The Effect of Process Variables on the Properties of Ketoprofen Loaded Solid Lipid Nanoparticles of Beeswax and Carnauba Wax

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ABSTRACT: Solid Lipid Nanoparticles (SLNs) have emerged as an alternative colloidal carriers for sustained release of lipophilic drugs with poor absorption and water solubility. This manuscript describes the effect of process variables on the production of Solid Lipid Nanoparticles (SLNs) from beeswax and carnauba wax and ketoprofen release from these carriers. It was found that by increasing drug content from 0.5 to 1.5% w/v the average particle size of SLNs increased from 82 to 116 nm and drug loading increased from 10.7 to 26.6% while entrapment efficiency remained almost constant (≈ 97%). Unexpectedly, the average size of SLNs increased from 82 to 150 nm by increasing homogenization time from 5 to 15 min. Increasing homogenization intensity from 11000 to 24000 rpm resulted in the particle size decrease from 108 to 82 nm. The rate of drug release from nanoparticles loaded with 0.5% w/v ketoprofen increased with increasing the ionic concentration of phosphate buffer solution from 0.05 to 0.1 M.

KEY WORDS: Solid lipid nanoparticles, Ketoprofen, Beeswax, Carnauba wax, Sustained release.

INTRODUCTION
In recent years, it has become more evident that the development of new drugs alone was not sufficient to ensure progress in drug therapy [1]. A promising strategy to overcome problems such as poor absorption, rapid metabolism and elimination, poor water solubility and high fluctuation of plasma levels, involves the development of suitable drug carrier systems [2].

Solid Lipid Nanoparticles (SLNs) introduced in 1991 [3], have emerged as an alternative colloidal carrier due to advantages such as improved physical stability, good tolerability, efficient incorporation of lipophilic drugs in lipid core of SLNs and ease of scale up and manufacturing [4]. These nanoparticles possess a solid lipid core matrix that is solublized by surfactants [5].

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Most lipid-based delivery technology research has focused on the delivery of hydrophobic small molecule drugs for human medical applications. Poorly water soluble Non-Steroidal Anti Inflammatory Drugs (NSAIDs) broadly used for administration via safe vehicles [6]. Ketoprofen [2-(3-benzoylphenyl) perpionic acid] is a non-steroidal anti-inflammatory and analgesic agent used to treat acute and chronic rheumatoid arthritis. Because of its gastrointestinal side effects [7] and short plasma elimination half-life (2-4h), it is considered as a good candidate to formulate sustained release drug delivery system [8].

Design of optimized carrier for slow and targeted release is depended on the overall formulation and process parameters. There is a complex and sensitive relationship between formulation chemistry and process parameters [9]. Therefore the process optimization efforts are necessary for each drug to be delivered by nanocarrier formulations.

The aim of the present study was to determine the effects of process variables such as drug content and homogenization time and speed on the characteristics of ketoprofen loaded SLNs using beeswax and carnauba wax as lipid core and combination of tween 80 and egg lecithin as emulsifier. The drug release behavior of the best formulation, based on particle size and drug loading, was also investigated in different dissolution media.

EXPERIMENTAL SECTION

Materials

Beeswax, carnauba wax, egg lecithin and ketoprofen were obtained from Sigma-Aldrich. Tween 80 was provided from Merck. PBS, acetonitrile and all other solvents used were of analytical grade.

Methods

Preparation of SLNs

SLNs were prepared using microemulsion method proposed by Gasco[10]. The specified amounts of 1:1 mixture of beeswax and carnauba wax, egg lecithin and drug were melted in a water bath at 90°C. Tween 80 was mixed with dionized water at 90°C and 2000 rpm for 2 min and then added to the molten ingredients. The primary emulsion was dispersed at different homogenization speeds and times using rotor stator homogenizer (Ultra-Turrax, IKA T18 basic). The resulting nanoemulsions then were dispersed gradually in 2°C cooled water at a ratio of 1:10 under stirring at 3000 rpm.

Particle size determination

The average particle size (Z average) and Poly Dispersity Index (PDI) of the lipid dispersion, obtained via various experiments were determined by Photon Correlation Spectroscopy (PCS) using nanosizer (Malvern Instruments, Nano-ZS). Measurements were carried with an angle of 90° at 25°C.

Entrapment efficiency and drug loading

Entrapment efficiency was estimated by measuring the free drug in the suspension. Free drug was separated from drug loaded nanoparticles by ultrafiltration method (Sartorious stedim biotech, Vivacell25, Germany) and its concentration was determined by HPLC method (UV detector and pump: Younglin Instrument, Korea; column: Nucleosil 100-5C18, 250×4.6 mm and 10 μm). A reverse phase HPLC method was used according to the literature [8,11].

The Entrapment Efficiency (EE%) and percent of Drug Loading (DL%) of drug loaded particles were calculated as follows:

\[
EE\% = \frac{\text{Total drug added} - \text{free drug}}{\text{total drug added}} \times 100
\]

\[
DL\% = \frac{\text{Total drug added} - \text{free drug}}{\text{total drug added} - \text{free drug} + \text{total amount of lipid + surfactants added}} \times 100
\]

Thermal behavior

Differential scanning calorimetry (DSC) was used to study the thermal behavior of SLNs. DSC thermograms of bulk materials and also the SLNs were recorded by Mettler Instrument (Mettler Toledo AG, Switzerland) after 45 days of sample preparation. For measurement, 6 mg of solid sample was placed in an aluminum pan and thermal behavior was determined in the range of 25-120°C at a heating rate of 5°C/min.

In-vitro drug release studies

In-vitro drug release experiments were performed in two different dissolution media of 0.05 and 0.1 M Phosphate Buffer Solution (PBS).

For this purpose, 35 mg of freeze dried sample was re-dispersed in 1 mL PBS and was charged into a cellulose acetate dialysis bag (MW Cut off:12 kDa). Dialysis bag was put into a glass receptacle which contained 30 mL PBS. The resulting dissolution medium was continually stirred...
with a small magnetic stirring bar at 37°C in order to be homogenized. 1 mL of a dissolution medium was withdrawn for determination of drug concentration by HPLC and 1 mL of fresh medium was fed into the receptacle.

**Effect of process variables**

To study the effect of drug content on the average particle size and drug encapsulation, 3 samples with different drug content (0.5, 1 and 1.5 %w/v), 1%(w/v) of 1:1 mixture of tween 80: lecithin and 3%(w/v) of 1:1 mixture of beeswax: carnauba wax were prepared. Homogenization time and speed of rotor stator were set at 5 min and 24000 rpm, respectively in the experiments.

The effect of homogenization time, varied from 5 to 15 min while homogenization speed was kept constant at 24000 rpm, was studied for a specified sample with 0.5%(w/v) drug content. Composition of other ingredients was the same as mentioned above.

At the next step the effect of homogenization speed which varied from 11000 to 24000 rpm, was investigated for a specified sample while homogenization time was kept at 5 min and the same composition of ingredients as before.

**RESULTS AND DISCUSSION**

**The effect of drug content on the particle size and drug encapsulation**

The effect of drug content on the particle size and drug encapsulation is shown in Figs. 1 and 2, respectively. By increasing total drug content, drug loading was increased while the entrapment efficiency remained constant. It suggests that of ketoprofen interacts favorably with lipid matrix. Lipid core of SLNs has high potential to solubilize hydrophobic ketoprofen even at high drug concentration, but excesses drug loading resulted in increased particle size as shown in Fig. 1. Increased solid content of the formulation due to the added drug resulted in the size increase of the primary emulsions and subsequent increased size of final nanoparticles. The next stage of the studies was followed by setting the composition of ingredients, namely 0.5% (w/v) drug content which resulted in SLNs with an average particle size of 82 nm.

**The effect of homogenization time on the particle size**

The effect of homogenization time on the particle size and polydispersity index is displayed in Fig. 3.
Table 1: Formulation and properties of the selected sample for DSC and drug release studies.

<table>
<thead>
<tr>
<th>1:1Lipid mixture of beeswax: carnauba wax (%w/v)</th>
<th>1:1Surfactant mixture of tween 80: lecithin (%w/v)</th>
<th>Drug content (% w/v)</th>
<th>Homogenization time (min)</th>
<th>Homogenization speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>5</td>
<td>24000</td>
</tr>
</tbody>
</table>

Average particle size (nm) Polydispersity index Zeta P(mv) EE (%) DL (%)

82± 2.43 0.26±0.02 -15.52±1.32 96.53±0.23 10.69± 0.06

Experiments were performed triplicate; average± S.D. of responses is reported (n=3).

Fig. 4: Particle size distribution at 15 min homogenization time. [1:1 mixture of tween 80: lecithin, 1:1 mixture of beeswax: carnauba wax, 0.5 % (w/v) drug content, homogenization speed: 24000 rpm].

Unexpectedly, increased homogenization time had adverse effect on the reduction of particle size. With increasing homogenization time, both particle size and polydispersity were increased. Although by increasing the time of homogenization shear force applied on the emulsion droplets breaks up them to smaller droplets but there is a high tendency for coalescence of droplets upon collision which increases by the increased surface area of primarily formed small droplets. According to the kinetic aspects, the small particles are driven together at relatively high speeds and more effectively interact with each other that contribute to the facile agglomeration process. The particle size distribution in Fig. 4 clearly shows that increased homogenization time resulted in the formation of nanoparticles with different size and broad distribution which in turn may contribute to further increase of particle size and polydispersity indices due to the agglomeration.

Fig.5: The effect of homogenization speed (rpm) on the particle size and distribution (PDI), [1:1 mixture of tween 80: lecithin, 1:1 mixture of beeswax: carnauba wax, 0.5 % (w/v) drug content, homogenization time: 5 min].

The effect of homogenization speed on the particle size

It is shown in Fig. 5 that by increasing homogenization speed, smaller particles with narrower distribution (lower PDI) were obtained. As the homogenization speed increases, the generated shear forces also increase. Increased shear force cause reduction of emulsion droplet size and consequent decrease of particle size. Decreased particle size with increasing shear force using rotor stator homogenizer (homogenization rpm), has been also demonstrated in the experiments performed by Maa & Hsu [12].

Further experiments were conducted by the samples with described properties given in Table 1.

Thermal behavior

Differential Scanning Calorimetry (DSC) was used to study the thermal behavior of SLNs. It is popular tool to investigate the crystallinity of colloidal SLN matrices [13]. DSC has also been used to evaluate the interaction of incorporated drugs with lipid matrix [14].
The crystallization behavior of ketoprofen loaded SLNs differs from the bulk lipid (Table 2). The DSC thermal behavior of the untreated lipid bulk is given as a reference for comparison. Mixture of beeswax and carnauba wax showed a double endothermic peak upon heating with two maximum at 69 and 83°C and enthalpies of 22.32 and 21.66 J/g (Fig. 6, graph 1). In contrast to the lipid mixture the melting point of drug loaded SLNs were decreased about 8°C in two mixture peaks (about 61 and 75°C) (Fig. 6, graph 2) and no peak for drug was detected. The absence of drug melting point has been observed for other drugs in previous studies. It suggests non-crystalline state of the incorporated drug. Although according to Westesen et al. [17] decline in melting point can be due to the colloidal dimension of particles (large surface to volume ratio), but high entrapment efficiency of ketoprofen (≥ 97%) and also little effusion of drug during the storage time (EE%≈ 96% after 45 days) can be assigned to the increase of lattice defects and physical stability of particles that permit better incorporation and storage of drug molecules.

**In vitro drug release behavior**

In vitro drug release profiles in 0.05 and 0.1 M PBS are shown in Fig. 7. At 0.1 M PBS, nanoparticles showed an initial burst release followed by a sustained release of ketoprofen, but no burst effect for drug release was observed in 0.05M PBS. This result indicates that concentration of buffer solution has significant effect on the hydrolytic degradation and hence on the rate of drug release. Diffusion and hydrolytic degradation (erosion) are two important mechanisms for drug release from SLNs. SLNs degrade in the presence of water by an ester hydrolysis reaction which is acid catalyzed and reversible [18]. Via hydrolysis of free fatty acids and hydroxyl group of wax structures long-chain esters degrade to carboxylic acids with short chains. Degradation products are acidic and the reaction also is autocatalytic. The possible explanation for the initial fast release in 0.1M PBS is the higher hydrolysis reaction rate of the acidic groups of lipid particles at the initial stage of drug release. The interaction between acidic products and salts of buffer resulted in the production of carboxylic acid sodium salts which are more soluble than the acid themselves and will tend to facilitate ester hydrolysis reaction. Enhanced hydrolytic degradation of SLNs in 0.1 M PBS increased lipid matrix porosity and drug release rate.

### Table 2: Thermal properties of lipid bulk mixture and SLNs containing 10.7% (w/w) ketoprofen.

<table>
<thead>
<tr>
<th></th>
<th>Lipid bulk mixture</th>
<th>SLNs with 10.7% (w/w) drug content</th>
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</thead>
<tbody>
<tr>
<td>T1(°C)</td>
<td>45±2.12</td>
<td>52±2.00</td>
</tr>
<tr>
<td>T2(°C)</td>
<td>69±0.71</td>
<td>61±0.58</td>
</tr>
<tr>
<td>H1 melting (J/g)</td>
<td>22.32±1.00</td>
<td>5.75±0.85</td>
</tr>
<tr>
<td>H2 melting (J/g)</td>
<td>74±0.71</td>
<td>70±0.58</td>
</tr>
</tbody>
</table>

*Mean ± S.D. (n=3).*
CONCLUSIONS

This study demonstrated that drug content and process variables have strong effect on the characteristics of SLNs and drug release profile. By increasing drug content of initial formulation entrapment efficiency remained constant while drug loading increased as expected. It suggests the potential of lipid core to entrap ketoprofen as a lipophilic drug even in high concentrations. On the other hand, the particle size increased by increasing drug content. Increasing homogenization speed resulted in a decrease of particle size due to the increase of the applied shear forces that contributed to the break up of the droplets with consequent reduction of particle size. But increasing homogenization time increased the particle size unexpectedly. The possible explanation for the reduction of particle size is the increased coalescence of primary small droplets with large specific surface area.

DSC thermograms of SLNs with 10.7% (w/w) drug content indicated molecular dispersion of ketoprofen in lipid core and physical stability of SLNs during storage time. The drug release behavior of SLNs in 0.05 and 0.1 M PBS indicated considerable effect of buffer salts concentration on the degradation rate of particles. This is of prime importance from practical point of view for administration of drug loaded SLNs that can be exposed to a variety of physiological solutions with different ionic strength in the body.

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