ABSTRACT

The present study envisages formulation of wax microparticulate drug delivery system. The objective of the present study was to minimise the unwanted side effects of Ketoprofen drug formulated by Congealable disperse encapsulation method using biodegradable waxes such as beeswax, ceresin wax microspheres using a wetting agent. Solid, discrete, reproducible free flowing microspheres were obtained. The yield of the microspheres was up to 92.0%. Microspheres prepared have smooth surfaces. These microspheres have free flowing and good packing properties and shows the characterization values well within the limit that are angle of repose, % Carr’s index and tapped density. More than 92.0% of the isolated spherical microspheres were in the particle size range of 29.50 µm as confirmed by scanning electron microscopy (SEM) photographs. The drug loaded in microspheres was found to be stable and compatible with waxes as confirmed by FTIR studies. The drug release from invitro study followed matrix model and it shows initial burst release followed by constant release up to 24hrs

Keywords: ketoprofen, bees wax, ceresin wax, melt solidification, dichloromethane.

INTRODUCTION

Ketoprofen, (RS)-2-(3-benzoylephn) propanoic acid is a non-steroidal anti-inflammatory drug used to treat rheumatoid arthritis, osteoarthritis analgesic, antipyretic and mild to moderate pain1. The GI irritation and ulcerogenic effect along with short half-life (2–2.5 h) has lead to the design of controlled release formulations of ketoprofen2. Due to its low melting point and hydrophobic nature many attempts have been made to develop wax based controlled release formulations. Melt dispersion technique has been reported for the development of ketoprofen microspheres3,4. Beeswax, carnauba wax, ceresine, microcrystalline wax, Pre- cirol ATO5, Gelucire 64/02 were evaluated as waxy carriers In these techniques ketoprofen-wax melt was emul- sified and then cooled to obtain microspheres5,7. The drug:wax ratios were significantly high from 1:3 to 1:5 (50–80% wax) with the drug loading in the range of 10–30%8–10. Ketoprofen forms a low viscosity melt, which due to its low melting point remains in liquid state for longer period of time. On the basis of these properties a melt solidification technique (MST) has been developed to obtain non disintegrating, excipient-free lipids of ketoprofen11,12. The drug release from lipids was significantly retarded, which may be attributed to the melt solidified bonds formed in the compact lipids13–15. In this technique molten ketoprofen was poured in to emulsifier maintained at temperature > 45°C, with agitation. The lipids obtained had poor sphericity and could controlled the release only up to 2.5 hr16–20. The objective of the present study was to develop controlled release ketoprofen lipids employing the strength of melt solidified bond and to impart sphericity with minimum amount of excipient. The lipids microspheres were characterized using scanning electron microscope (SEM) and FT-IR. The effect of variables on the yield, micromeritic properties, crushing strength and various release parameters was evaluated.
MATERIALS AND METHODS

Materials
Ketoprofen was kindly supplied by Themis Laboratories, Mumbai (India). All other reagents and chemicals used were of analytical grade.

Preparation of Wax Microspheres:
Weighed amount of Beeswax was melted separately in a china dish using water bath. Ketoprofen previously passed through sieve no.100 was dispersed in the melted wax mass evenly and stirred to obtain a homogeneous melt. These mixtures was poured into 200ml of mixture of dispersant medium containing 100ml of pH 7.4 Phosphate buffer solution (to minimize the solubility of drug) and 100ml of PVA (1%), which was previously heated to a temperature higher than melting point of wax (+5°C). Tween 80 (1.0-2.0% w/w) was added to the above mixture and was mechanically stirred at 900 rpm using a mechanical stirrer. Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The mixture was stirred continuously at 900 rpm at a higher temperature (+5°C) of the melting point of wax for 3 min. The temperature of the mixture in the beaker was cooled rapidly and brought down to 4°C by the addition of cold water. The resultant solid spheres collected by filtration were washed with water to remove any drug and surfactant residues. Air-drying was carried out at room temperature for 48 hr gave discrete, solid, free flowing microspheres. Similarly above process was carried out with Ceresin wax by melted in china dish at a temperature of 75°C. Total 6 formulations were prepared by varying concentration of both lipids as shown in table 1.

EVALUATION PARAMETERS

Particle Size Analysis of microspheres:
The size distribution of the Microspheres was determined using the particle size analyzer (Beckman Coulter, Delsanano C, Brea, USA) equipped with a dry accessory system. Sample was diluted with water and temperature maintained at 25°C. Sample was diluted with water and temperature maintained at 25°C. The bulk density, and tapped density were calculated by the following formulas:

\[ \text{Bulk density} = \frac{W}{V_o} \]

\[ \text{Tapped density} = \frac{W}{V_f} \]

Where, \( W \) = weight of the powder, \( V_o \) = initial volume, \( V_f \) = final volume

Drug Content
Ketoprofen drug incorporated wax microspheres of each batch was selected and powdered in a mortar. 100 mg of drug loaded wax microspheres was accurately weighed and added into 100mL volumetric flask. To this, 100mL DCM was added and stirred for 60 min, till the entire drug leached out. The solution was filtered and 1mL was withdrawn from this solution and added in to 10mL volumetric flask and volume was made to 10mL (10 g/mL) with phosphate buffer pH 6.8. Drug content was estimated UV spectrophotometrically at 259 nm using pH 6.8 phosphate buffer as a blank.

Encapsulation Efficiency
Encapsulation efficiency was calculated using the following formula

\[ \text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100 \]

Fourier Transform Infrared Spectroscopy (FTIR)
IR spectral analysis of pure drug, empty microspheres and drug loaded microspheres was carried out and observation was made whether
changes in chemical constitution of drug after combining it with the polymers occurred. The samples were crushed with KBr to get pellets by applying pressure of 600 Kg/cm².

**In-Vitro Dissolution Studies**

In-vitro dissolution studies of Ketoprofen Microspheres were performed using USP type-II (Paddle) dissolution test apparatus. 900ml of buffer is used as a dissolution medium. The medium was maintained at 37±0.5°C at a speed of 100rpm. The in-vitro dissolution studies were performed at different pH in 0.01N HCL for first 2 hrs simulated gastric fluid and remaining in simulated intestinal fluid up to 24hrs. An accurately weighed sample equivalent to 50mg drug was responded in dissolution medium consisting 900ml of buffer and dissolution was done up to 24hrs. At prefixed time intervals 1ml of sample was withdrawn and filtered through 0.4 m membrane filter. Then the withdrawn is diluted to 10ml. The volume of the dissolution medium was adjusted to 900ml at every sampling time by replace same 1ml of dissolution medium in order to maintain the sink condition. Then the samples were analyzed Spectrophotometrically at 259 nm.

**RESULT AND DISCUSSION**

For the preparation of microspheres of Ketoprofen bees wax or ceresin wax is used in varying concentration. Drug is insoluble in water. The volume of pH 7.4 phosphate buffer and PVA 1% used is about 200ml if the reduced volume is not sufficient for the formation of microspheres. If the volume is reduced irregular shaped particles are found as well clumps are formed. Tween80 is used as emulsifier in 2% concentration. Bees wax or ceresin wax is used as lipids in varying concentration just to check effect on particle size and drug release. Without emulsifier formulation is not possible. Speed is optimised at 900 rpm below that speed particle size is increased.

**Microsphere Size Analysis**

The particle size of the prepared Microspheres was determined by particle size analyzer (Beckman Coulter). The Average particle size of the Ketoprofen loaded Microspheres were found to be 29.25±29.5μm. Results are shown in Table 2. Size distribution plays a very important role in determining the release characteristics of the microspheres.

**Scanning Electron Microscopy:**

SEM photographs were taken using scanning electron microscope JEOL 5400, Tokyo, Japan, at suitable magnification at room temperature. By SEM observed the shape and surface characterization of microspheres and only Optimised batch is selected for SEM analysis. SEM showed that the lipid microspheres were spherical in nature, had a smooth surface. Result is shown in Figure 1.

**Angle of Repose:**

Tap density of the prepared microspheres was determined using tap density tester and % Carr's index was calculated and found to be satisfactory. Angle of repose was assessed to know the flowability of wax microspheres. All the formulations show good flow property. Results of all the formulations are shown in Table 2.

**Drug Content and Entrapment Efficiency**

Drug Content and Entrapment Efficiency was found in the range of 80-95%. After thorough mixing of drug with wax it shows uniform distribution and entrapment of drug. The drug is insoluble in water so the drug release during preparation is avoided. It was observed that the drug release from the formulations decreased with increase in polymer concentration. The decreased in vitro drug release from wax microspheres might be due to more hydrophobicity and influence of molecular weight of wax. The formulations F3 and F6 showed the longer duration of drug release for 24hrs in simulated intestinal fluid, in addition to completing retarding the drug release in gastric medium. This is due to the polymer Bees wax. The drug release from waxy microspheres was considerably retarded from the waxes. So that F6 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug. It was observed that the encapsulation efficiency increases with increase in polymer concentration; Formulation F6 shows maximum entrapment efficiency. Results of all the formulations are shown in Table 2.

**Fourier Transform Infrared Spectroscopy (FTIR)**

An FTIR spectrum shows that both the drug and polymer are compatible with each other. The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies. Figure 2. shows FTIR spectra of blank bees wax, ceresin wax microspheres, pure drug, formulation F3 and F6. IR spectra indicates that IR frequency bands of the -OH and C=O and groups having stretched at 2983 cm⁻¹ and 1635 cm⁻¹ respectively are not affected in the presence of Lipids.

**In-Vitro Dissolution Studies and Release kinetics**

From the release studies it was observed that, formulation F6 shows extended release up to 12 hrs. There is initial burst release followed by constant
It was observed that the drug release from the formulations decreased with increase in polymer concentration this is because more will be the wax concentration more time is taken to diffuse the drug molecule. Figure 3.

### Table 1
Formulation of Ketoprofen microspheres using Ceresin wax and Bees wax.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Quantity of Lipids</th>
<th>Drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ceresin Wax (mg)</td>
<td>Bees Wax (mg)</td>
</tr>
<tr>
<td>F1</td>
<td>150</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>F5</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>F6</td>
<td>-</td>
<td>250</td>
</tr>
</tbody>
</table>

### Table 2
Micromeritic properties of the drug loaded lipid microspheres

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Yield (%w/w)</th>
<th>Mean particle size (microns)</th>
<th>Angle of repose</th>
<th>Tap Density</th>
<th>Carr’s Index</th>
<th>Drug entrapment (%)</th>
<th>Drug Content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>83.92</td>
<td>56.6 ±1.4</td>
<td>22.10</td>
<td>0.987±0.006</td>
<td>11.486±0.553</td>
<td>42±0.34</td>
<td>10.64</td>
</tr>
<tr>
<td>F2</td>
<td>86.91</td>
<td>41.13 ± 1.9</td>
<td>26.37</td>
<td>1.243±0.006</td>
<td>12.869±0.809</td>
<td>55±0.27</td>
<td>11.13</td>
</tr>
<tr>
<td>F3</td>
<td>88.08</td>
<td>39.22 ± 2.1</td>
<td>28.06</td>
<td>1.477±0.006</td>
<td>14.670±0.982</td>
<td>64±0.73</td>
<td>10.73</td>
</tr>
<tr>
<td>F4</td>
<td>88.13</td>
<td>68.22±1.3</td>
<td>26.82</td>
<td>0.977±0.006</td>
<td>10.24±0.061</td>
<td>49±0.37</td>
<td>12.43</td>
</tr>
<tr>
<td>F5</td>
<td>89.32</td>
<td>44.50±1.25</td>
<td>25.97</td>
<td>1.243±0.005</td>
<td>12.331±0.903</td>
<td>64±0.15</td>
<td>12.88</td>
</tr>
<tr>
<td>F6</td>
<td>92.45</td>
<td>29.25±0.5</td>
<td>25.48</td>
<td>1.453±0.005</td>
<td>13.071±0.601</td>
<td>77±0.33</td>
<td>12.83</td>
</tr>
</tbody>
</table>

Figure 1
SEM Shows shape and size of microspheres.
Figure 2

Shows FTIR spectra of pure drug, formulation, bees wax and ceresin wax.
CONCLUSION
The Ketoprofen waxy microspheres were spherical with smooth surface and good micromeric properties. It can be concluded that there is no vigorous treatment to the formulation so the yield of the product is optimum as well as particle size can also be optimised. The formulations F3 and F6 showed the longer duration of drug release for 24hrs in simulated intestinal fluid, in addition to completing retarding the drug release in gastric medium. This is due to the polymer Bees wax. The drug release from waxy microspheres was considerably retarded from the waxes. So that F6 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug. The drug release from the formulations decreased with increase in polymer concentration. All the particles are having spherical shape. It releases the drug 92% upto 24hrs.so it can be assumed that it can be sustained release form.

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REFERENCES
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