

## Original Research Article

# 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, shape, 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. Introduction of 46 and 56 % of palm oil fractions yielded zeta potential of -42.8 and -50.7 mV, respectively, compared with -31.2 mV for the formulation without palm oil.

**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.

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## 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].

Diclofenac 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, BÜCHI) 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 on 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

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

$$EE(\%) = \{(C_i - C_f)/C_i\} \times 100 \dots\dots\dots (1)$$

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.

$$\text{Drug release (\%)} = (C_t/C_o) \times 100 \dots\dots\dots (2)$$

where  $C_t$  is the concentration of drug released at time  $t$  and  $C_o$  is the initial drug concentration

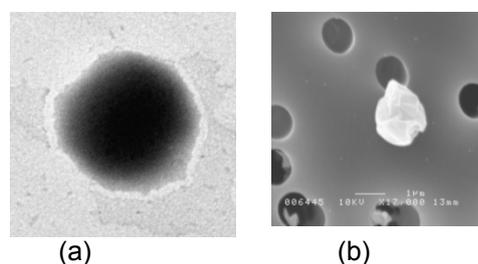
### Statistical analysis

Each value was expressed as the mean  $\pm$  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.



**Figure 1:** (a) TEM images of designed liposome, magnification 16000x; (b) SEM images of designed liposome magnification 17000x.

### 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

Formulation	Content of lipids (%w/w)					
	PC	CH	Palmitic acid	Oleic acid	Stearic acid	Linoleic acid
FI	66.67	33.33	-	-	-	-
FII	56.67	33.33	1.60	1.20	0.60	6.60
FIII	46.67	33.33	3.20	2.40	1.20	13.20
FIV	33.33	33.33	5.33	4.00	2.00	22.00
FV	20.00	33.33	7.47	5.60	2.80	30.80
FVI	10.00	33.33	9.07	6.80	3.40	37.40

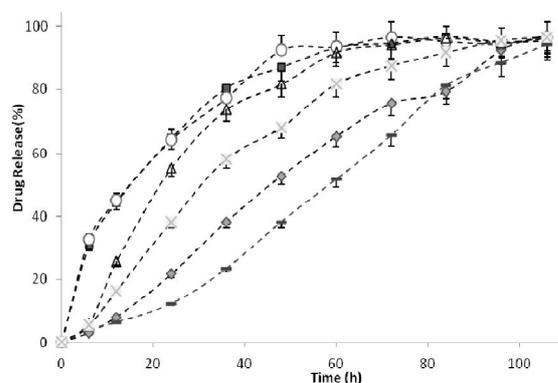
**Table 2:** Mean particle size, zeta potential and entrapment efficiency of the liposomes

Formulation	Mean particle size (nm, $\pm$ SD)	Mean zeta potential (mV)	Mean entrapment efficiency (% $\pm$ SD)
FI	439 $\pm$ 15	-31.2 $\pm$ 3.3	70.78 $\pm$ 0.41
FII	421 $\pm$ 12	-29.4 $\pm$ 2.0	60.14 $\pm$ 7.48
FIII	387 $\pm$ 13	-29.3 $\pm$ 2.4	55.63 $\pm$ 2.75
FIV	392 $\pm$ 21	-34.3 $\pm$ 1.4	30.98 $\pm$ 8.28
FV	360 $\pm$ 11	-42.8 $\pm$ 1.4	42.25 $\pm$ 4.43
FVI	300 $\pm$ 10	-50.7 $\pm$ 4.9	77.84 $\pm$ 5.85

efficiency and formulation FIV with an EE of 31.0 % had the least.

### *In vitro* drug release

Figure 3 illustrates the *in vitro* release through 106 hours for total release of DS from liposomes. DS release for FI was about 10 % through 12 h. It slightly increased until release was complete after 106 h. FII and FIII released drug of up to 44 and 46 %, after 12 h respectively, and DS release for both was complete after 72 h. FIV gradually released drug in 24 h but release increased more rapidly to 85 % after 80 h. There was a slow release of FV during the first 6 h but it was followed by a sharp rise reaching the 91 % in 48 h. DS release from FVI release rose progressively over 106 h.



**Figure 3:** Cumulative release of diclofenac sodium in PBS (pH 7.4). **Key:** F1 ( $\blacklozenge$ ), FII ( $\blacksquare$ ), FIII ( $\circ$ ), FIV ( $\triangle$ ), FV ( $\blacktriangle$ ), FVI ( $\times$ ); error bars indicate standard deviation n = 3.

## 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 decreased 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 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 [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 deformity 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 provides 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.

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