Section I/ Chapter 4

Optimization and development of quetiapine fumarate loaded chitosan nanoparticles for brain delivery via intranasal route

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Optimization and development of quetiapine fumarate loaded chitosan nanoparticles for brain delivery via intranasal route

Abstract

The objective of the present investigation was to optimize and develop quetiapine fumarate (QF) loaded chitosan nanoparticles (QF-NP) by ionic gelation method using Box-Behnken design. Three independent variables viz., $X_1$-Concentration of chitosan, $X_2$-Concentration of sodium tripolyphosphate and $X_3$-Volume of sodium tripolyphosphate were taken to investigate their effect on dependent variables ($Y_1$-Size, $Y_2$-PDI and $Y_3$-%EE). Optimized formula of QF-NP was selected from the design space and the average particle size, %EE and nasal diffusion were found to be 131.08 ± 7.45 nm, 89.93 ± 3.85% and 65.24 ± 5.26% respectively. Significantly higher brain/blood ratio and 2 folds higher nasal bioavailability in brain with QF-NP in comparison to drug solution revealed preferential nose to brain transport bypassing blood-brain barrier and prolonged retention of QF at site of action suggesting superiority of chitosan as permeability enhancer. Overall, the above finding shows promising results in the area of developing noninvasive intranasal route as an alternative to oral route for brain delivery.
4.1 Introduction to the development of QF-NP
Chitosan (CH) is a natural carbohydrate polymer with many ideal properties like bioadhesion, nontoxic and biodegradable. It has advantage of overcoming the natural defence mechanism known as mucociliary clearance due to mucoadhesion phenomenon and at the same time shows penetration enhancing properties. Presence of positively charged amino groups in CH interacts with negatively charged nasal mucosal surface thereby allowing prolonged residence time in the nasal cavity. This results in increased paracellular transport of drugs across membrane by transient opening of tight junctions (Casettati and Illum, 2014; Yeh et al., 2011)
Now a day’s use of natural polymeric nanoparticles in the area of nasal nanotherapeutics is increasing widely due to their desirable characteristics of biodegradability, biocompatibility and ease of preparation. Nanoparticles (NP) allow easy access across BBB by efficiently encapsulating drug molecules and increasing their diffusion across biological membrane (Costantino and Boraschi, 2012; Md et al., 2015). NP made up of natural polymer like CH have been reported by various scientists in improving nasal absorption and brain targeting of various neurotherapeutics (Bari et al., 2015; Mittal et al., 2016; Haque et al., 2014).
In the present investigation QF-NP were developed to evaluate the potential of developed system in enhancing the brain delivery via noninvasive intranasal route. QF-NP were formulated by ionic gelation method and characterized for physicochemical, solid state characterization, morphology and nasal diffusion evaluation. Pharmacokinetic studies were performed to measure brain targeting efficiency and direct transport percentage after IN and IV administration of QF-NP. Finally physicochemical, diffusion and pharmacokinetic parameters of QF-NP were compared with that of MME (CH-ME) to determine the potential of the carrier system.

4.2 Materials and methods
4.2.1 Materials
QF and risperidone were received as gift samples from Torrent Pharmaceuticals Ltd. (Ahmedabad, India). Sodium tripolyphosphate (TPP) was purchased from Sigma-Aldrich (Bangalore, India). Chitosan (CH) was obtained as a gift sample from Mahtani Chitosan Pvt. Ltd. (Veraval, India). All other chemicals and reagents were of highly purified grade and were used without further purification.
4.2.2 Development of QF-NP for intranasal delivery

4.2.2.1 Preparation and optimization of nanoparticles

NP were prepared by ionic gelation method which involves interaction of positively charged ions with negative charge. CH has positively charged amino groups which are believed to interact with negatively charged phosphate ions of sodium tri poly phosphate (TPP) and thus forming CH/TPP NP. Initially CH was dissolved in water for overnight and filtered to remove insoluble residues. TPP was also dissolved in distilled water and filtered to obtain clear solution. Preparation of NP involves mixing of polycation solution of CH with polyanion solution of TPP. Crosslinking solution of TPP was added dropwise to the CH solution and stirred on a magnetic stirrer at room temperature to form CH/TPP NP (blank NP). QF-NP were formulated by adding QF (1% w/v of total volume) into CH solution and was kept for overnight into which TPP solution was added and stirred on a magnetic stirrer. The obtained QF-NP suspension was centrifuged to obtain pellets, which were then washed with distilled water and freeze dried using mannitol for further characterization.

During preliminary optimization various process parameters were investigated which had crucial effect on the size, PDI and %entrapment efficiency (%EE) of QF-NP. Various trials were performed (CH volume was kept constant for each trial- 10 ml) to evaluate the effect of process parameters on the formation of QF-NP. Concentration of CH, concentration of TPP and volume of TPP had major effect on the quality of NP while stirring speed (700 rpm), time of stirring (15 min) and drug concentration (1% w/v) were set as fix parameters. Optimized formula for formation of QF-NP is depicted in Figure 38 in results and discussion.

4.2.2.2 Characterization of QF-NP (Shah et al., 2016c)

For characterization, three batches of optimized QF-NP were formulated and characterized for physicochemical, solid state characterization and nasal diffusion parameters. For solid state characterization blank NP and QF-NP were lyophilized using mannitol. QF-NP were also evaluated for in vivo pharmacokinetic parameters.

1). Physicochemical parameters

Below mentioned physicochemical parameters were performed in triplicate.
A). **Particle size, PDI and zeta potential measurements**: Particle size, PDI and zeta potential measurements were performed by photon correlation spectroscopy using Zetasizer. Particle size and PDI measurements were performed by taking 1 ml of formulation into polystyrene cuvettes and disposable folded capillary cell for zeta potential at 25 °C, respectively.

B). **%EE**: The %EE of formulated QF-NP was determined by centrifugation method. Samples were taken in centrifuge tubes and centrifuged at 15000 rpm for 30 min at room temperature in order to obtain pellet of QF-NP. After centrifugation supernatant was collected and analyzed for free drug content by HPLC. %EE was calculated by following equation:

\[
%EE = \frac{\text{Total amount of QF} - \text{Amount of free QF}}{\text{Total amount of QF}} \times 100
\]

C). **pH**: pH becomes very crucial aspect in case of formulation that comes in direct contact with nasal mucosa. pH of QF-NP was determined by taking 10 ml of formulation in a beaker. pH was measured at room temperature using a calibrated digital pH meter.

2). **Solid state characterization**

A). **Differential scanning calorimetry**: DSC analysis of QF, CH, mannitol, CH + mannitol, blank NP and QF-NP were performed using DSC Q20 instrument. Samples (3-5 mg) were sealed in standard aluminum pans and analysis was performed at heating rate of 10°C/min from 25 to 200°C under a nitrogen atmosphere with a flow rate of 50 ml/min. An empty sealed aluminum pan was used as reference.

B). **Fourier transform infrared spectroscopy (FTIR)**: FTIR spectrums were recorded for QF, CH + mannitol, blank NP and QF-NP using FTIR spectrophotometer. The samples were prepared by potassium bromide disc method and scanned in the range of 4000 cm\(^{-1}\) to 400 cm\(^{-1}\). IR spectrums were compared to investigate the interaction between NP and QF.

C). **X-ray diffraction (XRD) study**: X-ray diffraction measurement of QF, CH + mannitol, blank NP and QF-NP were performed using X-Ray diffractometer. Analysis was performed in continuous mode with step size of 0.017° over an angular...
range (2θ) of 3° to 50° and diffractograms were analyzed using JCPDF database diffractometry software.

3). Morphological characterization
A). Scanning electron microscopy (SEM): The shape and surface characteristics of QF-NP were determined by SEM (LEO make, 440i, UK) using gold sputter technique. Freeze dried QF-NP were dusted onto a double sided tape on an aluminium stub. The stubs containing the sample were coated with gold using a cool sputter coater (Fision SC 7610, Quorum Tech., UK). Photomicrographs were taken at the accelerated voltage of 10 KV and chamber pressure of 0.6 mmHg.

B). Transmission electron microscopy (TEM): The morphology of optimized QF-NP was carried out using TEM (Philips, Tecnai 20, Holland) at an acceleration voltage of 200 kV and viewed at a magnification of 50000x. QF-NP was diluted 10 times with distilled water and a drop of diluted sample was placed on a carbon-coated copper grid. Sample preparation and imaging process was followed as per procedure described in section 3.1.5.13.

4.2.2.3 Nasal diffusion study
Nasal diffusion was carried out using Franz diffusion cell with a receptor volume capacity of 12.5 ml using goat nasal mucosa as a dialyzing membrane. As per procedure described in section 3.1.5.9, comparative nasal diffusion study was performed in triplicate by taking QF-NP and QF solution (DS) and analyzed for drug content by HPLC at 250 nm. Percentage drug diffused (mean values) was plotted against time (h). Flux (µg/cm²/h) and diffusion coefficients (cm²/h) values were calculated from the slope of plot obtained between an amount of drug permeated/unit area (µg/cm²) of mucosal membrane versus time (h).

4.2.2.4 Pharmacokinetic study
All animal experiments were approved and performed in accordance with the guidelines of the Institutional Animal Ethics Committee as discussed earlier in section 3.1.5.17.

Animal handling drug administration: Sprague Dawley rats weighing between 250 and 300 g were selected for the biodistribution study. Animals were divided into three groups each consisting of 20 animals. First two groups (QF-NP and DS) received IN formulations and third group was administered QF-NP by IV route
equivalent to 2.3 mg/kg dose of QF respectively. For IN administration groups, formulations equivalent to 10 mg/ml of QF were prepared and 30 µl of formulation was administered into each nostril of anaesthetized rats. Rats were held from back in slanted position during IN administration. Similarly for IV administration group, 60 µl of QF-NP (10 mg/ml of QF) was injected through rat tail vein. At each of the following time points: 15, 30, 60, 120 and 240 min (n=4, each time point), blood samples were collected from retino-orbital vein into heparinized tubes. The rats were then sacrificed humanely by cervical dislocation method at said time intervals and brain tissue was collected and placed in a tube containing 1 ml of PBS pH 6.4.

**Extraction of QF from blood and brain samples:** As per method discussed in section 3.1.5.17.

**Pharmacokinetic analysis:** Concentration versus time profiles of QF for brain and plasma after IN and IV administration were determined. Pharmacokinetic parameters (C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-240</sub> and T<sub>1/2</sub>), DTP, DTE and nasal bioavailability were calculated as per methods and equations described in section 3.1.5.17.

### 4.2.3 Statistical analysis

All data are reported as mean ± S.D and the differences between the groups were tested using student’s t test (paired t test, two tailed) at the level of p<0.05 considering significant and p>0.05 non-significant. In case of multiple comparison, more than two groups were compared using ANOVA (mean of each column is compared with mean of control column using dunnnett test) and the differences were considered to be significant and highly significant at the level of p<0.05 and p<0.01 respectively with the help of GraphPad Pism Version 6.01 software.
4.3 Results and discussion

4.3.1 Optimization of CH/TPP NP
QF-NP were prepared by ionic gelation method. As discussed by Shah et al., 2016c, stirring time (15 min), speed (700 rpm) and amount of drug (1% w/v of total volume) were set as fix levels. CH concentration, TPP concentration and TPP volume showed major effect on the size, PDI and %EE of QF-NP, hence they were taken as independent variables and different experimental runs were conducted by them to optimize the variables and to attain lower size, PDI and higher %EE. Selection of variables and effect of individual variables on responses with detailed explanation is mentioned in the published paper as stated earlier in section 3.4. They have selected optimized formula from design space in the overlay plot. Selected optimized formula had three independent variables viz., CH concentration of 0.1% w/v, TPP concentration of 0.1% w/v and TPP volume of 2.08 ml which gave predicted values for three responses (size: 137.65 nm, PDI: 0.247 and %EE: 91.65). Using this optimized formula, three individual formulations of QF-NP were formulated.

4.3.2 Characterization of QF-NP
As shown in figure 38, optimized QF-NP were formulated and characterized for size, PDI, zeta potential, %EE and pH in triplicate. Particle size measurements were required to confirm the production of particles in nano-range. Particle size of 131.08 ± 7.45 nm with PDI of 0.252 ± 0.064 indicated narrow distribution with smaller size and hence it will have larger the surface area thereby increased rate of drug absorption. Zeta potential is an electrical charge on the particle surface and acts as a repulsive factor which imparts stability to formulation. The zeta potential value of 34.4 ± 1.87 mV was found to be positive revealing superior stability of the prepared QF-NP. The positive value for zeta potential could be attributed to cationic nature of CH and presence of residual amino group which are not neutralized by their interaction with TPP molecules. These amino groups prevent the anion adsorption and maintain the high value of electrical double layer thickness, thereby forming stable NP (Wang et al., 2008). %EE of 89.93 ± 3.85 could be indicative of higher encapsulation of QF in CH/TPP matrix. Another advantage of higher %EE is to obtain maximum drug concentration in polymer matrix which in turns reduces amount of dosing. pH value of 5.87 ± 0.11 for QF-NP was within pH range (5–6.5)

Figure 38. Optimized formula for QF-NP and characterization parameters (n=3).

4.3.3 DSC analysis

Figure 39 represents overlay DSC curve for QF, CH, mannitol, CH + mannitol, blank NP and QF-NP. DSC curves for QF, mannitol and CH + mannitol showed sharp endothermic melting events at 175.10°C, 168.62°C and 167.31°C respectively, indicating their crystalline nature. CH did not show any major event except a broad endothermic event at 85°C, which is attributed to moisture loss. In case of physical mixture containing CH and mannitol, the endothermic event (167.31°C) did not show any change when compared to CH and mannitol alone (168.62°C) indicating they are not interacting with each other, hence were taken in mixture for FTIR and XRD characterization. Blank NP and QF-NP showed presence of endothermic event
for mannitol at 165.62°C and 163.05°C respectively which signify crystalline nature of mannitol in both the systems. DSC curve of QF-NP did not show melting event for QF as compared to QF alone thereby suggesting reduction in degree of crystallinity for QF since maximum amount of QF was entrapped within the NP (%EE: 89.93 ± 3.85%). Slight higher enthalpy value of 96.62 J/g (QF-NP) compared to 83.70 J/g (blank NP) could be indicative of presence of small amount of unentrapped QF in QF-NP which would have melted along with mannitol and hence showed higher enthalpy.

Figure 39. Comparative DSC curves of QF, CH, mannitol, CH + mannitol, blank NP and QF-NP.

4.3.4 FTIR analysis

Overlay FTIR spectrum for QF, CH + mannitol, blank NP and QF-NP is shown in Figure 40. The characteristic peaks for QF at 3440, 2927, 2855, 1600 and 768 cm$^{-1}$ are assigned to stretching of OH, aromatic H, C-H, C-N, and C-S respectively. While the peaks for CH at 3402, 3289, 2920 and 2854 cm$^{-1}$ are due to stretching vibrations of OH group overlapped to the stretching vibration of N-H and C-H bond.
Figure 40. Overlay FTIR spectra of QF, CH + mannitol, blank NP and QF-NP.
in CH₂ and CH₃ groups, respectively. Peak at 1633 and 1540 cm⁻¹ are related to the vibrations of carbonyl bonds (C=O) of the amide group (secondary amide) and to the vibrations of protonated amine group (NH₂). Peaks at 1420, 1083, 1020 and 882 are attributed to vibration of OH group, C–O stretching vibration and wagging effect of saccharide in CH respectively (Silva et al., 2012). Shift of band from 1540 cm⁻¹ to 1524 cm⁻¹ and increase in intensity of amino group in case of QF-NP is indicative of NP formation as a result of ionic interaction of NH₂⁺ with negatively charged groups of TPP. The characteristic peaks for QF were absent in case of QF-NP when compared to the spectra of pure drug. This confirmed that the QF was entrapped into CH-TPP matrix.

4.3.5 XRD analysis

The comparative XRD of QF, CH + mannitol, blank NP and QFNP are shown in Figure 41. QF showed sharp peak at 2θ scattered angles of 16.13°, 20.01°, 21.03°, 22.15° and 23.19° exhibiting crystalline nature. CH + mannitol showed sharp peaks at 18.68°, 20.35°, 21.0° and 23.29° indicating crystalline nature of mannitol since CH is amorphous in nature which was in agreement with the result obtained from DSC thermogram of CH showing absence of endothermic events (Harris et al., 2010; Zhang et al., 2008). From Figure 41, it was observed that the XRD patterns of blank NP and QF-NP also showed peaks for mannitol indicating mannitol remained crystalline even in final NP system which was further in agreement with the DSC data showing sharp endothermic peak for mannitol in both the systems. Sharp peaks at 2θ scattered angles of 16.13° and 23.19° for QF were absent and/or showed very lower or negligible intensity in QF-NP indicating reduction in the crystallinity for QF. It is known that, broadening and lowering of intensities observed in QF-NP compared to individual XRD spectra are characteristic of poor crystalline nature. This could be also attributed to cross linking reaction taking place between CS and TPP into which QF was entrapped thereby showing lower or negligible intensities for QF in XRD spectra of QF-NP.
Figure 41. Comparative XRD pattern of QF, CH + mannitol, blank NP and QF-NP.
4.3.6 Morphological analysis
SEM images representing surface morphology of QF-NP in group as well as single NP are depicted in Figure 42. QF-NP were found to be spherical in shape with uniform distribution. SEM analysis revealed that the QF-NP had smooth surface and did not show any aggregation. Morphological characteristic of the QF-NP was analyzed using TEM (Figure 43). It was observed that QF-NP were spherical in shape with diameter in the range of 100–150 nm. This was further in agreement with the result obtained by photon correlation spectroscopy which showed an average diameter of 131.08 ± 7.45 nm. From Figure 43, it was illustrated QF-NP had core-shell structure with solid dense morphology.

![Figure 42. SEM images of QF-NP.](image)

![Figure 43. TEM images of QF-NP.](image)

4.3.7 Nasal diffusion study
Figure 44 depicts comparative nasal diffusion as percent drug diffused versus time (h) whereby the amount of percent QF diffused from QF-NP was highest at all the time points compared to QF-DS. It was observed that almost 60% of drug was diffused from QF-NP after 6 h, whereas an amount of drug diffused from QF-DS
was up to 40% only indicating enhanced diffusion of QF with NP system compared to pure drug solution. In addition to that, XRD and DSC data revealed crystalline nature of QF thereby showing poor solubility with lower permeation in case of QF-DS compared to QF-NP having lower and/or negligible amount of crystalline QF, hence higher permeation was observed. Amount of QF diffused from QF-NP and QF-DS after 8 h remains fairly constant up to 12 h. It was also observed that at the end of 12 h the difference in the amount of QF diffused from QF-NP and QF-DS was only 19.31%, which was not considered to be very large difference. Possible explanation behind lesser nasal diffusion could be lipophilic nature of nasal mucosa acting as rate limiting membrane from which QF did not diffuse well since it shows pH dependent solubility. Flux ($0.879 \pm 0.018 \, \mu g/cm^2/h$) and diffusion coefficient ($4.87 \pm 0.102 \times 10^{-4} \, cm^2/h$) values were found to be highest with QF-NP where as QF-DS showed 1.5 times lower value of flux ($0.618 \pm 0.012 \, \mu g/cm^2/h$) and diffusion coefficient ($3.34 \pm 0.069 \times 10^{-4} \, cm^2/h$). This could be explained by the fact that CH could have contributed for mucoadhesion and disruption and/or modulation of tight junction components on mucosa thereby decreasing strength of tight junction, thus resulting in an enhanced paracellular transport (Yeh et al., 2011).

![Ex vivo nasal diffusion of QF-NP and QF-DS in PBS pH 6.4 (n = 3).](image)

**Figure 44.** *Ex vivo* nasal diffusion of QF-NP and QF-DS in PBS pH 6.4 ($n = 3$).

### 4.3.8 In vivo pharmacokinetic study

Brain uptake studies following QF-NP (IN), QF-DS (IN) and QF-NP (IV) on Sprague Dawley rats were performed and the drug concentration in blood and brain were estimated at different intervals up to 4 h. Mean plasma and brain concentration
versus time profile of QF after IN and IV delivery were evaluated as shown in Figure 45 and 46 respectively, from which various pharmacokinetic parameters were calculated as shown in Table 20. Following IV administration of QF-NP, QF concentration was found to be significantly higher at all the time points in plasma compared to QF-NP and QF-DS administered intranasally where it was found lower (Figure 45).

Figure 45. Plasma concentration versus time profile following QF-NP (IN), QF-DS (IN) and QF-NP (IV) administrations (Data represents mean ± SD, n = 4).

Concentration for QF in the brain following IN administration of QF-NP was found to be significantly higher at all the time points which was followed by QF-NP (IV) and QF-DS (IN) respectively (Figure 46). This result indicated that IN administration of QF-NP ($C_{\text{max}}$: 169.27 ± 20.86 ng/gm) enhanced QF concentration upto 2.6 times in the brain compared to QF-DS ($C_{\text{max}}$: 65.44 ± 24.24 ng/gm) administered by IN route. This could be due to the fact that the QF-NP had a mucoadhesive nature due to presence of CH which acted as permeation enhancer and adhered for longer period of time at nasal mucosa which was not the case with QF-DS, since it got cleared rapidly by mucociliary clearance. This revealed that the addition of mucoadhesive agent plays a key role in improving brain uptake as a result of prolonged contact time of applied formulation with mucosa, which was in agreement with other findings (Haque et al., 2014). QF-NP administered by IV
route showed $C_{\text{max}}$ of 429.01 ± 24.24 ng/ml (plasma) and 83.21 ± 15.29 ng/gm (brain) indicating lesser amount of QF reaching at brain due to presence of higher positive surface charge of QF-NP (34.4 ± 1.87 mV), which was rapidly recognised by the reticuloendothelial system in blood (Md et al., 2013). As per Figure 46, amount of QF detected in the brain with QF-NP (IV) was slightly higher than QF-DS (IN), since QF-NP (IV) may have crossed BBB by opening tight junctions due to presence of CH compared to QF-DS.

![Graph showing brain concentration versus time profile following QF-NP (IN), QF-DS (IN) and QF-NP (IV) administrations.](image)

Figure 46. Brain concentration versus time profile following QF-NP (IN), QF-DS (IN) and QF-NP (IV) administrations (Data represents mean ± SD, $n = 4$).

Figure 47 depicted that the brain/blood ratio for QF-NP (IN) remained significantly higher at all the time points with respect to QF-DS (IN) and QF-NP (IV) indicating higher and prolonged retention of drug at site of action. The retention was around 2.5-5 times higher with QF-NP (IN) compared to QF-NP (IV) thus proving the superiority of CH as permeability enhancer for nose to brain delivery system. Another explanation could be presence of positively charged amino group of CH interact with the negatively charged sialic acid residue on the cell membranes of nasal mucosa thereby resulting into transient opening of tight junctions with higher permeation of drug across mucosal surface (Casettarti and Illum, 2014).
Figure 47. Brain/blood concentration ratio in rat at different time intervals following QF-NP (IN), QF-DS (IN) and QF-NP (IV) administrations
(Data represents mean ± SD, n = 4).

Various pharmacokinetic parameters are depicted in Table 20. $T_{\text{max}}$ value of 30 min ($C_{\text{max}}$: 248.19 ± 25.49 ng/ml) in plasma compared to $T_{\text{max}}$ of 15 min ($C_{\text{max}}$: 169.27 ± 20.86 ng/gm) in brain with QF-NP (IN) may be attributed to direct nose to brain transport following IN administration. The $\text{AUC}_{0\text{-}240}$ and $C_{\text{max}}$ values in the brain after IN and IV administration were in the order of QF-NP (IN) > QF-NP (IV) > QF-DS (IN), depicting significantly higher $\text{AUC}_{0\text{-}240}$ (13776.15 ± 2021.24 ng min gm$^{-1}$) with QF-NP (IN) compared to QF-NP (IV) (8152.12 ± 1835.11 ng min gm$^{-1}$) and QF-DS (IN) (6557.42 ± 726.22 ng min gm$^{-1}$). Nearly 2.1 folds higher $\text{AUC}_{0\text{-}240}$ in the brain with QF-NP (IN) compared to QF-DS (IN) could be attributed to presence of CH which reduced mucociliary clearance thereby providing longer residence time. %DTE and %DTP values were found to be 1.4 folds higher with QF-NP (374.93 ± 15.02% and 73.33 ± 4.14%) compared to QF-DS (259.14 ± 15.35% and 54.08 ± 5.17%) respectively indicating higher targeting efficiency by virtue of bioadhesion. Nasal bioavailability of QF in the brain was found to be 2 folds higher following IN administration of QF-NP (168.98 ± 9.27%) compared to QF-DS (83.15 ± 9.82%).
Table 20. Pharmacokinetic, brain targeting efficiency and direct transport percentage of QF following IN and IV administration (Data represents mean ± SD, n = 4).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tissue/organ</th>
<th>Formulation and route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>QF-NP (IN)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>Plasma</td>
<td>248.19 ± 25.49</td>
</tr>
<tr>
<td>(ng/gm)</td>
<td>Brain</td>
<td>169.27 ± 20.86</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>Plasma</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>15</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-240&lt;/sub&gt; (ng min ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Plasma</td>
<td>23458.42 ± 1672.37</td>
</tr>
<tr>
<td>(ng min gm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Brain</td>
<td>13776.15 ± 2011.24</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>Plasma</td>
<td>1.48 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>1.48 ± 0.12</td>
</tr>
<tr>
<td>DTE (%)</td>
<td>Brain</td>
<td>374.93 ± 15.02</td>
</tr>
<tr>
<td>DTP (%)</td>
<td>Brain</td>
<td>73.33 ± 4.14</td>
</tr>
<tr>
<td>Nasal bioavailability (%)</td>
<td>Plasma</td>
<td>58.15 ± 3.18</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>168.98 ± 9.27</td>
</tr>
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</table>
As shown in above Table 20, following IN administration of QF-NP, nasal bioavailability of QF in plasma (58.15 ± 3.18%) was found to be 2.9 times lower compared to brain (168.98 ± 9.27%) thereby showing larger difference among brain and plasma bioavailability. This can be explained by the fact that, part of nasally administered drug makes use of olfactory and trigeminal nerve for drug transport from nasal epithelium to brain, bypassing BBB, which may not occur in case of systemic circulation (Dhuria et al., 2010). Overall, combination of bioadhesion and paracellular transport effects had led to use of CH for the delivery of QF via IN route (Md et al., 2013).

4.4 Conclusion

The objective of the present investigation was to develop QF-NP by ionic gelation method and to evaluate its potential in enhancing the brain delivery in comparison to plain drug solution via intranasal route. Developed NP were characterized for size, zeta potential, %EE, pH, solid state characterization (DSC, FTIR and XRD), morphological parameters (SEM and TEM) and nasal diffusion study. QF-NP were successfully developed by ionic gelation method and they were found to be in the size of 130-150 nm with higher loading capacity for QF. Optimized formula of QF-NP showed average particle size, PDI, %EE and nasal diffusion of 131.08 ± 7.45 nm, 0.252 ± 0.064, 89.93 ± 3.85% and 65.24 ± 5.26% respectively. %EE of more than 85% indicated higher encapsulation of QF into CH/TPP matrix which in turns reduces the amount of dosing. XRD pattern of QF-NP showed reduction in crystallinity of QF thus indicating superior crosslinking between CH and TPP into which QF was entrapped. DSC and FTIR studies also showed reduced and/or negligible crystallinity for QF with QF-NP due to higher %EE. Morphological analysis revealed spherical NP with uniform distribution in the size range of 100-150 nm. pH value of 5.87 ± 0.11 showed neither toxicity nor structural damage on nasal mucosa upon histopathological examination indicating non-irritating nature of formulation. Significantly higher brain/blood ratio and 2 folds higher nasal bioavailability in brain with QF-NP in comparison to drug solution following intranasal administration revealed preferential nose to brain transport bypassing BBB and prolonged retention of QF at site of action. Overall in vivo biodistribution studies confirmed favourable effect of CH on modulation of tight junctions thereby suggesting the superiority of CH as permeability enhancer.
4.5 Outcome on the comparative characterization between CH-ME and QF-NP

From the above discussed two different carrier systems few observations were made on the preparation method, characterization parameters and they were compared between QF-NP and CH-ME. It was found that preparation of CH-ME was easier, quick and numbers of steps required were lesser. It was very difficult to solubilize QF while preparing QF-NP whereas with CH-ME, QF got solubilized easily and rapidly in the presence of oil, surfactant and cosurfactant. CH-ME as such was found to be stable over longer period of time while QF-NP requires additional step of lyophilisation to increase the stability of the formulation.

Characterization parameters depicted that QF-NP showed globule size of 131.08 ± 7.45 nm which was >100 and in case of CH-ME size was < 50 nm i.e. 35.31 ± 1.71 nm (Figure 48).

Ex vivo nasal diffusion showed 1.3 times higher diffusion with CH-ME when compared to QF-NP. Diffusion coefficient values for CH-ME and QF-NP were found to be $(8.32 ± 0.12) \times 10^{-4}$ cm$^2$/h and $(4.87 ± 0.102) \times 10^{-4}$ cm$^2$/h respectively, indicating 1.7 folds higher value with CH-ME. Probable explanation could be lipophilic nature of nasal mucosa which acts as rate limiting membrane in case of systems being hydrophilic in nature and thus hinders and/or slower the drug diffusion. In case of CH-ME higher diffusion could be due to lipophilic nature of the delivery system. Comparative in vivo pharmacokinetic data in rat brain between CH-ME and QF-NP is depicted in Table 21.
Table 21. Comparative pharmacokinetic parameters in rat brain between intranasally administered QF-NP and CH-ME.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QF-NP (IN)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/gm)</td>
<td>169.27 ± 20.86</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>15</td>
</tr>
<tr>
<td>AUC$_{0-240}$ (ng min gm$^{-1}$)</td>
<td>13776.15 ± 2021.24</td>
</tr>
<tr>
<td>DTE (%)</td>
<td>374.93 ± 15.02</td>
</tr>
<tr>
<td>DTP (%)</td>
<td>73.33 ± 4.14</td>
</tr>
<tr>
<td>Nasal bioavailability (%)</td>
<td>168.98 ± 9.27</td>
</tr>
</tbody>
</table>

1.4 folds higher $C_{\text{max}}$ value with CH-ME (IN) (243.19 ± 26.97 ng/gm) when compared to QF-NP (IN) (169.27 ± 20.86 ng/gm) could be attributed to smaller globule size of CH-ME. Globule size is one of the major factor for intranasal delivery of drugs to the brain, since globule size of CH-ME (35.31 ± 1.71 nm) was lower than that of axons in the filia olfactoria, leading to intracellular axonal transport into olfactory neurons by endocytic mechanism and thus resulting into enhanced therapeutic effect as reported by Bshara et al., 2014. Another explanation behind lower $C_{\text{max}}$ value in brain with QF-NP could be attributed to MCC and lower viscosity which might have cleared the instilled formulation rapidly, as a result of which $C_{\text{max}}$ in plasma was also found to be lesser. Hence it can be assumed that addition of mucoadhesive agent plays a key role in enhancing the viscosity thereby improving the brain uptake. DTE and DTP values were significantly higher for CH-ME. Nasal bioavailability was found to be 1.9 fold higher with CH-ME against QF-NP. This could be ascribed to dual nature of ME system which has both hydrophilic and lipophilic domains and thus can be transported easily through biological membranes which was not the case with NP. Additionally presence of CH altered the tight junction morphology and enhanced paracellular transport for QF. However in case of CH-ME drug is solubilized into mixture of oil (Capmul MCM EP),
surfactant (Tween80 and Labrasol) and cosurfactant (Transcutol-P), all of which are having permeation enhancing effect and reported to overcome biological obstacles upto some extent. As a result of it higher nasal diffusion, DTP and bioavailability was found with CH-ME over QF-NP. Since in case of QF-NP the drug was embedded into the matrix formed by crosslinking between positively charged amino group of CH and negatively charged phosphate group of TPP, the diffusion of QF depends upon the erosion of matrix and other physiological conditions.

Overall from the afore mentioned parameters it was concluded that the brain delivery of QF was superior with intranasal administration of CH-ME when compared to QF-NP, thus proving the potential of MME as a choice of delivery system in brain delivery of therapeutics via noninvasive intranasal route.