Study of Formulation and Evaluation of Chitosan Nanoparticles of Metformin in Nano Drug Delivery System

Aashish Pal¹ and Prince Shivhare ²

1, Radharaman Institute of Pharmaceutical Science, Bhopal, (M.P.) - India
2, Mahankal Institute of Pharmaceutical Science, Ujjain, (M.P.) - India

Abstract
Nanotechnology is considered as a promising area to develop targeted drug delivery systems using particulate systems as carrier for small and large molecules. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and bio degradability, and can be readily modified as a new drug delivery system. They have attracted increasing attention for their wide applications in loading protein drugs, gene drugs and anticancer chemicals drugs, and also provide versatile routes of administration including oral, nasal, intravenous and ocular. First part of the research is concerned with the cancer/diabetes treatment with nanoparticles. The subsequent section covers with characterizes of chitosan methods of targeting the cancer and applications.

Key Words: Nanoparticles, Metformin, Chitosan

Introduction
Metformin is a frequently used medication for patients with type-II diabetes mellitus (DM) that has received increased attention, because of a study from pharmacy and disease databases showing decreased cancer in individuals taking metformin. Metformin inhibits the growth of breast and prostate cancers cell lines. Various biologically active agents, such as antibiotics, contraceptives, enzymes, anticancer drugs, have been introduced to controlled release matrices. Chitosan is one of such biopolymer, reported to be non-toxic and bioabsorbable and has been explored for the release of many drugs. Chitosan is a fiber-like substance derived from chitin, a homopolymer of β-(1→4)-linked N-acetyl-D-glucosamine. Chitin is the second most abundant organic compound in nature after cellulose. Chitin is widely distributed in marine invertebrates, insects, fungi, and yeast. Chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules.

Material and Methods

Chemical and working equipment used
All chemicals and solvents were purchased from RFCL Ltd, New Delhi and HiMedia Laboratories Pvt. Ltd. Mumbai were of AR-grade purity. Metformin HCl was obtained as gift sample from Ipca Laboratories, Ratlam (M.P.), All reactions are carried out at laboratory Condition. Melting points were determined with capillary MP Apparatus; FT-IR spectra were recorded on a Bruker Germany. UV/Visible-1800 spectrophotometer were recorded on SHIMAZDU Japan, Mechanical stirrer, cooling centrifuge and separating funnel were recorded on remi India Pvt. Ltd Mumbai.

Preformulation studies
Preformulation studies for the selected drug Metformin HCl include test for identification (examination of melting point determination, IR spectroscopy, determination of absorption maxima), solubility studies and determination of partition coefficient.

Drug identification test
Melting point determination
A small quantity of powder was placed into a fusion tube. The tube was placed in the melting point determining apparatus. The temperature of the apparatus was gradually increased and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

IR Spectra of metformin
FT-IR spectroscopy was done using a Bruker-alpha FT-IR spectrophotometer and the KBr pellet method in the 4000-400 cm⁻¹ region at 4 cm⁻¹ resolution. The
sample were ground with KBr into fine powder by a pestle and mortar, the weight percent of sample in the KBr pellet was kept between 10%. The infrared spectrum of Metformin Hydrochloride was obtained and was compared with IP 1996.

**Solubility study**
Solubility may be defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. The solubility of Metformin HCl was tested in various solvents. A definite quantity (10 mg) of drug was dissolved in 10 ml of each investigated solvent at room temperature. The solubility was observed only by visual inspection.

**Quantitative estimation of drug**
**Determination of Absorption Maximum (λmax) of Metformin**
Absorption maximum was determined by dissolving 100 mg of Metformin HCl in 100 ml of distilled water. From this stock solution, 1 ml solution was added to the 10 ml of volumetric flask and volume was made up to 10 ml with distilled water. The solution was scanned in the range of 200 – 400 nm using Shimadzu- 1800 UV/Visible spectrophotometer. The scan was recorded in Figure 3.3.

**Preparation of Standard Curve**
**Preparation of Standard Stock Solution of Metformin Hydrochloride**
Metformin Hydrochloride (100 mg) was dissolved in 100 ml of distilled water to prepare stock solution with concentration of 1000 µg/ml.

**Preparation of Dilution**
For the preparation of calibration curve, a series of dilution with concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml were prepared by taking aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml of stock solution (1000 µg/ml) and diluted up to 10 ml with distilled water in 10 ml volumetric flask.

**Partition coefficient determination**
The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phase at equilibrium.

\[
P_{o/w} = \frac{C_{oil}}{C_{water}}\text{ equilibrium}
\]
Partition coefficient is a measure of drugs lipophilicity and an indication of its ability to cross biomembrane. The partition coefficient of Metformin HCl was determined in n-octanol: water system. Accurately weighed Metformin HCl (10 mg) was added to 10.0 ml each of n-octanol and aqueous phase. The mixture was put on mechanical shaker for 24 hours until equilibrium was reached. Phases were separated in a separating funnel and the aqueous phase was analyzed for amount of drug after appropriate dilution by UV spectrophotometer.

**Procedure**
10 ml of n-octanol and 10 mg of the metformin hydrochloride with 10 ml of water was taken in a separating funnel and allowed to stand for 24 hrs on mechanical shaker. After 24 hrs, the aqueous layer was separated out and measured absorbance after appropriate dilution by UV spectroscopy. 10 ml of n-octanol and 10 mg of the metformin hydrochloride with 10 ml of Phosphate buffer saline (pH 7.4) was taken in a separating funnel and allowed to stand for 24 hrs on mechanical shaker. After 24 hrs the aqueous layer was separated out and measured absorbance by UV spectroscopy.

**Drug polymer interaction study**
Drug polymer interaction study was done by incubating the drug solution with excess of polymer and recording the change in absorbance value. The absorption obtained for metformin was compared with absorption reading obtained for metformin - polymer combination.

**Preparation of chitosan nanoparticle**
Chitosan nanoparticle was prepared using the ionotropic gelation method as reported by Tiyaboonchai et al., 2010, with modifications. Briefly, Chitosan (40 mg) was allowed to swell in glacial acetic acid, the concentration range used for chitosan was 0.1%-0.75% w/v and that for glacial acetic acid was 1.5%v/v. Chitosan and acetic acid mixture was continuously stirred for 3 hours on a three blade Mechanical stirrer (Remi, India). Post three hours of stirring, tri-polyphosphate (0.10%-0.75% w/v) was added drop wise in chitosan solution. After that this chitosan mixture was centrifuge at 20000 rpm for 20 minutes, chitosan nanoparticle settle down in the centrifugal tube, it was washed carefully with water and separate out.

**Results and Discussion**
**Drug identification test**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Sample</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>220</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>222</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>225</td>
</tr>
</tbody>
</table>

Melting point of Metformin HCl was found to 220-225°C.

**FT- IR Spectra**
The IR spectrum of Metformin HCl (Sample) & IR spectrum of Metformin HCl (reference, IP .1996) are shown in fig 3.2 & 3.2. The characteristic absorption bands are recorded in Table 2.
Table 2: Interpretation of IR spectrum of Metformin HCl

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Functional Group</th>
<th>Range</th>
<th>Wave number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C-H Stretching</td>
<td>3500-3000 cm(^{-1})</td>
<td>3372.5336</td>
</tr>
<tr>
<td>2</td>
<td>N-H Stretching</td>
<td>3600-3200 cm(^{-1})</td>
<td>3295.7108</td>
</tr>
<tr>
<td>3</td>
<td>C-H Stretching</td>
<td>3200-3000 cm(^{-1})</td>
<td>3174.0850</td>
</tr>
<tr>
<td>4</td>
<td>O–H Stretching</td>
<td>3000-2500 cm(^{-1})</td>
<td>2692.4447</td>
</tr>
<tr>
<td>5</td>
<td>C≡C Stretching</td>
<td>2500-2000 cm(^{-1})</td>
<td>2214.0358</td>
</tr>
<tr>
<td>6</td>
<td>N–O asymmetric stretching</td>
<td>2000-1500 cm(^{-1})</td>
<td>1574.5393</td>
</tr>
<tr>
<td>7</td>
<td>C–N Stretching</td>
<td>1500-1000 cm(^{-1})</td>
<td>1416.8613</td>
</tr>
<tr>
<td>8</td>
<td>=C–H bending</td>
<td>1000-500 cm(^{-1})</td>
<td>934.9509</td>
</tr>
</tbody>
</table>
Quantitative estimation of drug
The λmax was found to be 233 nm (Figure 3.3).

Preparation of calibration curve
The absorbance value of standard concentration of 1-10 µg/mL were plotted (Figure 3.3) and linearity was observed with an $r^2 = 0.9997$ for Metformin HCl at 233 nm.

**Standard Calibration Curve of Metformin HCl**

Calibration curve for Metformin Hydrochloride in Water at 233 nm had an absorbance of 0.0809 µg/mL with a coefficient of correlation of 0.997.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>0.147</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>0.197</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>0.265</td>
</tr>
<tr>
<td>4.</td>
<td>4</td>
<td>0.361</td>
</tr>
<tr>
<td>5.</td>
<td>5</td>
<td>0.451</td>
</tr>
<tr>
<td>6.</td>
<td>6</td>
<td>0.537</td>
</tr>
<tr>
<td>7.</td>
<td>7</td>
<td>0.599</td>
</tr>
<tr>
<td>8.</td>
<td>8</td>
<td>0.695</td>
</tr>
<tr>
<td>9.</td>
<td>9</td>
<td>0.772</td>
</tr>
<tr>
<td>10.</td>
<td>10</td>
<td>0.855</td>
</tr>
</tbody>
</table>

Values represent Mean ±S.D, n=3
Fig. 3.5: Calibration curve of Metformin HCl in distilled Water

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drug + Excipients</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drug solution (5µg/ml)</td>
<td>0.451</td>
</tr>
<tr>
<td>2.</td>
<td>Drug solution + Chitosan (5µg/ml)</td>
<td>0.453</td>
</tr>
</tbody>
</table>

**Drug polymer interaction study**

**Table 3.4**: Interaction of Metformin HCl with excipients used in the formulations

References