Characterization of Diclofenac Liposomes Formulated with Palm Oil Fractions

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Abstract

Purpose: To characterize diclofenac sodium (DS) liposomes prepared using palm oil fractions. Methods: Reverse-phase evaporation method was used to prepare liposomes containing 10, 20, 30, 40 or 50% palm oil fractions. The effect of palm oil content on liposome formation, surface morphology, size and zeta potential of the liposomes were studied using scanning electron microscopy (SEM) transmission electron microscopy (TEM) and particle analyzer. Drug loading, entrapment efficiency and in vitro drug release were measured in phosphate-buffered saline (PBS, pH 7.4) by UV spectrophotometry.

Results: TEM and SEM images showed formation of liposomes for all formulations. However, increase in the proportion of palm oil in the formulations significantly reduced particle size and increased zeta potential. The effect on drug loading and drug release varied with palm oil fraction. The best release pattern with appropriate entrapment efficiency and stability was obtained with liposomes containing 33% palm oil fraction.

Conclusion: The results demonstrate the potentials of palm oil fractions in the preparation of suitable DS liposomes with good bioavailability.

Keywords: Liposome, Drug delivery, Palm oil, Diclofenac.

INTRODUCTION

Liposome is uni- or multi-layered spherical vesicles mainly composed of phospholipids [1]. They are used to deliver both hydrophilic and hydrophobic drugs by various routes of administration such as oral, intramuscular, intraperitoneal, subcutaneous, inhalation and ocular routes [2]. Furthermore, liposomal encapsulation of drugs can provide targeted drug delivery and sustained release effect besides reduction of toxicity and adverse effects [3].


Didclofenac sodium (DS) is widely used to treat mild to moderate pain and inflammations including those associated with osteo- and rheumatoid arthritis by inhibiting cyclooxygenase enzyme. Currently available DS dosage forms include gel, ophthalmic solution, immediate and controlled release tablets, suppositories and intramuscular injection [4]. Oral forms of DS are the most common in the market though they usually cause gastrointestinal problems such as abdominal cramps, nausea, constipation, diarrhea and peptic ulceration. Furthermore, 40% of the DS administered orally undergoes first-
pass metabolism and does not reach systemic
circulation [5]. Due to its poor solubility in water
and acidic medium in the stomach, it has a poor
oral bioavailability and a short half-life of about
two hours [5].

Intramuscular injection of DS avoids first pass
metabolism and achieves a faster therapeutic
effect [6]. However, the intramuscular injection
can cause cutaneous lesions at injection site.
This problem can be solved by encapsulation of
DS in liposomes where local tissue damage can
be reduced and the DS release rate can be
controlled [7].

Palm oil mainly consists of fatty acids like
palmitic, stearic, linoleic, oleic and myristic acids
[8]. It has been widely researched for its
nutritional and pharmaceutical properties,
including antioxidant activities, cholesterol
lowering, anti-cancer effects and protection
against atherosclerosis owing to the presence of
chemicals such as carotenes, tocopherols,
tocotrienols, terpenoids, tannins, flavonoids,
hydrocarbons and ketones [9].

The main objectives of this study was to
investigate the effect of palm oil fraction on
formation of liposomes, to assess the stability of
liposomal DS prepared by palm oil fraction, to
investigate the in vitro drug release rate from
new formulation of liposomal DS and to make
recommendation based on the best formulation
of liposomal DS prepared by palm oil fraction as
a parenteral drug delivery system.

EXPERIMENTAL

Materials
L-alpha-phosphatidylcholine (PC), cholesterol
(CH), linoleic acid, methanol and chloroform
were purchased from Sigma-Aldrich, Germany.
Diclofenac sodium (DS) was provided by Epic
Ingredients Sdn. Bhd (Malaysia). Palmitic acid,
Oleic acid, Stearic acid and linoleic acid which
are indicated as palm oil lipid portions were
purchased from Acidchem International Sdn.
Bhd (Malaysia). Sodium hydroxide and potassium
dihydrogen phosphate were purchased from
Merck, Germany.

Preparation of liposomes
Liposomes were prepared using conventional
method [10] as per the composition in Table 1.
Each formulation consisted of CH with varying
levels of PC and fatty acids. The lipid
components were dissolved in chloroform:methanol mixture (2:1, v/v) in a
round-bottom flask. The solvent was removed
under vacuum using a rotary evaporator
(Rotavapour R-124, BUCHI) at 50°C, 50 rpm.
After a thin lipid film was formed in the interior of
the flask, the system was purged with nitrogen to
remove organic solvent completely. The lipid film
was hydrated with 10 ml solution containing DS
(20 µg/L) in phosphate-buffered saline (PBS, pH
7.4). Finally, the mixture was sonicated for 15
min in a bath-type sonicator (Sonicor).
The liposomes were allowed to form at room
temperature and kept in the refrigerator overnight
[9]. The mixture was filtered through a syringe
filter (0.45 µm) 3 times.

Morphological studies
Transmission electron microscopy (TEM) and
scanning electron microscopy (SEM) were used to
characterize the surface and structure of the
liposome, respectively. The samples were
prepared for TEM, by applying a drop of the
mixture to a carbon-coated copper grid, and left
for one minute to allow some of the particles stick
to the carbon substrate. After removing the
excess of dispersion with a piece of filter paper a
drop of 1 % phosphotungstic acid solution was
applied for one minute, and left to air-dry. The
samples were then viewed under TEM
(ABFETEM Leo 9112). To observe the liquid
sample by SEM, special treatment was
necessary to dry up the wet samples before SEM
scan. As direct air-drying of the samples would
cause damage due to surface tension of the
evaporating water, critical point drying (CPD)
was performed to prevent such damage before
viewing under SEM (JSM 6400 SEM, Jeol) at
different magnification [11, 12].

Particle size distribution and zeta potential
measurement
Zeta potential (ZP) evaluates the stability of a
colloidal system. It is a measurement of the
repulsive forces between particles. Particles with
larger repulsive forces are less likely to
aggregate and would be more stable. Generally,
particles with ZP < -30 mV or > +30 mV are
normally stable [13]. Each sample solution was
inserted into the cell and ZP was measured with
a zetasizer (Zetasizer Nano Series, Malvern
Instrument). This procedure was repeated three
times and the result of the mean and standard
deviation recorded.

Construction of standard calibration curve
DS solutions (in fresh phosphate buffer, pH=7.4)
with a concentration range of 10 - 50 µg/mL were
prepared. Absorbance was measured by UV

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spectrophotometry (UV-1601 Shimadzu) at a wavelength of 300 nm and a standard calibration curve was constructed using Microsoft Excel 2007 program.

**Evaluation of entrapment efficiency**

To study entrapment efficiency of the liposome, the mixture was centrifuged (Universal 32) for 1.5 h at 9000 rpm, the supernatant was collected, and the absorbance measured by UV spectrophotometry at 300 nm [12]. The measurement was carried out in triplicate for each sample. Entrapment efficiency (EE) of the liposomes was determined as in Eq 1

\[
EE(\%) = \left(\frac{C_i - C_f}{C_i}\right) \times 100 
\]  

where \(C_i\) is the initial concentration of drug used in formulating the liposomes and \(C_f\) is the concentration of drug in the supernatant

**In vitro drug release study**

To measure *in vitro* release of DS from the liposomes, 100 mg of liposome was added into a dialysis bag (MW, 12000). The bag was closed at both ends and placed in 50 ml of fresh PBS medium (pH 7.4) at 37 °C at 60 rpm under sink conditions [14,15].

At predetermined time intervals, 2 mL of the medium was withdrawn and determined using UV spectrophotometer; the same volume of fresh fluid was used to replenish the release medium. The release test was continued for 120 h. The absorbance of the release samples, diluted as necessary with release medium, was measured spectrophotometrically at 300 nm. The results recorded are the mean values of triplicate measurements for each liposome batch. Cumulative DS released at various time points were plotted using Microsoft Excel 2007 and computed as in Eq 2.

\[
\text{Drug release (\%)} = \left(\frac{C_t}{C_0}\right) \times 100
\]

where \(C_t\) is the concentration of drug released at time \(t\) and \(C_0\) is the initial drug concentration

**Statistical analysis**

Each value was expressed as the mean ± SD. One-way analysis of variance (ANOVA) was applied to analyze the data, followed by Scheffe post-hoc test using SPSS 15 [for Windows 7]. Differences were considered significant at \(p < 0.05\).

**RESULTS**

**Morphology of liposomes**

TEM and SEM (Figure 1) images show that the liposomes were formed in all the formulations. FI and FII, which contained higher proportions of PC produced large and well-defined spherical vesicles; however, FIII, IV, V and VI which had palm oil fraction content of 20, 33.33, 46.67 and 56.67, respectively, produced smaller, slightly deformed vesicles. These results shows however there is possibility of preparation liposomal drug delivery systems with other natural products such as palm oil fractions, but the role of PC in formation, shape and size of liposomes is not negligible, formulation with higher percentage of PC produces more spherical vesicles in bigger size.

**Particle size and zeta potential of liposomes**

As it can be seen in Table 2, the particle size of the liposomes decrease from FI to FVI and the ZP values confirm the system stability. Results demonstrated in figure 2 shows zeta potential has close relationship with the percentage of PC and palm oil fractions in the formulations. While replacing palm oil fractions with PC, zeta potential changed to -50.7 from -31.2 mV. Introduction of 46 and 56 % of palm oil fractions significantly modified zeta potential to -42.8 and -50.7 mV, respectively.

**Calibration curve**

Based on the calibration curve of DS in PBS, the regression equation obtained was \(A = 0.0213 \times C + 0.056\), where \(A\) is the absorbance at the wavelength of 300nm, and \(C\) is the concentration of Diclofenac sodium (DS); the regression coefficient was \(R^2 = 0.9936\).

**Entrapment efficiency (EE)**

Table 2 shows that formulation FVI, with an EE of 77.8 % of DS, had the highest entrapment.
**Table 1: Composition of liposome formulations**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>PC (%w/w)</th>
<th>CH (%w/w)</th>
<th>Palmitic acid</th>
<th>Oleic acid</th>
<th>Stearic acid</th>
<th>Linoleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>66.67</td>
<td>33.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FII</td>
<td>56.67</td>
<td>33.33</td>
<td>1.60</td>
<td>1.20</td>
<td>0.60</td>
<td>6.60</td>
</tr>
<tr>
<td>FIII</td>
<td>46.67</td>
<td>33.33</td>
<td>3.20</td>
<td>2.40</td>
<td>1.20</td>
<td>13.20</td>
</tr>
<tr>
<td>FIV</td>
<td>33.33</td>
<td>33.33</td>
<td>5.33</td>
<td>4.00</td>
<td>6.00</td>
<td>22.00</td>
</tr>
<tr>
<td>FV</td>
<td>20.00</td>
<td>33.33</td>
<td>7.47</td>
<td>5.60</td>
<td>2.80</td>
<td>30.80</td>
</tr>
<tr>
<td>FVI</td>
<td>10.00</td>
<td>33.33</td>
<td>9.07</td>
<td>6.80</td>
<td>3.40</td>
<td>37.40</td>
</tr>
</tbody>
</table>

**Table 2: Mean particle size, zeta potential and entrapment efficiency of the liposomes**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean particle size (nm, ±SD)</th>
<th>Mean zeta potential (mV)</th>
<th>Mean entrapment efficiency (% ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>439 ±15</td>
<td>-31.2 ±3.3</td>
<td>70.78 ±0.41</td>
</tr>
<tr>
<td>FII</td>
<td>421 ±12</td>
<td>-29.4 ±2.0</td>
<td>60.14 ±7.48</td>
</tr>
<tr>
<td>FIII</td>
<td>387 ±13</td>
<td>-29.3 ±2.4</td>
<td>55.63 ±2.75</td>
</tr>
<tr>
<td>FIV</td>
<td>392 ±21</td>
<td>-34.3 ±1.4</td>
<td>30.98 ±8.28</td>
</tr>
<tr>
<td>FV</td>
<td>360 ±11</td>
<td>-42.8 ±1.4</td>
<td>42.25 ±4.43</td>
</tr>
<tr>
<td>FVI</td>
<td>300 ±10</td>
<td>-50.7 ±4.9</td>
<td>77.84 ±5.95</td>
</tr>
</tbody>
</table>

**Discussion**

DS liposomes obtained in this study were medium-sized, with values ranging from 300 to 439 nm depending on the proportion of PC in the system. This might be due to interactions between the lipid layers of liposome with DS. This is according to finding of Lopes which stated due to interaction of DS anion with the ammonium group of PC, the size of liposome that entraps DS reduced significantly, as the drug incorporated into the liposome [16]. Furthermore, large standard deviations found for mean particle size may be due to the broad heterogeneous sizes of the liposome. The ZP data indicate that there were repulsive forces between particles in colloidal systems and thus confirm the stability of the system. A ZP < -30 mV could result in particle aggregation and lead to caking and eventual spoilage. In this regard, FII and III with lower negative ZP would be less stable while FI, FIV, FV and FVI higher negative ZP would be more stable. A previous study suggests that interaction of DS with PC may adversely affect the structure and hence stability of the liposomes [13]. It was also observed that the liposomes were less stable the higher the proportion of PC, and more stable the higher the proportion of palm oil fractions.

Entrapment efficiency increased as the concentration of palm oil lipids increased. Hathout obtained a maximum entrapment efficiency of approximately 40 % and this is higher than the 30 % entrapment found in the...
present study [17]. Imura has stated that drug entrapment increases as PC concentration rises, and hence the lower entrapment found in our study may be due to the smaller level of PC in the formulations [18].

FII and FIII with lower negative ZP and would be less stable while F1, FIV, FV and FVI higher negative ZP would be more stable. A previous study suggests that interaction of DS with PC may adversely affect the structure and hence stability of the liposomes [16]. It was also observed that the liposomes were less stable the higher the proportion of PC, and more stable the higher the proportion of palm oil fractions.

Entrapment efficiency decreased as the concentration of palm oil lipids increased. The 30 % entrapment found in the present study is less than that earlier reported by Hathout et al [17]. Imura has stated that drug entrapment increases as PC concentration rises, and hence the lower entrapment found in our study may be due to the smaller level of PC in the formulations [18].

FII and FIII exhibited faster release among all formulations and achieved maximum 90 % release within 62 h. On the other hand, FIV and FI showed slower release after 6 h. Interaction of DS with PC might have caused deformation of the liposome besides affecting the stability and release pattern due to leakage of the drug from the unstable liposomes [16]. As the amount of PC decreased, the interaction is reduced and the stability of liposome increases. This may be the reason why FIV with a lower PC level exhibited slower release than FI which contained a higher PC content. Although FV and FVI contained higher concentrations of palm oil fractions, their liposomes were smaller.

The slower drug release obtained from FV and FVI, compared to FII and FIII, may be attributed to their higher stability and ZP [12,13] plus according to Yamauchi drug release rate will increase as liposomal size shrinks [19]. The slower drug release obtained from FV and FVI, compared to FII and FIII, may be attributed to their higher stability and ZP [12,13]; drug release rate increases as liposomal size shrinks [19].

CONCLUSION

The results of this study demonstrate the potential application of palm oil fractions in the preparation of liposomal DS. Since the amount of palm oil in formulation will significantly affect drug loading and drug release, this compound will provide the opportunity to prepare DS formulations with the desired bioavailability. By increasing the content of palm oil fraction from 10 to 33 %, more stable liposomes were obtained.

REFERENCES


