FORMULATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF KETOPROFEN USING DIFFERENT POLYMERS

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ABSTRACT

Microspheres have been explored extensively for their use in the field of drug delivery and various polymers have been utilized for the formulation of the microspheres, which in turn have been assessed for different purposes. Microspheres are one of the multiple unit dosage forms. Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. Microspheres are potential drug delivery carrier systems in the segment of novel drug delivery and are prepared using assorted polymers. Ketoprofen is a non-steroidal anti-inflammatory drug. The chemical name for Ketoprofen is 2-(3-benzoylphenyl)-propionic acid. It has a pKa of 5.94 in methanol: water (3:1) and an n-octanol: water partition coefficient of 0.97 (buffer pH 7.4). Ketoprofen is a white or off-white, odourless, nonhygroscopic, fine to granular powder, melting at about 95°C. It is freely soluble in ethanol, chloroform, acetone, and ether and soluble in benzene and strong alkali, but practically insoluble in water at 20°C. Ketoprofen is generally prescribed for arthritis-related inflammatory pains or severe toothaches that result in the inflammation of the gums. The present study was aimed to prepare and evaluated chitosan containing ketoprofen microspheres prepared by ionotropic gelation method for pulsatile release of drug at the part of GIT and decrease distinct tissue protection in the stomach. This method offers to prepare microspheres which are important in controlling the release rate and the absorption of aceclofenac from the intestinal region. Variation in polymer concentration was studied systematically for their influence on the encapsulation efficacy, particle size and in vitro drug release.
KEYWORDS: Ketoprofen, Aceclofenac, Microspheres.

INTRODUCTION
Microsphere small spherical particles, with diameters in the micrometer range and can be manufactured from various natural and synthetic materials.\(^1\)\(^-\)\(^4\) Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material.\(^5\)\(^-\)\(^7\) Ketoprofen (KP) are a non-steroidal anti-inflammatory drug (NSAID). It is widely used in medical care because of its potential on the treatment of inflammatory diseases and musculoskeletal injury, rheumatoid arthritis, osteoarthritis, and dysmenorrhea to relieve moderate pain.\(^8\) The main action of ketoprofen is the inhibition of the conversion of arachidonic acid to prostaglandins and thromboxane A2, compounds responsible for the inflammatory mechanism through the inhibition of cyclooxygenase (COX).\(^9\)\(^,\)\(^10\) Ketoprofen also acts as an inhibitor of cell growth in vitro and in vivo models of glioma tumors. Also, the characteristics of ketoprofen include quick absorption, easy metabolism and faster blood-brain hurdle crossing. The use of ketoprofen could result in an increased risk of serious side effects, abdominal pain, gastrointestinal erosions, and ulcers. The high dose of ketoprofen can cause ischemic stroke, myocardial infarction, and worsening of renal functions in humans. The various buoyant preparations include hollow microsphere (microballoons), granules powder, capsule, tablet (pills) laminated films. Based on the mechanism of buoyancy, two distinctly different types, i.e., non-effervescent and effervescent systems have been utilized in the development of FDDS. The various approaches used in and their mechanism of buoyancy are discussed in the following subsections.\(^11\)\(^-\)\(^12\)

MATERIAL AND METHODS
Ketoprofen was purchased from Ranbaxy Fine Chemical Ltd., India, Mumbai, India. Acetone, light liquid paraffin and petroleum ether were All other chemicals and reagents used were obtained from S.D. Fine chem, Mumbai, All other reagents and solvents used were of analytical grade.

Preparation of standard curve
Preparation of Standard Curve of Ketoprofen with 0.1 N HCl: 100 mg of Ketoprofen was accurately weighed and dissolved in a small portion of methanol and make the volume with 0.1 N HCl in a 100 ml volumetric flask then the volume was made up to 100 ml with 0.1 N.
HCl. This was the primary stock solution, contained concentration of 1000 μg/ml. From this primary stock solution 10 ml was accurately pipetted out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with 0.1 N HCl which contained the concentration of 100 μg/ml. From the second stock solution again 10 ml was pipette out and diluted up to 100 ml with 0.1 N HCl to get concentration of 10 µg/ml. From third stock solution aliquots equivalent to 1-10 µg was pipetted out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with 0.1 N HCl. The absorbance of these solutions was measured against the 0.1 N HCl as blank at 275 nm using UV-Visible double beam spectrophotometer.

**Preparation of chitosan microsphere of ketoprofen sodium**

Microspheres are matrix systems that contains drug throughout their structure and are potential candidates for oral controlled release. Microsphere can be defined as solid spherical particles ranging from one to 1000 μm in size. These particles consist of the drug which is the core material, and a coating material. The choice of methods for the preparation of microspheres depends on many factors such as the drug solubility, partition co-efficient, polymer composition, molecular weight etc. For instance, ionotropic gelation method may be a method of choice for the preparation of microspheres of water soluble drugs. Chitosan microspheres were prepared by ionotropic gelation method. In this method chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature. The drug Ketoprofen (1% w/v) was dissolved directly into the above prepared chitosan solution. 10 ml of this bubble free solution was dropped through a disposable syringe needle into a gently agitating 100ml of 2% (w/v) sodium tripolyphosphate solution. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were obtained which was air dried for twenty-four hours followed by oven drying for six hours at 40°C.[13]

**Evaluation of microspheres**

**Particle size analysis:**

Particle size analysis plays an important role in determining the release characteristics and chitosan property. Mean particle size for all formulation was determined by dividing the total weight size of formulation to % total weight of chitosan microspheres.
Determination of true density:
The true density of chitosan microspheres was determined by liquid displacement method using n-hexane as solvent. A pycnometer was used to determine true density. First of all, weight of pycnometer (a) was noted than 25 ml of n-hexane was added and weight (b) was noted. The pycnometer was emptied and weight amount of chitosan microspheres was added not weight (c) was noted. Now n-hexane was added to occupy the void spaces within the chitosan microspheres\[^{14}\]

Determination of tapped density:
It is the ratio between a given mass of chitosan microspheres and its volume after tapping. Tapped density of chitosan microspheres was determined by the tapping method. Accurately weighed quantity of chitosan microspheres was transferred in to a 10 ml measuring cylinder. After observing the initial volume of chitosan microspheres, the tapping was continued on a hard surface until no further change in volume was noted and the tapped density was calculated.\[^{15}\]

Percentage compressibility index:
The same tapping method was used to determine percentage compressibility index. The percentage compressibility index was calculated.\[^{15}\]

Angle of repose:
Flow property of chitosan microspheres is usually assessed by determining angle of repose of the chitosan microspheres. It is the maximum angle that can be obtained between the free chitosan surface of chitosan micro balloons heap and the horizontal plane. The angle of repose of chitosan microspheres was determined by fixed funnel method. The chitosan microspheres were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel.
The angle of repose $\theta$ was determined according to the following formula

$$\theta = \tan^{-1} \frac{h}{r}$$

Where, $h =$ height of pile; $r =$ radius of the pile formed by the chitosan microspheres.

Percentage yield:
The percentage yield of different formulations was determined by weighing the chitosan microspheres after drying. The percentage yield was calculated as follows.
Percentage Yield = \frac{\text{Total weight of chitosan microspheres}}{\text{Total weight of drug and polymer}} \times 100

**Shape and Surface characterization of chitosan microspheres by scanning electron microscopy:**

From the formulated batches of chitosan microspheres, formulation (F4) which showed an appropriate balance between the buoyancy and the percentage release were examined for surface morphology and shape using scanning electron microscope Hitachi, Japan, Trichy. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.\[16\]

**Drug entrapment:**

The various formulations of the chitosan microspheres were subjected for drug content. 50 mg of chitosan microspheres from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved with 10ml ethanol in 100ml volumetric flask and makeup the volume with 0.1 N HCl. This resulting solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 10 ml was taken out and diluted up to 100 ml with 0.1 N HCl. Again, from this solution 2 ml was taken out and diluted up to 10 ml with 0.1 N HCl and the absorbance was measured at 275 nm against 0.1 N HCl as a blank (17). The percentage drug entrapment was calculated as follows.

\[
\text{Calculated drug concentration} \times 100
\]

\[
\frac{\text{Theoretical drug concentration}}{\text{Calculated drug concentration}} \times 100
\]

\[\text{% Drug entrapment efficiency} = \frac{\text{Theoretical drug concentration}}{\text{Calculated drug concentration}} \times 100\]

**In vitro release studies:**

The drug release rate from chitosan microspheres was carried out using the USP dissolution paddle assembly. A weighed amount of microspheres equivalent to 100 mg drug were dispersed in 900 ml of phosphate buffer 6.8 maintained at 37 ± 0.5°C and stirred at 100 rpm. At preselected time intervals one ml sample was withdrawn and replaced with equal amount of phosphate buffer 6.8. The collected samples were suitably diluted with phosphate buffer 6.8 and analyzed spectrophotometrically at 275 nm to determine the concentration of drug present in the dissolution medium. The dissolution studies were repeated using phosphate buffer pH 6.8 as dissolution medium.\[17\]
**In-Vivo Anti-inflammatory study:**

It has been reported in the literatures that Ketoprofen may have therapeutic potential as anti-inflammatory agent either alone or in combination with non-steroidal anti-inflammatory drug. Therefore, the in-vivo 500 μg/kg of formulation release behavior of the best formulation F₄ was studied by measuring anti-inflammatory activity in adult male wistar rats using cotton pellet granuloma method. The experimental protocol was conducted in accordance with the internationally accepted principles for laboratory animal use and care as described by CPCSEA guideline after approval of 1698/PO/Re/S/13/CPCSEA, Patel college of Pharmacy, Bhopal (M.P.). The study was approved by institutional animal ethical committee. The male wistar rats were divided in to three groups, each group consisting of 6 animals. One group served as control, second group served as standard, received 500 μg/kg of Ketoprofen as solution in water in two divided doses orally. While third group received chitosan microspheres containing Ketoprofen orally require to release about 500 μg/kg of Ketoprofen) once daily during the experiment. The rats with an average weight of 150g were anaesthetized with ether. The cotton pellets each weighing 10 ± 1 mg were prepared and sterilized in hot air oven at 120°C for 3 hours. The abdomen was shaved cleanly, swabbed with 70% (v/v) ethanol and small incision was made in the lower abdomen of the rat. Using a blunt forceps, one sterile cotton pellet of known weight was placed in each aexilla and groin region and then incision was closed with sutures. On 8th day albino rat were sacrificed and four pellets were removed. The pellets were dried overnight at 55°C and weighed. The difference between the final weight of the pellet after drying and its initial weight was taken as the granuloma tissue weight. Results were expressed as percentage inhibition of granuloma in drug treated groups compared with the control group.[18]

**RESULT AND DISCUSSION**

**Particle size analysis:**

Particle size was determined by sieving method it plays important role in release corrected of drug from microspheres. If size of microspheres less than 500 μm so release rate of drug will be high, while microspheres range between 500μm – 1000μm, floating ability will be more and release rate will be in sustained manner. The mean particle size of microsphere was in range 613 – 869 μm.

**Drug entrapment:**

The drug entrapment efficacy of different formulations was in range of 43.14- 74.12 % w/w.
Drug entrapment efficacy slightly decrease Chitosan ratio in microspheres. This is due to the permeation characteristics of facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of microspheres.

**Percentage yield:**
Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 55.10 - 84.67%.

**True density:**
It is determined by liquid displacement method using n-hexane as solvent. The true density value of microsphere was ranges from 0.482-0.916 gm/cm$^3$ as shown in Table 8.6. The true density of microsphere was less than of gastric fluid (1.004 gm/cm$^3$) will suggest that it will exhibit good floating property.

**Tapped density:**
Tapped density was determined by tapping method. The tapped density value of different microspheres ranges from 0.201 - 0.405gm/cm$^3$ as shown in Table. The density value of microspheres was less than the density of gastric fluid (-1.004 g/cm$^3$) thereby, it will have good buoyancy property in stomach. It is determined by same tapping method and its range is 8.34 -17.45.

**Percentage compressibility index:**
The percentage compressibility value less than 20 for all formulation suggested excellent flow property.

**Angle of repose:**
Angle of repose of microspheres was determined by fixed funnel method. Angle repose of microspheres was in range of 24°.09’ - 38°.12’ as shown in Table. All formulation shown excellent flow ability as represented in term of angle of repose (<40°).

**Scanning electronic microscopy:**
Surface morphology of F8 examine at two different magnification 40X and 200X, which illustrate the smooth surface of floating microspheres and small cavity present in microsphere which is responsible for floating property.
Release kinetic:
Drug release pattern was evaluated in 0.1 N HCl and release rate of F1, F2, F3 formulations were found to be incomplete in dissolution medium. It was found that drug release rate increased by decreasing and increasing the ratio of Chitosan. Kinetics and mechanism of drug release from all formulation was evaluated on the basis of zero order, Higuchi equation and Peppa's model. Correlation coefficient \((r^2)\) and slop value for each equation was calculated from Microsoft excel. Zero order plots for all formulations were found to be linear in dissolution medium. That indicates it may follow zero order mechanism. Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Peppa’s plot was found good linear, \(n > 0.5\) for all formulations, indicated that drug release may follow anomalous diffusion. Zero order plot for F8 formulation was found to be linear in dissolution medium, it considered as a best fit for drug release. That indicates it may follow zero order mechanism.

*In-vivo* anti-inflammatory study:
Anti-inflammatory activity of F8 formulation was measured by cotton pellet granuloma method, in which inflammation and granuloma developed during period of 7 days. The effect of Ketoprofen treatment on the mean rate of granuloma was shown in Table 3. Weight of the granuloma pellet was less as compare to control and standard. This indicates that prepared microsphere exhibited better efficacy than standard preparation. It can be considered as a proof of continuous release of drug from formulation.

Stability study:
Stability study was carried out for the F8 formulation by exposing it to different temperature 5-8°C, 27°C and 45°C for 45 days. The sample was analyzed for drug content at the regular intervals. It was found that no remarkable change in the drug content of F4 formulation.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Formulation Code</th>
<th>Ketoprofen (gm)</th>
<th>Chitosan (gm)</th>
<th>Sod. TPP (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>0.1</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>0.1</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>0.1</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>0.1</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>0.1</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>0.1</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
</tr>
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</table>
Table 2: Characterization parameters of chitosan microspheres.

<table>
<thead>
<tr>
<th>Formula</th>
<th>True density (gm/cm³)</th>
<th>Tapped density (gm/cm³)</th>
<th>Percent Compressibility index</th>
<th>Angle of Repose</th>
<th>Mean particle size (µm)</th>
<th>Drug entrapment (% w/w)</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.482</td>
<td>0.201</td>
<td>8.34</td>
<td>24.09’</td>
<td>869</td>
<td>74.12</td>
<td>84.67</td>
</tr>
<tr>
<td>F2</td>
<td>0.513</td>
<td>0.22</td>
<td>9.67</td>
<td>26.22’</td>
<td>826</td>
<td>71.56</td>
<td>81.53</td>
</tr>
<tr>
<td>F3</td>
<td>0.584</td>
<td>0.256</td>
<td>10.56</td>
<td>27.98’</td>
<td>800</td>
<td>67.23</td>
<td>76.89</td>
</tr>
<tr>
<td>F4</td>
<td>0.647</td>
<td>0.279</td>
<td>11.23</td>
<td>29.38’</td>
<td>790</td>
<td>64.76</td>
<td>72.56</td>
</tr>
<tr>
<td>F5</td>
<td>0.672</td>
<td>0.301</td>
<td>13.89</td>
<td>32.09’</td>
<td>762</td>
<td>60.01</td>
<td>70.34</td>
</tr>
<tr>
<td>F6</td>
<td>0.711</td>
<td>0.356</td>
<td>12.87</td>
<td>35.61’</td>
<td>758</td>
<td>55.38</td>
<td>68.03</td>
</tr>
<tr>
<td>F7</td>
<td>0.852</td>
<td>0.378</td>
<td>16.23</td>
<td>36.34’</td>
<td>664</td>
<td>49.47</td>
<td>59.44</td>
</tr>
<tr>
<td>F8</td>
<td>0.916</td>
<td>0.405</td>
<td>17.45</td>
<td>38.12’</td>
<td>613</td>
<td>43.14</td>
<td>55.1</td>
</tr>
</tbody>
</table>

Table 3: Indicates Anti-inflammatory activity measurement.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Treatment</th>
<th>Dose (µg/kg)</th>
<th>Weight of dry cotton pellet granuloma (mg)</th>
<th>Weight of dry granuloma (mg)</th>
<th>Percentage decreases in granuloma (%) Before</th>
<th>After*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>10.1</td>
<td>84.72</td>
<td>74.62</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>500</td>
<td>10.2</td>
<td>67.98</td>
<td>57.73</td>
<td>22.63</td>
</tr>
<tr>
<td>3</td>
<td>F8 formulation</td>
<td>500</td>
<td>10.1</td>
<td>51.17</td>
<td>40.07</td>
<td>45.49</td>
</tr>
</tbody>
</table>

Table 4: Stability Study Data for F4 Formulation.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Days</th>
<th>Percent drug residual at 5-8°C</th>
<th>Percent drug residual at 27±2°C</th>
<th>Percent drug residual at 45±2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100±00</td>
<td>100±00</td>
<td>100±00</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>99.6±0.015</td>
<td>99.9±0.003</td>
<td>99.4±0.041</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>99.5±0.013</td>
<td>99.8±0.027</td>
<td>99.2±0.036</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>99.4±0.15</td>
<td>99.6±0.012</td>
<td>99.1±0.02</td>
</tr>
</tbody>
</table>

* Values are mean ± S.D.

Figure 1: Standard Curve in Phosphate Buffer pH 6.8.
SUMMARY AND CONCLUSION

In the present study, microsphere of Ketoprofen, mean particle size range for all formulation was varied from 613.74 to 869.1 μm, due to change in drug and polymer ratio. Drug entrapment of all formulation was found in range of 60.14 to 75.12% w/w and its efficiency slightly decreases with increasing the HPMC content. Angle of repose (<40) for all formulation showed excellent flowability. Shape of the microsphere was found to be spherical by SEM study. Percent drug release rate of F1, F2, F3 formulations (89.1%, 87.51%, 83.16%) in 5-6 hours, which is incomplete drug release. F4, F5, F6 formulations showed high release rate (98.32%, 92.12, 89.23%) in 10 hours and F7, F8 formulations showed high release rate (91.87%, 87.23%) in 12 hours. The in-vitro release data was applied to various kinetic models to predict the drug release kinetic mechanism. The zero order plots for all formulation were found linear in dissolution medium. Result shows that, drug release rate may follow zero order mechanism. Higuchi and Peppas plot were found good linear,
which indicates diffusion may be the mechanism of drug release and n>0.5, that indicated drug release may follow anomalous diffusion. In-vivo anti-inflammatory efficacy was studied for F8, using cotton pellet granuloma method. It shown better efficacy compared to standard preparation which can be considered as continuous release of drug from formulation. In stability study, there was no remarkable change in content of F8 formulation during 45 days in which it was stored at various temperatures. The stability study was carried out at room temperature and at 45 0C and 75% RH for 45 days. The results showed that, non-significant difference was observed between the release pattern of fresh and stored microspheres. The optimized multiple-unit microspheres delivery system is expected to provide clinicians with a new choice of an economical, safe and more bioavailable formulation.

REFERENCES


