Formulation development and characterization of Chitosan nanoparticles bearing Rizatriptan Benzoate for intranasal delivery

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Abstract

The present investigation was aimed to formulate and characterize chitosan nanoparticles (CS-NPs) loaded with Rizatriptan Benzoate (RIZ) for intranasal administration for brain targeting. The NPs were prepared using inotropic gelation method. Optimization of the process and formulation parameters was done using the Box-Behnken design (BBD). The optimized batch having the concentrations of chitosan and sodium tri-polyphosphate were found to be 0.4 %w/v and 0.3 %w/v respectively while the Zeta potential and Z-average size were also found to be +49 mV and 201 nm with poly dispersity index of 0.2 respectively. Drug entrapment efficiency (% EE) and drug loading (% DL) were of 63.5±1.61% and 17.3±1.38 % w/w respectively. Moreover, improved in-vitro and ex-vivo release profile of CS-NPs were observed in comparison to drug solution. In a nutshell, the developed formulation holds a great promise in overcoming the limitation associated with currently marketed RIZ formulations and illustrates the potential use of CS-NPs to administer the drug by nasal route for brain targeting.

Keywords: Chitosan nanoparticles (CS-NPs), Ionotropic gelation, Rizatriptan benzoate, Brain delivery, Migraine

1. Introduction

Drug delivery through nasal route has been potentially explored as an alternative route for the administration of vaccines and bio molecules such as proteins, peptides and non-peptide drugs that are susceptible to enzymatic or acidic degradation and first-pass hepatic metabolism. The nasal mucosa is one of the most permeable and highly vascularized sites for drug administration ensuring rapid absorption and onset of therapeutic action. Many drugs are not effectively and efficiently delivered to the brain using conventional drug delivery approaches. Transport of drugs from systemic circulation into the central nervous system (CNS) is restricted by the BBB and blood-CSF barrier. While the BBB serves to protect the brain spinal cord from a variety of pathogens and toxic substances, it also presents a significant barrier to treating CNS disorders. Mainly charged, hydrophilic, water soluble substances, and large therapeutic agents are inhibited or prevented from entering the
brain by the BBB [1]. BBB penetration is extremely difficult and one of the critical challenges facing in pharmaceutical therapeutics for effective drug targeting to the brain. CS-NPS physicochemical characteristics (hydrophilicity and low particle size) are particularly regarded in order to address the critical issues related to the suitable brain targeting formulations. CS-NPS accumulation in the brain by nasal administration for brain targeting via olfactory nerve is new approach. This can help in by passing the BBB for polar drugs.

Thus, targeting the drug to the brain would decrease its distribution to other organs and thereby reduce the side effects. It would also result in lowering of dose and related toxicity. One of the approaches for brain targeting is intranasal administration. Drug delivery by intranasal route has numerous advantages like; BBB can be bypassed, non-invasiveness and ease of administration, smaller amounts of the drug are required to produce the desired pharmacological effect, avoidance of the gastrointestinal tract (GIT) side effects and first pass metabolism. The intranasal administration could enable drugs to directly enter the central nervous system (CNS) by means of an olfactory route [2-6]. However, conventional intranasal formulations such as drops and sprays have poor residence time because of rapid clearance. The residence time in the nasal cavity can be increased by using mucoadhesive materials such as chitosan. In addition, chitosan can also facilitate paracellular transport of polar drugs across nasal mucosa [7, 8]. Out of the various commercially available bioadhesive materials that have been used for intranasal delivery of drugs, chitosan (CS) a bioadhesive, a biocompatible and biodegradable copolymer of glucosamine and N-acetyl glucosamine [7] have been widely used. Moreover, it provides a controlled and sustained drug release for prolonged period of time and also imparts a mucoadhesiveness which delays the elimination of drug/formulation by nasal clearance [9]. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both transcellular and paracellular drug targeting to the site of action in controlled or sustained manner for longer period of time so as to achieve enhancement in the therapeutic efficacy of drug with decrease in the side effects [10].

Rizatriptan is a 5-HT1 agonist triptan drug used in the treatment of migraine headaches. Rizatriptan has mean oral absolute bioavailability of about 45%, and mean plasma half-life of 2-3 hrs. Thus, the present investigation was aimed to formulate chitosan nanoparticles (CS-NPs) of Rizatriptan Benzoate (RIZ) targeted to brain via intranasal instillation which overcomes the above problems such as low bioavailability due to first-pass metabolism, adverse effects etc. and furthermore provide quick onset of action along with prolonged release of drug.

2. Materials and Methods

2.1 Materials

Rizatriptan Benzoate (RIZ) was obtained as a gift sample from Dr. Reddy’s Lab, Hyderabad, India. Chitosan (CS) with medium molecular weight (Mw = 750kDa) having a degree of deacetylation about 75-85 % and sodium tri-polyphosphate (TPP) were purchased from the Sigma-Aldrich (USA). Glacial acetic acid (GAA) and Isopropyl alcohol (IPA) were obtained from Rankem chemical, Vadodara, India. Methanol HPLC grade,
Acetonitrile HPLC grade and ortho-phosphoric acid (OPA) HPLC grade were purchased from Spectrochem Pvt. Ltd., Mumbai, India. All other reagents of analytical grade (AR) were used in the study.

2.2 Methods

2.2.1 Preparation of CS-NPs

CS-NPs were formulated by ionotropic gelation method [11-15]. In brief, blank NPs were prepared by injecting TPP aqueous solution (0.2% w/v, 0.25% w/v, 0.3% w/v in distilled water) with the help of insulin U-40 syringe (2 ml, Hi-Tech medical products Ltd., India) to a 10 ml CS solution (0.2% w/v, 0.3% w/v, 0.4% w/v, 0.5% w/v in 1% v/v acetic acid) with continuous stirring (1800-1900 rpm) on magnetic stirrer at the room temperature for 30 min. The rate of injection of TPP solution (4 ml) was 1 ml/min. RIZ loaded NPs were prepared similarly with the addition of RIZ (10 mg) in CS solution before adding TPP solution. The resulting nanoparticulate dispersion was centrifuged at 17000 rpm, 4°C for 1 hr. A pellet of NPs settled down and the supernatant was separated out with the help of micropipette and evaluated for the free drug content and a pellet of NPs was further resuspended in 1% v/v acetic for the determination of % EE.

2.2.2 Optimization of CS-NPs by design of experiment

Optimization carried out by changing one variable at a time is a complex method to evaluate the effects of different variables on an experimental outcome. Response surface methodology (RSM) is an approach to evaluate the impact of the independent variables on the dependent variables by varying all the important factors simultaneously in a systemic manner. A Box- Behnken design matrix was generated by design expert software 7.0.3 for the optimization of CS-NPs due to its multiple advantages [16-18]. Here, chitosan (A) and TPP (B) were selected as the independent variables and % entrapment efficiency (Y1) and particle size (Y2) as dependent variables. Statistical analysis was also carried out by the design expert software 7.0.3 to understand the response coefficient significance and interaction of the independent factors.

2.2.3 Characterization

2.2.3.1 Particle size distribution and Zeta potential

Particle size distribution and zeta potential determination were carried out by using zetasizer Nano ZS (Malvern Instruments Ltd, UK). The analysis was performed with 5 mW He-Ne laser (632.8 nm) at a scattering angle of 90° at 25°C. Samples (1 ml) were diluted (10X) were placed in zeta cell and the particle size and zeta potential were measured as a function of dynamic light scattering (DLS) and electrical surface charge respectively.

2.2.3.2 Scanning Electron Microscopy (SEM)

For studying the surface morphology of RIZ loaded CS-NPs, Scanning Electron Microscopy (EVO-18, Zeiss, Germany) was used. After lyophilization of the sample, it was gold coated using sputter coater (Emitech) for 4 min at 10 mA current. After the gold coating, samples were attached to the aluminum stubs and then viewed using an accelerating voltage of 15.00 kV at the different magnifications.
2.2.3.3 % Entrapment Efficiency (% EE) and Drug Loading (% DL)

In order to determine the % EE of CS-NPs, the formulation was centrifuged at 17,000 rpm for 1 hr (Cooling centrifuge, Sigma instruments, Germany) and the NPs pellets and supernatant were separated. Analysis of RIZ from CS-NPs was done by breaking pellets of CS-NPs in 1 % v/v acetic acid. The pellets were probe sonicated for 3 cycles (0.8 cycle/min x 80% amplitude x 30 sec). After that, the absorbance of the entrapped drug was determined by taking 1% v/v acetic acid as a blank using UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) at 225 nm. To ensure the mass balance of drug, the supernatant was also analyzed for the free drug content using UV-Visible spectrophotometer at 225 nm. The % EE and % DL were calculated by using following equations [19, 20].

\[
\text{% EE} = \frac{\text{Amount of Entrapped drug}}{\text{Total drug added}} \times 100
\]

\[
\text{% DL (} \% \text{ w/w)} = \frac{\text{Drug loaded (mg)}}{\text{Total weight of NPs (mg)}} \times 100
\]

2.2.3.4 In-vitro drug release study

In-vitro release study of RIZ loaded CS-NPs was carried out by using dialysis bag diffusion technique [21]. The drug release study was performed using pH 7.4 phosphate buffered saline (PBS). The drug release for Rizatriptan benzoate solution and Rizatriptan benzoate loaded CS-NPs/gels were determined. The nanoparticulate dispersion/gel equivalent to 2 mg of Rizatriptan benzoate were placed in a dialysis bag, which was previously dipped overnight in diffusion medium, cleaned and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 30 ml of pH 7.4 PBS, which was stirred at 100 rpm and maintained at 37±2°C. The receptor compartment was covered to prevent the evaporation of release medium. Samples (1 ml) were withdrawn at regular time intervals from the receptor compartment and the same volume (1 ml) was replaced by fresh diffusion medium. The samples were analyzed using UV-Visible Spectrophotometer at 225 nm. Rizatriptan benzoate solution in distilled water (2 mg/ml) was also evaluated for in-vitro drug release study.

2.2.3.5 Ex-vivo drug permeability study

Freshly excised goat nasal mucosa was collected from the slaughter house and dipped immediately in PBS pH 7.4. The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using phosphate buffer PH 7.4 in both the compartments (donor and receptor) and stirred for 15 min using a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh PBS PH 7.4 was filled in the acceptor compartment. The temperature of receiver chamber, containing 8 ml of diffusion media was controlled at 37±2°C under continuous stirring using teflon coated small magnetic bead at a constant rate (100 rpm), in a way that the nasal membrane surface just in contact with the diffusion fluid. At the predetermined time intervals (0,1,2,3,4,5,6,7…28 hr), samples (1 ml) from the receptor compartment were withdrawn and replaced by an equal volume of PBS pH 7.4. The samples were analyzed using UV-Visible Spectrophotometer at 225 nm [22].
2.2.3.6 Stability study

Stability study of the optimized batch of RIZ loaded CS-NPs was conducted at different storage conditions for 2 months as per the ICH guidelines. The optimized batch was then characterized for particle size, % EE and zeta potential at the time interval of 15 days, 30 days and 60 days [23-26].

3. Results

3.1 Preparation and optimization of RIZ loaded CS-NPs

RIZ loaded CS-NPs were prepared by ionic gelation technique as already reported [11-14]. Preliminary optimization was carried out on the basis of the concentration of CS, TPP and the CS/TPP volume ratio. From the preliminary results, optimized formulation parameters like concentration of CS and TPP were found to be 0.4 %w/v and 0.30 %w/v respectively. While the optimized volume ratio of the CS/TPP was found to be 2.5:1. The optimized process parameters like rate of addition of TPP solution into CS solution, stirring speed and stirring time were found to be 1 ml/min, 1800-1900 RPM and 30 min respectively. By applying BBD design matrix, the process parameters and formulation parameters affecting the CS-NPs optimization were optimized. Total 17 runs with 5 centre points were obtained from the BBD design matrix and the results are shown in table 1.

Table 1 Optimization of Rizatriptan Benzoate Loaded CS-NPs

<table>
<thead>
<tr>
<th>Run</th>
<th>Chitosan (% w/v)</th>
<th>TPP (% w/v)</th>
<th>Drug (mg)</th>
<th>% EE</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.30</td>
<td>0.25</td>
<td>10</td>
<td>61.43</td>
<td>164.8</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.25</td>
<td>10</td>
<td>60.74</td>
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<tr>
<td>3</td>
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<td>0.20</td>
<td>10</td>
<td>60.71</td>
<td>246.3</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>0.25</td>
<td>10</td>
<td>62.95</td>
<td>222.5</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>0.20</td>
<td>10</td>
<td>52.31</td>
<td>218.5</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
<td>0.25</td>
<td>10</td>
<td>59.25</td>
<td>240.3</td>
</tr>
<tr>
<td>7</td>
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<td>10</td>
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<td>154.2</td>
</tr>
<tr>
<td>8</td>
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<td>0.30</td>
<td>10</td>
<td>63.12</td>
<td>201.2</td>
</tr>
<tr>
<td>9</td>
<td>0.50</td>
<td>0.30</td>
<td>10</td>
<td>66.78</td>
<td>223.7</td>
</tr>
<tr>
<td>10</td>
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<td>0.30</td>
<td>10</td>
<td>61.28</td>
<td>189.7</td>
</tr>
<tr>
<td>11</td>
<td>0.30</td>
<td>0.20</td>
<td>10</td>
<td>49.68</td>
<td>168.4</td>
</tr>
<tr>
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<td>0.25</td>
<td>10</td>
<td>60.54</td>
<td>225.3</td>
</tr>
<tr>
<td>13</td>
<td>0.50</td>
<td>0.25</td>
<td>10</td>
<td>69.7</td>
<td>241.2</td>
</tr>
<tr>
<td>14</td>
<td>0.50</td>
<td>0.20</td>
<td>10</td>
<td>59.49</td>
<td>262.4</td>
</tr>
<tr>
<td>15</td>
<td>0.30</td>
<td>0.30</td>
<td>10</td>
<td>59.23</td>
<td>152.1</td>
</tr>
<tr>
<td>16</td>
<td>0.40</td>
<td>0.25</td>
<td>10</td>
<td>60.88</td>
<td>217.6</td>
</tr>
<tr>
<td>17</td>
<td>0.40</td>
<td>0.25</td>
<td>10</td>
<td>61.56</td>
<td>221.6</td>
</tr>
</tbody>
</table>

3.1.1 Statistical analysis

The values of the two independent variables {% EE (Y1), particle size (Y2)} were incorporated into the design expert software 7.0.3 and to evaluate the effect of the formulation variables on the response variables, polynomial equations were generated using design expert software 7.0.3.
% EE = +61.32+3.54*A+3.53*B-2.72*C-0.56*A*B-1.40*A*C+2.58*B*C-0.082*A²-2.46*B²+0.51*C²

Particle Size = +220.57+40.29*A-16.11*B-3.45*C-5.61*A*B+2.38*A*C+9.83*B*C-16.84*A²-3.09*B²-4.55*C²

The interaction between the various combinations of the independent variables that can affect the response parameters were studied and are represented graphically as response surface plots (figure 1).

Figure 1 A) Response surface (3D) showing combined effect of Chitosan and TPP concentration on % EE, B) Contour plot (2D) showing combined effect of TPP and Chitosan concentration on % EE, C) Response surface (3D) showing combined effect of Chitosan and TPP concentration on particle size, and D) Contour plot (2D) showing combined effect of TPP and Chitosan concentration on particle size

3.2 Characterization of optimized RIZ loaded CS-NPs

Zetasizer Nano ZS (Malvern Instruments Ltd, UK) was used to measure the particle size and zeta potential of the optimized RIZ loaded CS-NPs. Optimized particle size and zeta potential of the RIZ loaded CS-NPs were found to be 201 nm (PDI values of 0.201) and 49.3 mV respectively (figure 2). Surface morphology of RIZ loaded CS-NPs was evaluated by SEM. It revealed that the RIZ loaded CS-NPs had a smooth surface and were spherical in shape with desired particle size shown in figure 2. The mean % EE and % DL obtained during the optimization of CS-NPs of RIZ were found to be 63.5 ± 1.6% and 17.3 ±1.4 %w/w respectively. From the results, it was observed that with an increase in CS concentration, there was an increase in % EE and particle size of CS-NPs.
Figure 2 Particle size and zeta potential of optimized Rizatriptan Benzoate CS-NPs
The results of in vitro drug release studies for RIZ solution and RIZ loaded CS-NPs are shown in figure 4. It can be seen that within 2 hr, 95% drug released from the plain drug solution, whereas only 25% drug release from the CS-NPs occurred, reaching 84% after 28 hr, indicating sustained release. The data obtained from the in-vitro drug release studies was fitted to the zero order, first order, higuchi model and korsmeyer-Peppas model. From the regression coefficient values of these models, the plot of Log % CDR v/s log time i.e. korsmeyer-Peppas model for CS-NPs was found to be 0.997 which is higher than others. Hence, we can conclude that the RIZ loaded CS-NPs followed korsmeyer-peppas model and the value of n (release exponent) was found to be 0.4653 i.e. 0.45<n<0.89 indicates the anomalous diffusion/non-fickian diffusion.
The % cumulative drug permeated through nasal mucosa from the drug solution and RIZ loaded CS-NPs are shown in figure 5. From the results, it was found that 28 % drug released from the plain drug solution within 5 hr, while in the case of RIZ loaded CS-NPs, faster diffusion was clearly observed (54% in 5 hr).

![Figure 5 Ex-vivo release profile of optimized Rizatriptan Benzoate CS-NPs](image)

The results of stability study at different temperature are shown in table 2 and were predicted with respect to particle size (nm), % EE, zeta potential (mV). From the results, it was found that particle size of CS-NPs dispersion increased both at room temperature and refrigerated temperature. However, increase in particle size of CS-NPs stored at refrigerated temperature was found to be relatively less than that of CS-NPs stored at room temperature. Stability of CS-NPs was also assessed by % EE and zeta potential, showed that at room temperature, reduction in % EE and zeta potential was faster than the refrigerated temperature due to the hydrophilic nature of drug which has a tendency to migrate out from the NPs. Thus, to avoid an increase in particle size and reduction in % EE and zeta potential, CS-NPs should be stored under refrigerated conditions.

Table 2 Results of stability study of RIZ loaded CS-NPs

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Storage conditions for RIZ loaded CS-NPs</th>
<th>Refrigerated temperature (2-8°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room temperature (25-40°C)</td>
<td></td>
</tr>
<tr>
<td>Particle size (nm)</td>
<td>Initial</td>
<td>201.2±1.71</td>
</tr>
<tr>
<td></td>
<td>After 15 days</td>
<td>218.5±1.28</td>
</tr>
<tr>
<td></td>
<td>After 30 days</td>
<td>222.5±2.01</td>
</tr>
<tr>
<td></td>
<td>After 60 days</td>
<td>234.3±2.96</td>
</tr>
<tr>
<td>% EE</td>
<td>Initial</td>
<td>64.19±0.20</td>
</tr>
</tbody>
</table>
After 15 days & 52.71±0.51 & 63.82±0.07 \\
After 30 days & 44.72±1.03 & 63.51±0.15 \\
After 60 days & 35.38±1.19 & 63.07±0.14 \\

<table>
<thead>
<tr>
<th>Zeta potential (mv)</th>
<th>Initial</th>
<th>After 15 days</th>
<th>After 30 days</th>
<th>After 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>49.3±0.83</td>
<td>33.4±1.66</td>
<td>31.2±0.74</td>
<td>30.6±0.58</td>
</tr>
<tr>
<td></td>
<td>49.3±0.83</td>
<td>47.8±0.47</td>
<td>44.6±1.12</td>
<td>42.3±0.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total drug content (% assay)</th>
<th>Initial</th>
<th>After 15 days</th>
<th>After 30 days</th>
<th>After 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.20±0.17</td>
<td>96.83±0.86</td>
<td>93.49±1.09</td>
<td>91.31±1.38</td>
</tr>
<tr>
<td></td>
<td>99.20±0.17</td>
<td>98.84±0.54</td>
<td>97.20±0.88</td>
<td>96.47±0.75</td>
</tr>
</tbody>
</table>

4. Discussion

CS-NPs is one of the types of polymeric nanoparticulate systems, used to deliver the drug in controlled manner for prolonged period of time resulting in reduced in dosing frequency thus enhancing the patient compliance. Moreover, RIZ has a short t½ (2-3 hr) and is to be given frequently (1-2 times a day) and also has a low molecular weight (<400 Da), which makes RIZ a promising candidate for formulating as CS-NPs.

Ionotropic gelation method was used to formulate the RIZ loaded CS-NPs. From the preliminary optimization of CS-NPs, the effect of various formulation and process parameters on the formation of CS-NPs were evaluated. A BBD design matrix was generated by design expert software 7.0.3 and was used in the optimization of the RIZ loaded CS-NPs. Response surface graph (Figure 1) shows the significant impact of each independent variables on the response parameters i.e. % EE and particle size. In brief, from the results (table 1) the rate of addition of TPP solution to CS solution of 1 ml/min was chosen due to high % EE (61.43 ± 1.9 %) and desired particle size (164.8 ± 5.2 nm) because at low rate (0.5 ml/min) of addition of TPP, the % EE was 19.92 ± 2.2 % and particle size was 329.5 ± 6.4 nm while in the case rapid injection of TPP solution the masses of polymer formed. The reason for this may be the rapid gelation leading to aggregation of the polymer mass. While in the case of stirring speed, at low RPM (800-900) the formed CS-NPs were having less % EE and higher particle size and polydispersity index (PDI) values. However, table 1 shows as increase in the CS concentration leads to linear increase in the particle size but at higher concentration of CS (0.5 %w/v), % EE increased but particle size was greater than 200 nm which is not desirable because NPs less than 200 nm have better brain targeting efficiency via olfactory pathway [27]. 0.3 %w/v of TPP concentration was chosen because of the better % EE and desirable particle size (around 200 nm). The CS/TPP volume ratio was varied (3.3:1 to 2.5:1) and from the preliminary optimization CS/TPP volume ratio of 2.5:1 was chosen due to desired particle size and % EE.
The particle size of CS-NPs indicates that the particles formed were monodisperse across the size range, i.e. the particle size distribution was uniform. This high zeta potential value also indicates an electric repulsion among the particles which avoids particle aggregation contributing to the stability of CS-NPs. The positive surface potential may be attributed to positively charged amino groups of chitosan which facilitate the adhesion and transport properties of the CS-NPs due to the electrostatic attractions between the positively charged formulation and the negatively charged cell membrane [28]. While SEM images of the RIZ loaded CS-NPs revealed that CS-NPs had a smooth surface and were spherical in shape. It was also observed that the field showed desired particle size of CS-NPs, which will facilitate the transport of CS-NPs transcellularly through neuronal cells in the olfactory region [29, 30]. The size of the RIZ loaded CS-NPs was around 200 nm (figure 2) which was in concurrence with the results obtained in particle size analysis.

Due to the hydrophilic nature of drug, faster drug release occurs from the plain drug solution. It was assumed that drug release from CS-NPs followed the biphasic model, with an initial burst release attributed to the drug associated near particle surface. Also, particles of nano size range led to a shorter average diffusion path for the matrix-entrapped drug molecules, thereby causing faster diffusion. Thereafter, the release rate of the drug was decreased, reflecting the release of drug entrapped in the polymer matrix. The release rate in the second phase is assumed to be controlled by the diffusion of the drug across the polymer matrix. After fitting this data into the various models, $r^2$ of the korsmeyer- peppas model for CS-NPs was found to be 0.997 and also the value of $n$ indicates the anomalous diffusion/non-fickian diffusion of the CS-NPs, refers to the combination of both diffusion and erosion controlled rate release [31-33].

*Ex-vivo* drug permeability study shows the increased permeation of CS-NPs due to the mucoadhesive nature of CS and also the inherent mechanism of CS to interact with protein kinase C which could be resulted in a strong ionic interaction of a positively charged amino group of CS with negatively charged sites on the cell membranes leading to an opening at the tight junctions of epithelial cells, increasing the permeation of polar drugs via paracellular transport [34]. The probable reason for lesser permeation of the pure drug solution in comparison to CS-NPs could be the hydrophilic nature of drug. Since due to the high solubility of polar drugs in the mucus, their passage through the nasal membrane is very poor. [35-37].

5. Conclusion

In the present investigation, RIZ loaded CS-NPs were formulated by ionotropic gelation technique and optimized using Box-Behnken design. It was further characterized for particle size, zeta potential, % EE, % DL, and the *iv-vitro* studies supported the controlled drug release of RIZ loaded CS-NPs for prolonged period of time. The present investigation demonstrates that intranasal RIZ loaded CS-NPs can potentially transport RIZ to the brain via olfactory route and can serve as a noninvasive alternative for the delivery of RIZ to the brain.

Declaration of interest

The authors declare that they have no conflict interests.
References