DEVELOPMENT AND CHARACTERIZATION OF QUETIAPINE LOADED CHITOSAN NANOPARTICLE


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ABSTRACT
Quetiapine Fumarate (QTF) belongs to the class of second generation (atypical) antipsychotic used in the treatment of psychotic disorders. The atypical antipsychotic drugs electively bind to central dopamine D$_2$ and serotonin (5-HT$_2$C) receptor, appear more effective on the associated negative symptoms of schizophrenia and have a lower propensity of causing extra pyramidal symptoms. It has poor bioavailability due to hepatic first-pass metabolism and low permeability into the brain due to efflux by P-glycoproteins. The present investigation aimed to prepare a nanoparticulate drug delivery system of QTF using chitosan for direct nose-to-brain delivery to provide brain targeting and sustained release. The Quetiapine-loaded chitosan nanoparticles (CS-NPs) were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP), and characterized by entrapment efficiency, particle size, zeta potential, and SEM studies. The NP was evaluated for in vitro release, and ex vivo diffusion studies. The NP were 237.5 nm in diameter and had entrapment efficiency 94.81±2.11%. In vitro drug release showed sustained release (81.02±0.0035 % after 48 h), following the non-Fickian diffusion-based release mechanism. Ex vivo diffusion through goat nasal mucosa showed 63.799% of drug diffusion in 48h from NP.. These results proved that QTF could be transported directly to the brain after Intra Nasal delivery, enhanced drug concentration in the brain and would therefore be effective in improving the treatment of Schizophrenia.

Key Words: Quetiapine; Chitosan; TPP; Nanoparticles; Intra-Nasal, Ex-Vivo Study.

INTRODUCTION
Schizophrenia is a chronic and severe brain disease occurs due to alteration in neurotransmitters. People with schizophrenia often suffer terrifying symptoms such as hearing internal voices not heard by others, or believing that other people are reading their minds, controlling their thoughts, or plotting to harm them$. These symptoms may leave them fearful and withdrawn. Their speech and behavior can be so disorganized that they may be incomprehensible or frightening to others. Schizophrenia is caused by an overactive dopamine system in the brain; excessive dopamine and reduced striatal activity can disrupt all aspects of motor, cognitive and emotional functioning and can
result in an acute schizophrenic psychosis. An excessive dopamine concentration in the
brain of people with a schizophrenic disorder was originally thought to be associated with
increased activity of the D2 class of dopamine receptors in the prefrontal cortex.

**Quetiapine Fumarate (QTF)** belongs to the class of second generation (atypical)
antipsychotic used in the treatment of psychotic disorders. The atypical antipsychotic
drugs electively bind to central dopamine D$_2$ and serotonin (5-HT$_2$C) receptor, appear
more effective on the associated negative symptoms of schizophrenia and have a lower
propensity of causing extra pyramidal symptoms. It has poor bioavailability due to
hepatic first-pass metabolism and low permeability into the brain due to efflux by P-
glycoproteins.

The development of effective drug delivery systems that can transport and deliver a drug
precisely and safely to its site of action is becoming a highly important research area for
pharmaceutical researchers. The use of NPs has been regarded as having great potential
for the targeted drug delivery. The term NPs refers to well defined particles ranging in
size approximatively from 10 to 1000 nm (1μm) with a core-shell structure (nanocapsules) or a continuous matrix structure (nanospheres).

CS-NPs have gained more attention as drug delivery carriers because of their better
stability, low toxicity, simple and mild preparation method, and providing versatile routes
of administration. Their sub-micron size not only suitable for parenteral application, but
also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa,
which are non-invasive route$^{40}$. It also facilitated by CS absorption enhancing effect.

**MATERIALS AND METHODOLOGY**

**Materials :**

<table>
<thead>
<tr>
<th>Category</th>
<th>Name of Materials</th>
<th>Name of Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Quetiapine</td>
<td>Gift sample from Zydustr cadila</td>
</tr>
<tr>
<td>Polymer</td>
<td>Chitosan</td>
<td>Balaji drugs</td>
</tr>
<tr>
<td>Ion gelating agent</td>
<td>TPP</td>
<td>Krishna chem</td>
</tr>
<tr>
<td>Solvent</td>
<td>Acetic acid</td>
<td>Sulab chem</td>
</tr>
</tbody>
</table>

**Preparation Of Nanoparticles By Ionotropic Gelation Method:**

Chitosan was dissolved in aqueous acetic acid (2%) at fixed concentration 5mg/ml.
Under magnetic stirring at room temperature, 12ml of TPP solution of different
concentration (2mg/ml, 4mg/ml, 6mg/ml) was added dropwise using syringe needle to 30ml of chitosan solution containing drug (P:D – 1:1, 1:0.5, 1:0.25). The stirring was continued for about 30 minutes. The resultant nanosuspension was centrifuged till particles get settled down and clear supernatant solution appears. The particles obtained after centrifugation are finally lyophilized.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug (mg)</th>
<th>Polymer (mg)</th>
<th>P:D ratio</th>
<th>TPP (mg/ml)</th>
<th>Acetic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>37.5</td>
<td>150</td>
<td>1 : 0.25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>75</td>
<td>150</td>
<td>1 : 0.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>F3</td>
<td>150</td>
<td>150</td>
<td>1 : 1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>F4</td>
<td>37.5</td>
<td>150</td>
<td>1 : 0.25</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F5</td>
<td>75</td>
<td>150</td>
<td>1 : 0.5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F6</td>
<td>150</td>
<td>150</td>
<td>1 : 1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F7</td>
<td>37.5</td>
<td>150</td>
<td>1 : 0.25</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>F8</td>
<td>75</td>
<td>150</td>
<td>1 : 0.5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>F9</td>
<td>150</td>
<td>150</td>
<td>1 : 1</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

**Drug Entrapment:**

In this method, analysis of QTF from CS-NPs was done by dissolving CS-NPs dispersion in 0.1N HCl. The dispersion was then allowed to stand for overnight for complete dissolution of drug. Then, absorbance was taken against 0.1N HCl as a blank on UV-Visible Spectrophotometer (at 290 nm). The % entrapment was calculated by using following equation.

\[
\text{% Drug Entrapment} = \left( \frac{\text{Drug entrapped in NPs}}{\text{Total drug taken}} \right) \times 100
\]

**Particle Size and Zeta Potential:**

The average diameter (Z-AVE), and zeta potential of optimized Quetiapine nanoparticle was determined by photon correlation spectroscopy (PCS) (Zeta- sizer Nano ZS, Malvern Instruments, UK) at room temperature. Nanosuspension was added to the sample
dispersion unit (deionized water) and stirred at 2000 rpm with magnet in order to reduce
the inter-particulate aggregation. The samples were adequately diluted with deionized
water and placed in an electrophoretic cell. The average particle size was measured after
performing the experiment in triplicates.

**Scanning Electron Microscopy (SEM):**

The morphology of optimized batch Quetiapine nanoparticle was determined using
scanning electron microscopy (SEM). Prior to examination, the samples were mounted
on to metal stubs using a double sided adhesive tape under vacuum. The scanning
electron microscope was operated at an acceleration voltage of 20 Kv.

**In-Vitro Drug Diffusion:**

In vitro release of QTF NPs was evaluated by the dialysis bag diffusion technique
reported by Yang et al. The studies of release of QTF from NP were performed in
phosphate buffered (PBS) (pH 6.4) to create a perfect sink condition, since has limited
solubility in buffer. The aqueous nanoparticulate dispersion equivalent to 1 mg of QTF
was placed in a dialysis bag, which was previously soaked overnight in water,cleaned
next morning and sealed at both ends. The dialysis bag was immersed in the receptor
compartment containing 50 ml of PBS (pH 6.4), which was stirred and maintained at 37
± 2 _C. The receptor compartment was covered to prevent the evaporation of release
medium. Samples were withdrawn at regular time intervals, and the same volume was
replaced by fresh release medium. The samples were analyzed spectrophotometrically at
290 nm. All the experiments were performed in triplicate and the average values were
taken.

**Ex vivo diffusion studies:**

The use of natural membranes is very important for predicting the potential drug release
characteristic. Freshly excised goat nasal mucosa was dipped immediately in phosphate
buffer (pH 6.4).Cartilages were removed properly, and the mucosal membrane was
isolated and washed with phosphate buffer (pH 6.4). Ex vivo drug diffusion study was
performed using a Franz-type diffusion cell. The tissue was stabilized under phosphate
buffer (pH 6.4) in both donor and acceptor compartments. Solution from both
compartments was removed, and the acceptor compartment was filled with fresh
phosphate buffer (pH 6.4). QTF NPs, the lyophilized QTF-loaded NP (equivalent amount
of 1 mg drug) reconstituted with phosphate buffer (pH 6.4) were used. Samples from the receptor phase were withdrawn at periodic time intervals, and analyzed using a UV–Visible spectrophotometer at 290 nm. Each removed sample was replaced by an equal volume of diffusion medium. Percentage drug diffusion was calculated from the calibration curve of QTF in phosphate buffer (pH 6.4).

**DRUG RELEASE KINETIC**

The data obtained from drug release studies were fitted to various models like- Zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell cube root models to understand the mechanism of drug release from the QTF.

**STABILITY STUDIES:**

The nanoparticles containing the batch of formulations viz F-6 was subjected to the accelerated stability testing to find their stability and efficacy. Each formulation was divided into three portions of which, one portion was kept at room temperature, second at 45°C and third at 4°C for a period of two month. At 15 days intervals, samples were determined spectrophotometrically.

**RESULT AND DISCUSSION:**

**PREFORMULATION STUDY:**

**Organoleptic properties:**

The drug sample was evaluated for its color and odor and appearance.

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Parameter</th>
<th>Quetiapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>White to Off white</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>3</td>
<td>Appearance</td>
<td>Crystalline powder</td>
</tr>
</tbody>
</table>

**Determination of melting point:**

<table>
<thead>
<tr>
<th>Reported Melting Point</th>
<th>Observed Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>172 – 174 °C</td>
<td>173 -175 °C</td>
</tr>
</tbody>
</table>
Solubility Studies:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility in mg/ml</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>3.8</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>35.6</td>
<td>Soluble</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>16.8</td>
<td>Soluble</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>28.4</td>
<td>Soluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>31.4</td>
<td>Soluble</td>
</tr>
<tr>
<td>pH 4.5 acetate buffer</td>
<td>6.1</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>pH 6.4 PBS</td>
<td>2.6</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>pH 7.4 PBS</td>
<td>2.8</td>
<td>Slightly soluble</td>
</tr>
</tbody>
</table>

**ANALYTICAL METHOD:**

**Calibration curve of Drug**

Linearity was observed between 10-40 μg/ml and therefore the drug obeys Beer's law in the range of 10-40 μg/ml.
EVALUATION OF NANOPARTICLES:
% yield, Drug loading and Entrapment Efficiency of QTF nanoparticles:
Prepared nanoparticles were evaluated by percentage yield, drug loading, entrapment efficiency and In-vitro release studies. All the batches (F1-F9) shows significant %yield (64.39 ± 3.80 – 87.47 ± 4.76) and drug loading (23.96 ± 4.8 – 61.01 ± 1.4) but the entrapment efficiency was vary from batch F1-F9.
Based on these results, F6 batch shows the significant entrapment efficiency (94.81±2.11%) with P:D ratio (1:1) and TPP concentration (4 mg/ml). The drug loading of F6 batch was found to be 58.67 ± 1.5 , % yield was found to be 86.22 ± 1.4 and the in-vitro release was 77.427 ± 0.0027% in 48 hr.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Yield (% w/w)*</th>
<th>Drug loading (%±SD)*</th>
<th>Entrapment efficiency (%±SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>72.18±1.67</td>
<td>54.89±2.1</td>
<td>23.70±1.4</td>
</tr>
<tr>
<td>F2</td>
<td>81.22±1.09</td>
<td>61.01±1.4</td>
<td>33.08±4.19</td>
</tr>
<tr>
<td>F3</td>
<td>64.39±3.80</td>
<td>57.34±3.9</td>
<td>77.35±2.34</td>
</tr>
<tr>
<td>F4</td>
<td>86.33±2.34</td>
<td>56.23±1.7</td>
<td>43.97±4.78</td>
</tr>
<tr>
<td>F5</td>
<td>80.44±2.49</td>
<td>43.37±3.9</td>
<td>81.71±3.21</td>
</tr>
<tr>
<td>F6</td>
<td>86.22±1.4</td>
<td>58.67±1.5</td>
<td>94.81±2.11</td>
</tr>
<tr>
<td>F7</td>
<td>87.47±4.76</td>
<td>57.34±4.1</td>
<td>10.62±2.18</td>
</tr>
<tr>
<td>F8</td>
<td>74.18±3.24</td>
<td>41.86±2.8</td>
<td>16.48±3.33</td>
</tr>
<tr>
<td>F9</td>
<td>70.22±3.12</td>
<td>23.96±4.8</td>
<td>70.49±1.42</td>
</tr>
</tbody>
</table>

Surface Morphology:
Surface morphology was analysed using scanning electron microscope and it confirmed that the particles were almost spherical in shape.
Particle Size:

Size Distribution Report by Intensity

Sample Details

Sample Name: F6
SOP Name: mansettings.nano
General Notes:

File Name: Sumandeep-Mahesh
Dispersant Name: Water
Record Number: 1
Dispersant Ri: 1.330
Material Ri: 1.59
Viscosity (cP): 0.6872
Material Absorption: 0.010
Measurement Date and Time: Tuesday, April 30, 2013 11:37...

System

Temperature (°C): 25.0
Count Rate (kcps): 147.4
Cell Description: Disposable sizing cuvette

Duration Used (s): 80
Measurement Position (mm): 4.65
Attenuator: 10

Results

Size (d.nm): 237.5
% Intensity: 100.0
Width (d.n.m): 11.40

Peak 1: 215.0
Peak 2: 0.000
Peak 3: 0.000

Z-Average (d.n.m): 237.5
Pdi: 0.570
Intercept: 0.876

Result quality: Good

Size Distribution by Intensity
Zeta Potential:

**Zeta Potential Report**

**Sample Details**
- **Sample Name:** a-1 zeta 1
- **SOP Name:** mansettings.nano
- **General Notes:**

**File Name:** a-1.dts
- **Dispersant Name:** Water
- **Record Number:** 7
- **Dispersant RI:** 1.330
- **Date and Time:** Tuesday, April 30, 2013 12:15:21
- **Viscosity (cP):** 0.8872
- **Dispersant Dielectric Constant:** 78.5

**System**
- **Temperature (°C):** 25.0
- **Zeta Runs:** 100
- **Count Rate (kcps):** 6.5
- **Measurement Position (mm):** 2.00
- **Cell Description:** Clear disposable zeta cell
- **Attenuator:** 11

**Results**

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta Potential (mV)</td>
<td>27.198</td>
</tr>
<tr>
<td>Zeta Deviation (mV)</td>
<td>6.71</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>0.698</td>
</tr>
<tr>
<td>Peak 1 (mV)</td>
<td>27.198</td>
</tr>
<tr>
<td>Peak 2 (mV)</td>
<td>0.00</td>
</tr>
<tr>
<td>Peak 3 (mV)</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean (%)</td>
<td>100</td>
</tr>
<tr>
<td>Width (mV)</td>
<td>5.61</td>
</tr>
</tbody>
</table>

**Result quality:** Good

**Zeta Potential Distribution**

![Zeta Potential Distribution Graph](image-url)
In-vitro Drug Release studies:
The *in vitro* drug release of drug QTF from the various nanoparticles formulations was carried out by using dialysis method in 6.4 pH phosphate buffer for 48 h. The cumulative percentage release of Quetiapine from the prepared nanoparticles varied from $59.79\pm0.0027$ to $81.02\pm0.0035$ % which depended upon the drug concentration in the prepared formulations for 48 h.
**Release profile of batch F7 – F9.**

**Ex- VIVO STUDIES:**

The *ex-vivo* drug release of drug QTF from the nanoparticles formulation (F6) was carried out by using Franz diffusion cell in 6.4 pH phosphate buffer for 48 h. The cumulative percentage release of Quetiapine from the prepared nanoparticles was found to be 63.799±0.0035 % which depended upon the drug concentration in the prepared formulations for 48 h.

**Ex-vivo Studies**

Ex- vivo studies of F6
RELEASED KINETICS:

The data obtained from the *ex-vivo* release studies were fitted to various kinetic models. Higher $r^2$ values obtained from zero order and Higuchi models revealed that the drug release was zero order diffusion controlled. The $n$ value obtained from Korsmeyer-Peppas model confirmed non-Fickian type drug release from the nanoparticles.
Stability Studies:
Stability studies of QTF loaded nanoparticles for optimized batch (F6) were conducted over a period of 2 months. Drug stability in the nanoparticles formulations was assessed by calculating the % drug remaining of stored formulation. There was no effective change in the QTF content in the formulation stored at 25°±2°C/60%±5% RH at the end of 60 days of stability studies. However, the samples kept at 40°±2°C/75%±5% RH, significant reduction in amount of QTF was detected at the end 60 days.

Table: Stability studies of batch F6 stored at 25° C ± 2° C (60%± 5%RH)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Tested after time (days)</th>
<th>Physical appearance</th>
<th>% of Drug Remaining± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch F6</td>
<td>15</td>
<td>No change</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>No change</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>No change</td>
<td>99.59 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>No change</td>
<td>98.55 ± 0.05</td>
</tr>
</tbody>
</table>

Table: stability studies of formulation batch F6 stored at 4° C ± 2° C

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Tested after time (days)</th>
<th>Physical appearance</th>
<th>% of Drug Remaining ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch F6</td>
<td>15</td>
<td>No change</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>No change</td>
<td>99.62 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>No change</td>
<td>98.57 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>No change</td>
<td>98.49 ± 0.62</td>
</tr>
</tbody>
</table>

Table: stability studies of batch F6 stored at 40° C ± 2° C (75%± 5%RH)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Tested after time (days)</th>
<th>Physical appearance</th>
<th>% of Drug Remaining ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch F6</td>
<td>15</td>
<td>No change</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>No change</td>
<td>98.99 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>No change</td>
<td>98.51 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>No change</td>
<td>97.46 ± 0.05</td>
</tr>
</tbody>
</table>
CONCLUSION
From results, it can be concluded that the prepared quetiapine loaded nanoparticle was capable of exhibiting sustained release over a period of 48 hr. This may reduce concentration of drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability, and increase the effectiveness of the drug. The Ex-vivo results obtained shown effective permeation.

Future work proposed:
- In-vivo studies
- Scale-up studies of the ideal formulation
- Bioavailability studies
- Clinical trials if the earlier works yield encouraging results

REFERENCES


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