DESIGN AND EVALUATION OF KETOPROFEN LOADED ALBUMIN MICROSPHERES

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ABSTRACT

To design and formulate Ketoprofen loaded albumin microspheres for effective management of chronic pain without side effects associated with NSAIDs, Ketoprofen was encapsulated within bovine serum albumin microspheres for sustained delivery for long duration of time. The microspheres were prepared by solvent evaporation technique. The microspheres were found to have incorporation efficiency of 48% to 79%. The effect of albumin concentration was evaluated with respect to entrapment efficiency, particle size, surface characteristics and in vitro release behaviours. The results of FTIR spectral showed that there was no significant interaction between the drug and polymer. These results indicated that Ketoprofen loaded albumin microspheres could be prepared providing a sustained release property. Albumin microspheres showing smooth surface were confirmed by Scanning Electron Microscopic (SEM) study. The mean particle size and entrapment efficiency were found to be varied by changing various formulation parameters. The in vitro release profile could be altered significantly by changing various formulation parameters to give a sustained drug release from the microspheres. The microspheres were in the suitable particle size range of 27.5-203µm. The release kinetics studies were found to fit into zero order and Non Fickian diffusion controlled mechanism was observed. The drug was released continuously for a period of 12 hours with a maximum release of 90.5%. From the preliminary trials it was concluded that it is possible to formulate sustained release Ketoprofen loaded albumin microspheres.

Key words: Microspheres, drug delivery, Ketoprofen, drug release, albumin.

INTRODUCTION

Ketoprofen is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations (EC50 2µg/l). It produces reversible COX inhibition by competing with the substrate, arachidonic acid, for the active site of the enzyme. This inhibition results in a reduction in the tissue production of prostaglandins such as PGE2 and PGF2α. In addition to its effect on
cyclooxygenase, Ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. Ketoprofen is also a powerful inhibitor of bradykinin, an important chemical mediator of pain and inflammation. It also stabilizes lysosomal membranes against osmotic change and prevents the release of lysosomal enzymes that mediate tissue destruction in inflammatory reactions.

Ketoprofen is a NSAID readily absorbed from the gastrointestinal tract and peak plasma concentrations occurs in about 0.5–2 h after dosing, but it causes certain level of irritation in the gastrointestinal mucous membrane. The half-life of Ketoprofen in plasma is about 2–2.5 hours. The short half-life and the low single dose administration makes Ketoprofen a very good candidate for the formulation of sustained release dosage forms. On the other hand, albumin is a major plasma protein constituent, accounting for 355% of the total protein in human plasma. The accumulation of albumin in solid tumours forms the rationale for developing albumin-based drug delivery systems for tumour targeting. Thus it has been used as a carrier for targeting drugs to tumours, and since the synovium of the rheumatoid arthritis patients shares various features observed in tumours, albumin-based delivery systems can be used to target drugs to the inflamed joint. Furthermore, the solvent evaporation technique, which was used in this study, is a simple process that is also inexpensive enough for scaling up to a commercial level. The purpose of the present study was to prepare Ketoprofen microspheres by using solvent evaporation method and to study the effect of drug-polymer concentration on drug release. The release mechanism of Ketoprofen from microspheres was also discussed.

MATERIALS AND METHODS

Ketoprofen drug was obtained as gift sample from PPI Ltd (India); Bovine serum albumin was purchased from Rolex chemical industries, Mumbai, India. Tween 80, Ethanol, petroleum ether was purchased from S D Fine chemical Ltd, Mumbai, India. All other chemical used were of AR grade.

Preparation of microspheres:

Bovine serum albumin (BSA) microspheres containing Ketoprofen were prepared by solvent evaporation method. Different concentrations like 20, 40 and 60% solutions of BSA was made to which Ketoprofen drug was added and used as the aqueous phase. The
oil phase composed of 100ml sunflower oil and 10ml petroleum ether with 0.5ml Tween 80 as emulsifier. The aqueous phase was added drop wise to 100ml of sunflower oil preheated to 60°C and stirred on a magnet stirrer at 600 rpm for 30 minutes to form the initial emulsion and to allow the formation and solidification of microspheres. The microspheres suspension was decanted and the settled micro-spheres were washed three times with petroleum ether to remove traces of oil on microsphere surfaces. The microspheres were vacuum dried in desiccators overnight and stored in dark. [6] Three batches of microspheres were prepared by the above mentioned method and marked as KP1, KP2 and KP3. The microsphere formulations were carried out in different ratios as per the Table1.

Evaluation of Microspheres

Drug Polymer Interaction Studies

Drug-polymer interactions were studied by FT-IR spectroscopy. The spectra were recorded for Ketoprofen, bovine serum albumin and physical mixture of Ketoprofen: albumin (1:1). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 τ cm-2 for 3 minutes. The scanning range was 400–4000 cm-1 and the resolution was 4cm-1. [7]

Scanning electron microscopy

SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry Ketoropofen loaded albumin microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Ketoprofen loaded albumin microspheres were taken by random scanning of the stub. It can be clearly observed from the photographs of the Ketoprofen loaded albumin microspheres, prepared by solvent evaporation technique that the microspheres are smaller and have spherical shape shown in figure 4. [8]

Size distribution analysis

The mean particle size of microspheres was shown in figure 1. Various manufacturing parameters (apparatus design, type of stirrer, stirring speed, and viscosity of emulsion phases) affect particle size. In this study only the effect of polymer concentration, thus the inner phase viscosity, on particle formation and particle size, while keeping the other
parameters constant, was investigated. Increasing the Polymer: Drug ratio caused the mean microspheres size to shift towards a higher particle size. Higher concentration of polymer produced a more viscous dispersion which formed larger droplets and consequently larger microspheres. Size distribution of Ketoprofen loaded albumin microspheres was shown in figure 3. [9]

**Percentage yield**

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of microspheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared microspheres was determined by using the formula.

\[
\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
\]

The prepared microspheres were then characterized for their various properties.

**Determination of drug content**

Practical drug content was determined by taking a weighed quantity of albumin microspheres (approximately 10 mg) in a 100-mL volumetric flask. Sufficient quantity of water was added to make the volume 100 ml. The suspension was shaken vigorously and then left for 24 hours at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by UV spectrophotometry at suitable wavelength (256 nm) using a shimadzu UV visible spectrophotometer (SHIMADZU, Spectrascan-2200, Japan). [10]

**Drug loading and incorporation efficiency of microspheres**

The high content of Ketoprofen in microspheres was believed to be due to the poor solubility of drug in poor solvent. These suggested that the present method was suitable for the preparation of microspheres of a poorly water-soluble drug, such as Ketoprofen.

\[
\text{Drug loading (\%)} = \frac{M_{\text{actual}}}{M_{\text{weighed quantity of powder of microspheres}}} \times 100
\]

(1)
Where M actual is the actual Ketoprofen content in weighed quantity of powder of microspheres and M theoretical is the theoretical amount of Ketoprofen in microspheres calculated from the quantity added in the fabrication process.

**In vitro release studies of microspheres**

The drug release studies of the microspheres were carried out for 12 h at 100 rpm by using USPXXIII dissolution test apparatus by paddle method. The temperature of the dissolution medium was controlled at 37 ± 0.1°C. A quantity of microspheres equivalent to 100mg of drug was weighed. The dissolution medium was 900 ml of Phosphate buffer (pH 7.2±0.2). Five millilitres of the dissolution fluid was withdrawn at regular time interval and was replaced with fresh quantity of dissolution fluid. The samples were filtered, and the filtrate was assayed spectrophotometrically at 256nm to determine the dissolved drug concentration using a spectrophotometer (Model 1601 Shimadzu, Japan).

**Kinetics of drug release**

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q₀-Q) v/s t], Higuchi’s square root of time (Q v/s t^(1/2)) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q₀-Q) is the cumulative percentage of drug remaining after time t.

In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows:

- Cumulative percentage drug release Vs. Time (zero order rate kinetics) shown in Fig 6
- Log cumulative percentage drug retained Vs. Time (first order rate kinetics) shown in Fig 7
- Cumulative percentage drug release Vs. √T (Higuchi’s classical diffusion equation) shown in Fig 8
- Log of cumulative percentage drug release Vs. log Time (Peppas exponential equation) shown in Fig 9
RESULTS AND DISCUSSION

Preparation of albumin microspheres

Evidence have shown in the recent years that Bovine serum albumin (BSA) have the physical properties and behaviour suitable to prepare sustained drug delivery systems which are biocompatible, and biodegradable microspheres to release the entrapped drug. In the present study, solvent evaporation method was employed using BSA to entrap the drug, furthermore various parameters were characterized for drug and albumin ratio, stirring speed, amount of surfactant, temperature of the oil phase. Therefore the influences of the above parameters were highlighted. When the temperature of the external phase was more than 60°C the albumin formed gel like substance and thus devoid of encapsulation. The maximum drug load was obtained at 40-60°C for KP1, KP2 and KP3 formulations. The present study reveals that 1:6 (drug:polymer) ratio was suitable for producing the ideal spherical microspheres. Resultant microspheres did not have any surface irregularities and are non aggregated. As the drug:polymer ratio was decreased, the yield was reduced and the resultant microspheres were irregularly shaped and were highly aggregated in nature and highly impossible to distinguish as individual microspheres. In order to avoid the formation of irregularly shaped larger particles, in the present method, 1:6 drug:polymer ratio was best used. Incorporation of drug into BSA microspheres required the addition of Tween 80 as a surfactant, at an optimum concentration to reduce the interfacial tension between the internal aqueous phase and external hydrophobic phase. An attempt was made to incorporate drug in the albumin microspheres without the addition of a surfactant. But the process failed, as it resulted in an aggregate cake like mass during the solidification of albumin. This may be due to repulsion resulting from high interfacial tension between the hydrophobic oil material and the internal aqueous phase. It was found that Tween 80 having hydrophobicity and lipophobicity balance (HLB) value of 15 was suitable to increase substantially dispersion of albumin material in external oil phase and promote drug incorporation in the albumin microspheres. The batches were tested to obtain an optimal surfactant concentration; various concentrations ranging from 20-60 % (w/w) of the total formulation were selected. Discrete microspheres with good flow properties using an optimum concentration of surfactants 0.5 ml (Tween 80) were used. Concentrations of Tween 80
of 0.25, 1, 1.5, 2 and 2.5ml failed to produce reproducible microspheres. The resultant albumin microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. From SEM studies it was observed that the resultant microspheres were free from surface irregularities, except some wrinkles. In the present study, to produce the spherical discrete microspheres, an optimum drug to BSA ratio of 1:6 w/w was used. It was found that lower the amount of drug to BSA ratio (1:2) produces aggregate masses during the cooling process. 

The resultant microspheres were unsuitable for pharmaceutical uses. Hence an optimum 1:6 ratio was used to prepare microspheres (Table 1). [11] The average size of the microspheres ranged between 27.5 to 203µm. A stirring speed of 600 rpm and stirring time of 30 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 600 to 1000 rpm there was a decrease in the formation of the spheres and recovery yield of the microspheres. It is due to small sized albumin microspheres, which were lost during successive washings. When the stirring speed was lower than 600 rpm, gel like slime were formed. When the stirring time was lower than 30 minute, little amount of melted material adhered to the sides of the beaker during the cooling process results in reduction of yield.

### TABLE 1: FORMULATION COMPOSITION OF KETOPROFEN ALBUMIN MICROSPHERES

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug-Polymer ratio</th>
<th>Drug-Polymer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug-Polymer ratio</td>
<td>Drug-Polymer ratio</td>
</tr>
<tr>
<td>KP1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>KP2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>KP3</td>
<td>1</td>
<td>6</td>
</tr>
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</table>

**Drug loading and incorporation efficiency of microspheres**

The high content of Ketoprofen in microspheres was believed to be due to the poor solubility of drug in solvent. These suggested that the present method was suitable for the preparation of microspheres of a poorly water-soluble drug, such as Ketoprofen. The experiments were carried out and the results of % yield of albumin microspheres
were 83.86% to maximum of 96.99%. The maximum yield was obtained with formulation KP3. The average diameter of the albumin microspheres were found to be in the range of 27.5-203µm shown in fig 1. On further analysis of drug encapsulation of albumin microspheres, the encapsulation efficiency was found to be between 48% - 79% shown in fig 2. As the polymer concentration increases the drug encapsulation was found to be increasing in albumin microspheres. Also percentage yield, average particle size also increased along with increase in polymer concentration. The results of percentage yield, drug entrapment, average particle size, and % cumulative drug release were shown in table 2.

**TABLE 2: DATA FOR % YIELD, DRUG ENCAPSULATION EFFICIENCY, AVERAGE PARTICLE SIZE, % CUMMULATIVE DRUG RELEASE OF KETOPROFEN ALBUMIN MICROSPHERES**

<table>
<thead>
<tr>
<th>Formula Code</th>
<th>Percentage yield (%)</th>
<th>Drug encapsulation efficiency (%)</th>
<th>Average particle size (µm)</th>
<th>% Cumulative drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP1</td>
<td>83.86</td>
<td>48.00±2.30</td>
<td>27.5±2.5</td>
<td>90.5</td>
</tr>
<tr>
<td>KP2</td>
<td>90.40</td>
<td>55.60±1.50</td>
<td>110.5±3.5</td>
<td>85.4</td>
</tr>
<tr>
<td>KP3</td>
<td>96.99</td>
<td>79.00±3.50</td>
<td>203.0±5.5</td>
<td>79.4</td>
</tr>
</tbody>
</table>

SD = Standard deviation (n=3)
Figure 1
Average diameter of Ketoprofen loaded albumin microspheres

Figure 2
Drug entrapment efficiency of Ketoprofen loaded albumin microspheres
Figure 3
Frequency distribution curves of Ketoprofen loaded albumin microspheres prepared by Solvent evaporation method.

Scanning electron microscopy (SEM)
SEM photographs showed that the albumin microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage, which is due to the collapse of the wall of the microspheres (Fig.4). Photographs reveal that as the concentration of the polymer was increased the surface of the microspheres was found to be smooth and the surface shrinkage reduced, indicating uniform distribution of the drug within the microspheres. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product. [12]
In vitro studies

Hence based on this study, the drug release profile of all the formulations were carried out in Phosphate buffer pH 7. The dissolution studies revealed that albumin microspheres released the drug completely within 10 h at lower drug to polymer ratio. At ratio of more than 1:4, the drug release was sustained over a period of 12 h shown in fig 5. The data for In-vitro drug release profile of Ketoprofen albumin microspheres is shown in table 3.

**TABLE 3: REGRESSION CO-EFFICIENT (R²) VALUES OF DIFFERENT KINETIC MODELS AND DIFFUSION EXPONENT (N) OF PEPPAS MODEL FOR KETOPROFEN MICROSPHERES**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi Matrix</th>
<th>Peppas plot</th>
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<tr>
<td></td>
<td>r² value</td>
<td>‘n’ value</td>
<td></td>
<td>r² value</td>
</tr>
<tr>
<td>KP1</td>
<td>0.9860</td>
<td>0.8572</td>
<td>0.9464</td>
<td>0.9717</td>
</tr>
<tr>
<td>KP2</td>
<td>0.9827</td>
<td>0.9479</td>
<td>0.9536</td>
<td>0.9752</td>
</tr>
<tr>
<td>KP3</td>
<td>0.9774</td>
<td>0.9759</td>
<td>0.9472</td>
<td>0.9769</td>
</tr>
</tbody>
</table>
Figure 5

*In vitro* release of Ketoprofen loaded albumin microspheres.

Figure 6

Zero order release mechanism of Ketoprofen loaded albumin microspheres.
Figure 7
Release kinetics profile of Ketoprofen albumin microspheres according to First order

Figure 8
Release kinetics profile of Ketoprofen albumin microspheres according to Higuchi matrix diffusion
CONCLUSION

The purpose of present work was to develop sustained release microspheres of water insoluble drug, Ketoprofen, using bovine serum albumin as the drug carrier, by solvent evaporation method. From the results of characterization and drug release studies of microspheres it was concluded that this method was ideal for manufacturing sustained-release microspheres. On the basis of release studies it was indicated that albumin enhances the solubility of Ketoprofen from microspheres, hence presenting a suitable method for preparing the sustained-release albumin microspheres for poorly water-soluble drug Ketoprofen.

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REFERENCES


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