and effect relationship between process variables and output. A 2-factor 3-level factorial central composite experimental design technique was employed to investigate the variables. This technique was applied to quantify the influence of operating parameters on the particle size and entrapment efficiency of microspheres. The dependent variables were polymer concentration and stirring rate. The factorial design parameters and experimental condition are shown in table 1. Various batches of rizatryptan benzoate loaded microspheres were prepared based on the 3\* factorial designs. The independent variables were polymer concentration 1 to 3% (X 1) and stirring rate 2000 to 3000 rpm (X 2) and their levels were shown in table 1.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Coded value</th>
<th>Drug-polysaccharide ratio (X 1)</th>
<th>Stirring rate in rpm (X 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>1:1</td>
<td>2000</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1:2</td>
<td>2500</td>
</tr>
<tr>
<td>3</td>
<td>+1</td>
<td>1:3</td>
<td>3000</td>
</tr>
</tbody>
</table>

**Optimization data analysis and model validation**

A nonlinear quadratic polynomial model was generated to more precisely evaluated effect of independent variables on dependent variables using Design Expert v10 software (Stat-Ease, Inc. Minneapolis, MN).

\[
Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 + \beta_6 X_1^3 + \beta_7 X_2^3 + \ldots \ldots \ldots \ldots (1)
\]

Where \( Y_i \) is the level of response variable, \( \beta_0, \beta_1, \beta_2, \beta_3, \beta_4 \) and \( \beta_5 \) is the regression coefficient; \( X_1, X_2 \) stands for the main effect; \( X_1X_2 \) is the interactions between the main effects, and \( X_1, X_2 \) are quadratic terms of the independent variables that are used to simulate the curvature of the designed sample space. The \( X_1 \) and \( X_2 \) were termed as codes for the concentration of polysaccharide and stirring rate.

The polynomial equation was used to draw conclusions after considering the magnitude of coefficients, and the mathematical sign it carries, i.e., positive or negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect. In the model analysis, the responses: the particle size of the microsphere and entrapment efficiency of all model formulations were treated by Design Expert® software. The best-fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient \( R^2 \), adjusted multiple correlation coefficient (adjusted \( R^2 \)), and the predicted residual sum of square (PRESS), provided by Design Expert® software. Level of significance was considered at p<0.05. Three-dimensional response surface plots resulting from equations were obtained by the Design Expert® software [12].

**Characterization of microspheres**

**Production Yield**

The production yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for the preparation of microspheres [13].

\[
\text{Yield (\%)} = \frac{\text{Weight of microspheres}}{\text{Total expected weight of drug and polymer}} \times 100 \ldots (2)
\]

**Particle size analysis**

The geometric particle size of microspheres was measured using a phase contrast microscope (Radical Instruments, India). Suspension of microspheres was prepared in paraffin oil. Two or three drops of the suspension were transferred onto a glass slide and covered with a coverslip. The geometrical diameter of 300 microspheres of each batch was separately measured, and the average diameter was calculated [14].

**Entrapment efficiency**

For the estimation of encapsulation efficiency and drug content, accurately weighed a sample of microspheres (20 mg) was dispersed in 20 ml phosphate buffer (pH 6.8). The dispersion was sonicated for 30 min and kept overnight for the complete erosion of microspheres. Then the sample was centrifuged (Remi), filtered and analyzed using a UV-visible spectrophotometer (Shimadzu 1700, Japan) at 225 nm. After that encapsulation efficiency and drug content of microsphere were calculated by the following equation (3).

\[
\text{Encapsulation efficiency (\%)} = \frac{\text{Actual amount of drug}}{\text{Theoretical amount of drug}} \times 100 \ldots (3)
\]

**Fourier transforms infrared spectroscopy (FTIR) studies**

To determine any possible changes in the chemical nature of the drug during the preparation of microspheres, FTIR spectra of drug, polysaccharide, and the microspheres were recorded by using the FTIR spectrophotometer (Shimadzu, Kyoto, Japan) following the potassium bromide disc method.

**Scanning electron microscopy (SEM)**

The surface morphology of the microspheres was studied using SEM (Leo, 435 VP, Milpitas, CA). Microspheres were placed on an aluminum stub with double-sided adhesive tape and were subjected to gold sputtering before SEM analysis.

**In vitro drug release**

The in vitro release of the drug from the microspheres was studied using the modified dissolution method [15]. An accurately weighed amount of microspheres equivalent to 10 mg of drug was suspended into 10 ml of phosphate buffer (pH 6.8) in a beaker containing 0.1% v/v of Tween 80. The microspheres were packed into a small pouch of filter paper, which was kept immersed in the dissolution medium.

The medium was maintained at 37±1°C and continuously stirred at 50 rpm using a magnetic stirrer. Samples (200 ml) were withdrawn at the time interval of 5 min., diluted, filtered and centrifuged at 4000 rpm for 15 min. The supernatant was collected and estimated for the drug content using UV spectrophotometer at 225 nm. Quantitative estimation of released drug was done using a calibration curve of the drug in a phosphate buffer of pH 6.8 in the concentration range of 1–10 µg/mL.
Drug release kinetics

To study the release kinetics of rizatriptan benzoate from Trigonella foenum-graecum microspheres, dissolution data were fit according to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas equations. Value of kinetic rate constant (K), mucoadhesive microspheres of *Trigonella foenum-graecum* correlation coefficient (r²) and release exponent (n) were calculated to find out the best fit model. To investigate the drug release mechanism, the release data were fitted to model representation: Zero order (see equation 4) as cumulative amount of drug released vs. time, First order (see equation 5) as log cumulative percentage of drug remaining vs. time and Higuchi’s model (see equation 6) as cumulative percentage of drug released vs. square root of time [12].

**Zero-order**

\[
Q_t = K_0 t 
\]  
(4)

Where \(K_0\) is the zero-order rate constant expressed in units of concentration/time and \(t\) is the time in minutes. A graph of concentration vs. time would yield a straight line with a slope equal to \(K_0\) and intercept the origin of the axes.

**First order**

\[
\log C = \log C_0 - K_0 t / 2.303 
\]  
(5)

Where \(C_t\) is the initial concentration of the drug, \(K_0\) is the first order constant and \(t\) is the time.

**Higuchi model**

\[
Q_t = K_0 t^{1/2} 
\]  
(6)

Where \(Q_t\) is the amount of drug release in time \(t\), \(K_0\) is the kinetic constant and \(t\) is the time in minutes.

**Korsmeyer-peppas model**

A more stringent test was used to distinguish between the mechanisms of drug release. The release data were fitted to the Peppas exponential model as a cumulative log percentage of drug released vs. log time (equation 7). The release exponent \(n\) and \(K\) value were calculated through the slope of the straight line.

\[
M_t / M_\infty = K t^n 
\]  
(7)

Where \(M_t\) represents an amount of the released drug at time \(t\), \(M_\infty\) is the total amount of drug released after an infinite time, \(K\) is the diffusion characteristic of drug polysaccharide system constant and \(n\) is an exponent that characterizes the mechanism of drug release. The value of \(n\) indicates the drug release mechanism from the delivery system. If the exponent \(n\) is 0.43 then the drug release mechanism is Fickian diffusion, if 0.43<n<0.85 then it is non-Fickian or anomalous diffusion, if \(n\) is 0.85 mechanism is non-Fickian case II diffusion [16, 17].

**Stability studies**

Stability study was carried out for rizatriptan benzoate loaded microspheres as per ICH guidelines. The best batch of microspheres was sealed in an ambered coloured bottle and stored at 25±2°C/60±5%, 40±2°C/75±5% relative humidity (RH) for 90 d. The sample was evaluated for physical appearance and entrapment efficiency [22].

### RESULTS AND DISCUSSION

**Formulation of microspheres**

Nine formulations of rizatriptan benzoate loaded mucoadhesive microspheres were prepared by emulsion technique using factorial design, in which the independent variables of drug polysaccharide ratio \(1\) to 3% (\(X_1\)) and stirring rate \(X_2\) 2000 to 3000 rpm and particle size (\(Y_1\)) and % entrapment efficiency (\(Y_2\)) were taken as the response parameters as the dependent variables as shown in table 2.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug polysaccharide ratio ((X_1))</th>
<th>Stirring rate in rpm ((X_2))</th>
<th>Particle size ((\mu m + SD))</th>
<th>Entrapment efficiency (%+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
<td>40.82±0.12</td>
<td>60.70±0.20</td>
</tr>
<tr>
<td>F2</td>
<td>-1</td>
<td>0</td>
<td>55.82±0.50</td>
<td>62.60±0.10</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>+1</td>
<td>62.48±0.41</td>
<td>65.07±1.30</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>-1</td>
<td>49.15±1.02</td>
<td>67.46±0.50</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
<td>49.15±0.70</td>
<td>68.66±0.41</td>
</tr>
<tr>
<td>F6</td>
<td>0</td>
<td>+1</td>
<td>49.98±1.20</td>
<td>69.64±0.8</td>
</tr>
<tr>
<td>F7</td>
<td>+1</td>
<td>-1</td>
<td>60.81±0.50</td>
<td>70.84±1.2</td>
</tr>
<tr>
<td>F8</td>
<td>+1</td>
<td>0</td>
<td>57.48±0.80</td>
<td>76.83±0.3</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>46.65±0.20</td>
<td>79.22±0.2</td>
</tr>
</tbody>
</table>

\(\text{mean}+\text{SD}, (n=3), n= \text{ number of observation, F = formulation code of microspheres} \)

**Optimization data analysis and model-validation**

**Fitting of data to the model**

The two factors with lower, middle and upper design points in coded and encoded values are shown in table 1. The ranges of responses \(Y_1\) and \(Y_2\) were 40.82±0.12-62.48±0.41 \(\mu m\) and 60.70±0.2-79.22±0.2%, respectively. Response observed for all the prepared batches were fitted to various models using Design-Expert® software. It was observed that the best-fitted models were F1 for particle size and linear for entrapment efficiency. The values of R², adjusted R², predicted R², SD and % CV are given in table 3.

The result of ANOVA in table 4 for the dependent variables demonstrates that the model was significant for both the response variables. It was observed that independent variables drug polysaccharide ratio \(X_1\) and stirring rate \(X_2\) had a positive effect on particle size (\(Y_1\)) and entrapment efficiency (\(Y_2\)).

**Table 2: Formulation of microspheres using 3² factorial designs**

**Table 3: Summary of results of regression analysis for response \(Y_1\) and \(Y_2\)**
Table 4: Results of analysis of variance for a measured response

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size Model</td>
<td>337.98</td>
<td>3</td>
<td>112.66</td>
<td>8.54</td>
<td>0.0206 significant</td>
</tr>
<tr>
<td>Residual</td>
<td>65.94</td>
<td>5</td>
<td>13.19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>403.92</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. E. Model</td>
<td>284.45</td>
<td>2</td>
<td>142.22</td>
<td>66.23</td>
<td>&lt;0.0001 significant</td>
</tr>
<tr>
<td>Residual</td>
<td>12.89</td>
<td>6</td>
<td>2.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>297.33</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Regression equation

Particle size \( (Y_1) = 11.94 + 18.88X_1 + 19.29X_2 - 8.95X_1X_2 \) \( .... (8) \)

Entrapment efficiency \( (Y_2) = 51.18 + 6.42X_1 + 2.48X_2 \) \( .... (9) \)

From the above equation 8 it was confirmed that the major factor which is influencing the particle size of the film is stirring rate \((X_2)\). The interaction factor between both the independent variables is very less. The concentration of Trigonella foenum-graecum \((X_1)\) also has an effect on particle size of Trigonella foenum-graecum microsphere. In case of entrapment efficiency, the concentration of Trigonella foenum-graecum \((X_1)\) also has an effect on entrapment efficiency of microsphere because the higher the amount of polysaccharide, the more will be entrapment efficiency because of the more availability of polysaccharide to encapsulate the drug.

Table 5: Regression coefficient and \( r^2 \) values for Trigonella foenum-graecum microspheres containing rizatriptan benzoate

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
<th>( \beta_1 \beta_2 )</th>
<th>( r^2 )</th>
<th>F-values for the model (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size</td>
<td>11.94</td>
<td>18.88</td>
<td>19.29</td>
<td>-8.95</td>
<td>0.8368</td>
<td>8.54 (0.0206)</td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>51.18</td>
<td>6.42</td>
<td>2.48</td>
<td>0</td>
<td>0.9567</td>
<td>66.23 (&lt;0.0001)</td>
</tr>
</tbody>
</table>

Response surface plot analysis

Three-dimensional response surface plots generated by the Design Expert® software are presented (in fig. 1 and 2) for the studied responses, i.e., particle size and entrapment efficiency. The (fig. 1) depicts response surface plot of drug polysaccharide ratio \((X_1)\) and stirring rate \((X_2)\) on particle size, which indicate that \(X_1\) and \(X_2\) show linear effect i.e., when increased polysaccharide concentration and stirring rate from 2000 to 3000 the value of particle size was decreased. The (fig. 2) represent response surface plot of the effect of drug polysaccharide ratio \((X_1)\) and stirring rate \((X_2)\) on entrapment efficiency which indicate a linear effect. This explains that the higher the amount of polysaccharide, the more will be entrapment efficiency because of the more availability of polysaccharide to encapsulate the drug.

Optimization and validation

The result in table 6 illustrates the comparison between the observed values of both the response \( Y_1 \) and \( Y_2 \) for all the batches were presented. It can be seen that in all cases there was a reasonable agreement between the experimental values. The equations describe the influence of the selected independent variables on the responses under study adequately. This indicates that the optimization technique was appropriated for optimizing the rizatriptan benzoate loaded Trigonella foenum-graecum microsphere. The low magnitudes of error in the present investigation prove the high prognostic ability of the optimization technique by factorial design.

Fig. 1: Response surface plots for the \( X_1 \) and \( X_2 \) on particle size \((Y_1)\), where \( X_1 \) = drug polysaccharide ratio, \( X_2 \) = stirring rate
Fig. 2: Response surface plots for the $X_1$ and $X_2$ on entrapment efficiency ($Y_2$), where $X_1$ = drug polysaccharide ratio and $X_2$ = stirring rate.

Table 6: The predicted and observed response variables of the rizatriptan benzoate loaded microspheres

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Actual and predicted values for Particle size ($Y_1$)</th>
<th>Actual value</th>
<th>Predicted value</th>
<th>Residual</th>
<th>Entrapment efficiency ($Y_2$)</th>
<th>Actual value</th>
<th>Predicted value</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>40.82</td>
<td>41.16</td>
<td>-0.34</td>
<td>60.70</td>
<td>60.08</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>55.82</td>
<td>51.50</td>
<td>4.32</td>
<td>62.60</td>
<td>62.56</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>62.48</td>
<td>61.84</td>
<td>0.64</td>
<td>65.07</td>
<td>65.04</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>49.15</td>
<td>51.09</td>
<td>-1.94</td>
<td>67.46</td>
<td>66.50</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>49.15</td>
<td>52.48</td>
<td>-3.33</td>
<td>68.66</td>
<td>68.98</td>
<td>-0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>49.98</td>
<td>53.87</td>
<td>-3.89</td>
<td>69.64</td>
<td>71.46</td>
<td>-1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>60.81</td>
<td>61.02</td>
<td>-0.21</td>
<td>70.84</td>
<td>72.92</td>
<td>-2.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>57.48</td>
<td>53.46</td>
<td>4.02</td>
<td>76.83</td>
<td>75.40</td>
<td>1.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>46.65</td>
<td>45.90</td>
<td>0.75</td>
<td>79.22</td>
<td>77.88</td>
<td>1.34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F=$ formulation code of microspheres

Characterization of microspheres

Percentage yield

The percentage yield of production was found in the range 64.5±0.2% to 77.6±0.6% (Table 7). These respectively low values could be attributed mainly to the loss of fines particles during the washing of microspheres with Isopropyl alcohol.

Particle size

The mean particle size of microspheres ranged from 40.82±10 µm to 62.48±6.56 µm (Table 7), indicating a narrow size distribution (Fig. 1). Such a particle size distribution was considered favorable for intranasal administration. The particle size of microsphere was increased with an increase in the drug polysaccharide ratio. The formation of larger droplets during emulsification due to the higher viscosity of polysaccharide solution at higher concentration. Increase stirring rate also contributed to decreasing the particle size but not much significantly and this may be due to the narrower range being selected to focus this parameter [20].

Encapsulation efficiency result

The percentage encapsulation efficiency was found to increase as the drug polysaccharide ratio increased (Table 7). It was 60.7±0.2% in a batch F1 (1:1 drug polysaccharide ratio) and increased subsequently, to 79.22±0.22% in a batch F9 (1:3 drug polysaccharide ratio). The reason behind the increased value of percent encapsulation efficiency is the more availability of Trigonella foenum-graecum polysaccharide as a carrier for the encapsulation of drug. At high concentration of polysaccharide, highly viscous aqueous droplets are formed during emulsification, which makes a complex network of polysaccharide and prevents the migration of drug into surrounding media [21]. In a batch F1 with drug polysaccharide ratio 1:1, drug loading was less because most of the drug remained unentrapped due to an insufficient amount of carrier polysaccharide.

Table 7: Mean value of percentage yield, particle size and entrapment efficiency of microspheres

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Percentage yield ($%$+SD)</th>
<th>Particle size ($\mu$m+SD)</th>
<th>Entrapment efficiency ($%$+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>64.5±0.2</td>
<td>40.82±0.12</td>
<td>60.70±0.2</td>
</tr>
<tr>
<td>F2</td>
<td>70.4±0.5</td>
<td>55.82±0.50</td>
<td>62.60±0.10</td>
</tr>
<tr>
<td>F3</td>
<td>76.2±0.1</td>
<td>62.48±0.41</td>
<td>65.07±1.30</td>
</tr>
<tr>
<td>F4</td>
<td>74.5±0.2</td>
<td>49.15±1.02</td>
<td>67.46±0.50</td>
</tr>
<tr>
<td>F5</td>
<td>69.7±1.3</td>
<td>49.15±0.70</td>
<td>68.66±0.41</td>
</tr>
<tr>
<td>F6</td>
<td>72.3±0.1</td>
<td>49.98±1.20</td>
<td>69.64±0.8</td>
</tr>
<tr>
<td>F7</td>
<td>77.6±0.6</td>
<td>60.81±0.50</td>
<td>70.84±1.2</td>
</tr>
<tr>
<td>F8</td>
<td>71.4±1.4</td>
<td>57.48±0.80</td>
<td>76.83±0.3</td>
</tr>
<tr>
<td>F9</td>
<td>73.1±0.2</td>
<td>46.65±0.20</td>
<td>79.22±0.2</td>
</tr>
</tbody>
</table>

$*mean$±$SD (n=3), n = no of observation, $F=$ formulation code of microspheres
FTIR spectroscopy

Interpretation of FTIR spectrum of *Trigonella foenum-graecum* polysaccharide (fig. 3(a)) was: -OH stretch (3656.78), -H (3150.50), C=C (2313.46), -H bend (1636.49), -H rock (1617.20), -H bend out of plane (1029.45) and FTIR spectrum of rizatriptan benzoate (fig. 3(b)) was: -NH stretching (3446), -CH₃ stretching (2947), -CH₂ stretching (2893), -C=C stretching (1608), -C=N stretching (1506), -NH bending (1570), -CH₂ bending (1458), -CH₃ bending (1375), -CN (1016, 1140, 1296).

The spectrum of physical mixture indicates that there was no evidence of any possible interaction between the drug and the polysaccharide and characteristic peaks of the pure drug were found present in the spectrum of mixture (fig. 3 (c)), which confirmed the absence of interaction between a drug and the polysaccharide [10, 18-19].

Fig. 3: IR spectra of *Trigonella foenum-graecum* polysaccharide (a), Rizatriptan benzoate (b) and Physical mixture (Rizatriptan benzoate and *Trigonella foenum-graecum* polysaccharide) (c)
Surface morphology
The morphology of all the batches was examined by a Scanning Electron Microscopy (SEM) shown in fig. 4. Microspheres are spherical and possessed a smooth surface also they had no rupture on the surface; such morphology would result in slow clearance and good deposition pattern in a nasal cavity [16].

![SEM images of different batches at 100×](image)

**Fig. 4: SEM image all batches at 100×**

Result of in vitro drug release
The release profiles of the drug from different batches of *Trigonella foenum-graecum* microspheres are shown in fig. 5. Batches of microspheres were found to release 50% drug within 15 min., and the whole drug content within 60 min. time except batch F7, which might due to high concentration of *Trigonella foenum-graecum* polysaccharide retarded the drug release from the microspheres, because of the viscous three-dimensional network at higher concentration of polysaccharide, which reduced the diffusion of drug.

Result of release kinetics
The release constant was calculated from the slope of the zero order, first order, Higuchi plots and determined the regression coefficient (R²) in the range of 0.70-0.98. It was found that the in vitro drug release of all the nine batches was best explained by zero order kinetic, as plot show the highest linearity, R² (see table 8), indicating diffusion controlled drug release. The corresponding plot log of cumulative percentage drug release vs. log time of the Korsmeyer-Peppas equation indicated good linearity of regression coefficient (R²); 0.27-0.97. The release exponent (n) values for all the nine
Hence, it was concluded that the F7 batch of microspheres has good efficiencies ranged from 60.7±0.2% to 79.2±0.2% and mean size concentration of polysaccharide ratio influences the entrapment microspheres were spherical and free-flowing. The entrapment even after exposing to stress conditions at a different temperature. There was no significant change in physical appearance and entrapment efficiency. Stability studies for the optimized batch (F7) were carried out at a different temperature for 90 d. The formulation was evaluated for controlling polymer for the effective treatment of migraine patients.

CONCLUSION

Stability studies for the optimized batch (F7) were carried out at a different temperature for 90 d. The formulation was evaluated for physical appearance and entrapment efficiency even after exposing to stress conditions at a different temperature. Hence, it was concluded that the F7 batch of microspheres has good stability during their shelf life.

The rizatriptan benzoate loaded polysaccharide based microspheres can be successfully prepared by double emulsion techniques. A full 3 factorial design was applied taking drug polysaccharide ratio (X) and stirring rate (X) as two independent variables than the dependent variables were evaluated. The prepared batches of microspheres were spherical and free-flowing. The entrapment efficiencies ranged from 60.7±0.2% to 79.2±0.2% and mean size was in the range of 40.8±0.12 µm to 62.4±0.41 µm. The concentration of polysaccharide ratio influences the entrapment efficiency. Thus, the investigation indicates a promising potential of control release rizatriptan benzoate loaded microspheres whereas the Trigonella foenum-graecum polysaccharide used as rate controlling polymer for the effective treatment of migraine patients.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declare none

REFERENCES


