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.

INTRODUCTION

In a novel drug delivery system, various route of administration may be used to achieve the controlled and targeted drug delivery [1]. A nasal cavity, as a site of drug delivery, have many advantages such as avoid first-pass metabolism, applicability for long-term treatment and because of the large surface area for absorption it may provide ease of administration. In a novel drug delivery system, microspheres are used to improve the bioavailability and stability of a drug. Intranasal drug administration is receiving increased attention as a delivery method for bypassing the blood-brain barrier (BBB) and rapidly targeting therapeutics to the central nervous system (CNS) [2]. There are two classes of nasal delivered therapeutics are in the market. The first one comprises with low molecular weight and hydrophobic drugs mostly useful in the treatment of the nasal mucosa and sinus. Whereas the second class of drugs having poor absorption properties, instability in a gastrointestinal tract (GIT) and have a sufficient nasal absorption to display systemic effects [3]. Single emulsion technique is a well-established process for microspheres preparation. The microparticulate carriers of natural polysaccharides i.e., those of proteins and carbohydrates are prepared by a single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in a non-aqueous medium like oil. In the next step, the cross-linking of the dispersed globule is carried out. The cross-linking can be achieved either by heat or by using the chemical crosslinkers [4].

Rizatriptan benzoate is a selective serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptor agonist ("trip坦") commonly used for the treatment of a migraine and completely absorbed from GIT, but absolute bioavailability (as conventional tablet) is 4.5%, indicating the first-pass metabolism, which is due to the metabolism of the drug by monoamine oxidase A isoenzyme (MAO-A) to an inactive indole acetic acid metabolite. Rizatriptan benzoate has also relatively short elimination half-life (about 3 h) and the prolonged drug release is needed [5, 6].

The aim of this research was, to formulate and evaluated rizatriptan benzoate loaded polysaccharide based microspheres for the delivery via the nasal route with aim to avoid hepatic first-pass metabolism, and enhance residence time. The natural polysaccharide was extracted from the seeds of Trigonella foenum-graecum L. (Fenugreek seeds, Family: Fabaceae) as mucoadhesive excipient [7]. In traditional Chinese medicine, it is also used for kidney problems and conditions affecting the male reproductive tract. The recent researches have proved it beneficial for atherosclerosis, constipation, diabetes, high cholesterol and hypertriglyceridemia [8].

MATERIALS AND METHODS

Materials

Rizatriptan benzoate was obtained as a generous gift from Cipla (Raigarh, India). Castor oil, epichlorohydrin, Isopropyl alcohol and acetone were purchased from the CDH, (New Delhi, India). All other reagents used were of analytical grade.

Extraction of fenugreek polysaccharide

Extraction of polysaccharide was done using a reported method [9, 10] with minor modification. The plant materials of Trigonella foenum-graecum (fenugreek seeds, NHPG/NBPGR/2014-16) were collected from the local market in Meerut (India), and were washed with water to remove dirt, dried and crushed them to convert into powdered form. The powder was soaked in water for 5-6 h, boiled for 30 min. and allowed to stand for 1 hr so that all the mucilage was released into the water. Then the materials were squeezed from muslin bag to remove the marc from the solution. After that 1500 ml of acetone was added to the filtrate to precipitate the mucilage. The mucilage was separated, dried in an oven at a temperature less than 50 °C, and the dried powder was passed through a sieve no. 80 and retained on sieve no. 100. It was stored in desiccators.

Preparation of microspheres by emulsion technique

Microspheres of Trigonella foenum-graecum polysaccharides were prepared using an emulsification technique. Aqueous
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 complete hydration of polysaccharide. An aqueous phase was emulsified into a 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. The emulsion was homogenized for 5 min. with the addition of 0.2 ml H2SO4 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 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 rizatriptan benzoate loaded microspheres were prepared based on the 3² factorial designs. The independent variables were polymer concentration 1 to 3% (X1) and stirring rate 2000 to 3000 rpm (X2) and their levels were shown in table 1.

### Table 1: Factorial design parameters and experimental condition

| S. No. | Coded value | Actual value | Drug polysaccharide ratio (X1) | Stirring rate in rpm (X2) |
|--------|-------------|--------------|--------------------------------|--------------------------|
| 1      | -1          | 1:1          | 1:1                            | 2000                     |
| 2      | 0           | 1:2          | 1:2                            | 2500                     |
| 3      | +1          | 1:3          | 1:3                            | 3000                     |

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).

\[ Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \]  

Where \( Y_i \) is the level of response variable, \( \beta_0 \) (\( \beta_1 \), \( \beta_2 \), \( \beta_3 \), \( \beta_4 \), and \( \beta_5 \)) are the regression coefficient; \( X_1 \) and \( X_2 \) are the main effects; \( X_1 X_2 \) is the interactions between the main effects, and \( X_1^2 \) and \( X_2^2 \) 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 (R²), adjusted multiple correlation coefficient (adjusted R²), and the predicted residual sum of square (PRESS), provided by Design Expert® software. Level of significance was considered at \( p < 0.05 \).

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].

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

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 spectrometer (Shimadzu 1700, Japan) at 225 nm. After that encapsulation efficiency and drug content of microsphere were calculated by the following equation (3).

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

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 pock 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

\[ C = K_0 t \]  \hspace{1cm} (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

\[ \log C = \log C_0 - K_0 t/2.303 \] \hspace{1cm} (5)

Where \( C_0 \) is the initial concentration of the drug, \( K \) is the first order constant and \( t \) is the time.

Higuchi model

\[ Q_t = K_{1/2} \] \hspace{1cm} (6)

Where \( Q_t \) is the amount of drug release in time \( t \), \( K \) 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.

\[ M_t/M_\infty = K_n t^n \] \hspace{1cm} (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 \( n=0.43 \) then the drug release mechanism is Fickian diffusion, if \( 0.43<n<0.85 \) then it is non-Fickian or anomalous diffusion, if \( n=0.85 \) 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/75±5% 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 dependent variables as shown in table 2.

| Batch code | Drug polysaccharide ratio (X_1) | Stirring rate in rpm (X_2) | Particle size (µm+SD)* | Entrapment efficiency (%+SD)* (Y_2) |
|------------|---------------------------------|-----------------------------|-------------------------|-------------------------------------|
| F_1        | -1                              | -1                          | 40.82±0.12              | 60.70±0.20                          |
| F_2        | -1                              | 0                           | 55.82±0.50              | 62.60±0.10                          |
| F_3        | -1                              | +1                          | 62.48±0.41              | 65.07±1.30                          |
| F_4        | 0                               | -1                          | 49.15±0.12              | 67.46±0.05                          |
| F_5        | 0                               | 0                           | 49.15±0.70              | 68.56±0.41                          |
| F_6        | 0                               | +1                          | 49.98±1.20              | 69.64±0.8                           |
| F_7        | +1                              | -1                          | 60.81±0.50              | 70.84±1.2                           |
| F_8        | +1                              | 0                           | 57.48±0.80              | 76.83±0.3                           |
| F_9        | +1                              | +1                          | 46.65±0.20              | 79.22±0.2                           |

*mean±SD, (n= 3), n= 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±0.41µm 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 4: Results of analysis of variance for a measured response

| Parameters | SS   | DF | MS   | F     | Significance p         |
|------------|------|----|------|-------|------------------------|
| Particle size | 337.98 | 3 | 112.66 | 8.54 | 0.0206 significant    |
| Residual   | 65.94 | 5 | 13.19 | -     | -                      |
| Total      | 403.92 | 8 | -    | -     | -                      |
| E. E.      | -    | - | -    | -     | <0.0001 significant    |
| Model      | 284.45 | 2 | 142.22 | 66.23 | -                      |
| Residual   | 12.89 | 6 | 2.15 | -     | -                      |
| Total      | 297.33 | 8 | -    | -     | -                      |

Regression equation

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

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

From the above equation 8 it was confirmed that the major factor which is influencing the particle size of the film is stirring rate \( (X_2) \).

Regression equation and \( r^2 \) values for \( Trigonella foenum-graecum \) microspheres containing rizatriptan benzoate

| Independent variables | \( \beta_0 \) | \( \beta_1 \) | \( \beta_2 \) | \( \beta_3 \) | \( r^2 \) | F-values for the model (p-value) |
|-----------------------|------------|------------|------------|------------|---------|-------------------------------|
| Dependent variables   |            |            |            |            |         |                               |
| Particle size         | 11.94      | 18.88      | 19.29      | -8.95      | 0.8368  | 8.54 (0.0206)               |
| Entrapment efficiency | 51.18      | 6.42       | 2.48       | 0          | 0.9567  | 66.23 (<0.0001)           |

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) reprent 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. 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

| Formulation code | Actual and predicted values for Particle size ($Y_1$) | Entrapment efficiency ($Y_2$) |
|------------------|-----------------------------------------------------|-------------------------------|
|                  | Actual value | Predicted value | Residual | Actual value | Predicted value | Residual |
| F1               | 40.82        | 41.16           | -0.34    | 60.70        | 60.08           | 0.62     |
| F2               | 55.82        | 51.50           | 4.32     | 62.60        | 62.56           | 0.04     |
| F3               | 62.48        | 61.84           | 0.64     | 65.07        | 65.04           | 0.03     |
| F4               | 49.15        | 51.09           | -1.94    | 67.46        | 66.50           | 0.96     |
| F5               | 49.15        | 52.48           | -3.22    | 68.66        | 68.85           | -0.22    |
| F6               | 49.98        | 53.87           | -3.89    | 69.64        | 71.46           | -1.82    |
| F7               | 60.81        | 61.02           | 0.21     | 70.84        | 72.92           | 2.08     |
| F8               | 57.48        | 53.46           | 4.02     | 76.83        | 75.40           | 1.43     |
| F9               | 46.65        | 45.90           | 0.75     | 79.22        | 77.88           | 1.34     |

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 distributions were considered favorable for intranasal administration. The particle size of microsphere was to increase 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

| Batch code | Percentage yield* (%±SD) | Particle size* (µm±SD) | Entrapment efficiency* (%±SD) |
|------------|--------------------------|------------------------|------------------------------|
| F1         | 64.5±0.2                 | 40.82±0.12             | 60.70±0.20                   |
| F2         | 70.4±0.5                 | 55.82±0.50             | 62.60±0.10                   |
| F3         | 76.2±0.1                 | 62.48±0.41             | 65.07±1.30                   |
| F4         | 74.5±0.2                 | 49.15±1.02             | 67.46±0.50                   |
| F5         | 69.7±1.3                 | 49.15±0.70             | 68.66±0.41                   |
| F6         | 72.3±0.1                 | 49.98±1.20             | 69.64±0.8                    |
| F7         | 77.6±0.6                 | 60.81±0.50             | 70.84±1.2                    |
| F8         | 71.4±1.4                 | 57.48±0.80             | 76.83±0.3                    |
| F9         | 73.1±0.2                 | 46.65±0.20             | 79.22±0.2                    |

*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), -C-H bend (1636.49), -C-H rock (1617.20), -CH 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=O 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].

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 79.22+0.2% and mean size efficiency. Thus, the investigation indicates a promising potential of 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 physical appearance and entrapment efficiency. There was no different temperature for 90 d. The formulation was evaluated for dependent variables were evaluated. The prepared batches of control release rizatriptan benzoate loaded microspheres whereas can be successfully prepared by double emulsion techniques. A full 3x factorial design was applied taking drug polysaccharide ratio (X 1 ) and stirring rate (X 2 ) 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 79.22±0.2% and mean size was in the range of 40.82±0.12 μ to 62.48±0.41 μ. 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.

Stability studies
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.

CONCLUSION
The rizatriptan benzoate loaded polysaccharide based microspheres can be successfully prepared by double emulsion techniques. A full factorial design was applied taking drug polysaccharide ratio (X 1 ) and stirring rate (X 2 ) 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 79.22±0.2% and mean size was in the range of 40.82±0.12 μ to 62.48±0.41 μ. 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
1. Chandra A, Batra D, Kakar S, Singh R. A review on target drug delivery: magnetic microspheres. Journal of Acute Disease 2013;2:189-95.
2. Pathaka R, Dashab RP, Misraa M, Nivs, Arkarb M. Role of mucoadhesive polysaccharides in enhancing delivery of nimodipine microemulsion to brain via intranasal route. Acta Pharm Sin B 2014;4:151–60.
3. Dhakar GC, Mauya SD, Tilak VK, Gupta AK. A review on factors affecting the design of nasal drug delivery system. Int J Drug Delivery 2011;3:194-208.
4. Kadam NR, Suwanna V. Microspheres: a brief review. Asian J Biomed Pharma Sci 2015;5:13-9.
5. Khedkar A, Rajendra V, Kulkarni A, Dehghan MH, Sufee M, Lahoti S. Spectrophotometric method for analysis of rizatriptan benzoate. Int J Pharm Sci 2009;1:307-13.
6. Prasanta A, Padma MS. Estimation of rizatriptan benzoate tablet by using uv spectrophotometric methods. Int J Pharm Sci Res 2013;1:9-100.
7. Basch E, Ulbricht C, Grace K, Pharm D, Philippe S, Smith M. Therapeutic applications of fenugreek. Altern Med Rev 2003;8:20-7.
8. Indian Herbal Pharmacopoeia. Indian drugs manufacturing association Mumbai. Regional Research Laboratory Jammu Tawi 1999;1:33.
9. Suruse PB, Shivhare UD, Mathur VB, Meshram KL, Patadh AK. Development of microcapsules of glimepiride using fenugreek seed extract. Int J Pharm Pharmpacoil 2013;3:212-5.
10. Jani GK, Prajapati VD. Gums and mucilage: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci 2009;4:399-23.
11. Sharma N, Kulkarni GT, Sharma A, Bhatnaagar A, Kumar N. Natural mucoadhesive microspheres of abelmoschus esculentus polysaccharide as a new carrier for nasal drug delivery. J Microencapsul 2013;30:589–99.
12. Mahajan HS, Tattiya BV, Nerkar PP. Ondansetron loaded pectin based microspheres for nasal administration: in vitro and in vivo studies. Pharm Dev Technol 2012;17:1:9-10.
13. Kellaway W, Hamed MD. Preparation and in vitro characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. Eur J Pharm Biopharm 1997; 44:53–60.
14. Gavini R, Rasso G, Carnelli V, Spada G, Cossu M, Giunchedi P. Mucoadhesive drug delivery systems for nose-to-brain targeting of dopamine. J Nanonanosci 2012;2:47–55.
15. Rawat A, Majumder QH, Ahsan F. Inhalable large porous microspheres of low molecular weight heparin: in vitro and in vivo evaluation. J Controlled Release 2008;128:224–32.

16. Gavini E, Hegge AB, Rassu G, Sanna V, Testa C, Pirisino G, et al. Nasal administration of carbamazepine using chitosan microspheres: in vitro/in vivo studies. Int J Pharm 2006;307:9–15.

17. Kar K, Pal RN, Bala NN. Preparation, characterisation, and evaluation of ropinirole hydrochloride loaded controlled release microspheres using solvent evaporation technique. Int J Pharm Pharm Sci 2018;10:57-67.

18. Kumar R, Patil S, Patil MB, Patil SR, Paschapur MS. Isolation and evaluation of disintegrant properties of fenugreek seed mucilage. Int J Pharm Tech Res 2009;1:982-96.

19. Sunder Raj TJ, Bharathi CH, Kumar MS, Prabahar J, Kumar PN, Sharma HK, et al. Identification, isolation, and characterization of process-related impurities in rizatriptan benzoate. J Pharm Biomed Anal 2009;49:156–62.

20. Patil SB, Sawant K. Mucoadhesive chitosan microspheres as a delivery system for nasal insufflation. Colloids Surf B 2011;84:384-9.

21. Yang YY, Chung TS, Ng NP. Morphology, drug distribution, and in vitro release profiles of biodegradable polysaccharides microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 2001;22:231–41.

22. Deshmukh M, Mohite S. Formulation and characterization of olanzapine-loaded mucoadhesive microspheres. Asian J Pharm Clin Res 2017;10:249-55.