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INTRODUCTION

Liposomes have been developed as transdermal drug delivery carriers because there are numerous study focusing on the use of liposomes for enhancing skin permeation of various drugs including hydrophilic drugs (sodium fluorescein [1], carboxyfluorescein [2]), lipophilic drugs (retinoic acid [3], tretinoin [4]), genes [5], proteins [6], and macromolecules [7]. Several studies reported that elastic vesicles were more efficient in enhancing the transport of drugs than rigid vesicles. Accordingly, new categories of liposome with high elasticity have been introduced. The elastic liposome mainly consists of phospholipids and various types of penetration enhancer (e.g., edge activator, single-chain surfactant, etc.), which only a specially designed of liposome was shown to be able to allow outstanding transdermal drug delivery carriers. Recently, various kinds of elastic liposomes have been developed for enhancing the skin permeation of various drugs. The effectiveness of liposome and their physicochemical stability have been question and not yet fully understood. For example, ethosomes are novel lipid carrier that composed of phospholipid, water and high ethanol content (20-45%). This type of elastic liposome could enhance skin permeation of various drugs e.g., testosterone [8], 5-fluorouracil [9] and 5-aminolevulinic acid [10]. The high concentration of ethanol might improve the effectiveness of skin permeability. However, it might also limit their safe and stability for transdermal delivery carriers. The intensive studies of liposomal systems suggested that the liposome composition directly affected the physicochemical characteristics and their stability. The physicochemical characteristics and the stability of liposome formulation might be a major factor that should be concerned in development of novel liposome carriers. The most effectiveness for skin delivery with instability formulation was not suitable for transdermal delivery carriers. Meloxicam (MX), a nonsteroidal anti-inflammatory drug (NSAID) as a preferential COX-1 inhibitor. MX is often used clinically but oral and injectable administrations of MX are not appropriate for peptic ulcers and patient compliance. Moreover, MX has no available option for transdermal delivery. Therefore, MX is suitable for development as a transdermal delivery candidate.

The objectives of this study were to develop and investigate the effect of liposome composition on physicochemical characteristics (i.e., vesicle size, zeta potential, elasticity and drug content) and the stability (i.e., vesicle size, zeta potential and drug content) of liposome formulation. Three types of liposome formulations i.e., cationic, neutral and anionic liposome were optimized for becoming optimal transdermal delivery carrier for meloxicam. This knowledge provides the useful information for optimizing the novel liposome formulation for enhancing skin delivery of meloxicam, especially liposomes containing surfactant systems.

MATERIALS AND METHODS

Preparation of liposome formulation Three types of liposome formulations i.e., cationic liposome (cLP), neutral liposome (nLP) and anionic liposome (aLP) were prepared. The liposome formulations containing phosphatidylcholine (PC), cholesterol (Chol), cationic surfactants (cetylpyridium chloride; C16), anionic surfactants (sodium hexadecyl sulfate; A16) and meloxicam (MX) were formulated. As shown in Table 1, liposome formulations were prepared by the sonication method. Briefly, lipid mixtures of PC, Chol, surfactant and MX were dissolved in chloroform/methanol (2:1 v/v ratio), and the solvent was evaporated under nitrogen gas stream. The lipid film was dried in a desiccator for 6 h to remove the remaining solvent. The dried lipid film was hydrated with acetate buffer solution: ABS (pH 5.5). Liposomes were subsequently sonicated for 30 min using a bath-type sonicator and then duplicated sonicated in ice-bath using probe sonicator (Sonics Vibra Cell™, Newtown, CT, USA) for 30 min. An excess amount of lipid composition and MX was removed by centrifugation at 4°C using 15,000 rpm for 15 min, and the supernatant was collected. All formulations were freshly prepared or stored in airtight containers at 4 °C prior to further studies.

Measurement of physicochemical characteristics The MX loaded liposome formulations were characterized for vesicle size, zeta potential and MX content. Vesicle size and zeta potential of liposome

formulations were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, UK). Twenty µL of the liposome formulations were diluted with 1480 µL of deionized water. All measurement samples were performed at room temperature, at least three independent samples were taken, and the vesicle size and zeta potential were measured in triplicate (n=3).

Measurement of elasticity The elasticity value of the lipid bilayer of the vesicles was directly proportional to $J_{Flux} \times (r_v/r_p)^2$:

$$\text{Elasticity value } (\text{mg}\cdot\text{sec}^{-1}\cdot\text{cm}^{-2}) = J_{Flux} \times \left(\frac{r_V}{r_P}\right)^2 \qquad (1)$$

Where J_{Flux} is the rate of penetration through a permeable barrier ($\text{mg}\cdot\text{sec}^{-1}\cdot\text{cm}^{-2}$), r_v is the size of the vesicles after extrusion (nm), and r_p is the pore size of the barrier (nm) [11]. To measure J_{Flux}, the vesicles were extruded through a polycarbonate membrane (Nuclepore, Whatman Inc., MA, USA) with a pore diameter of 50 nm (r_p), at a pressure of 0.5 MPa. After 5 min of extrusion, the extrudate was weighed (J_{Flux}), and the average vesicle diameter after extrusion (r_v) was measured by Zetasizer Nano ZS instrument.

Stability study of liposome formulation The MX loaded liposome formulations were kept in glass bottle with plastic plugs and stored at 4±1 °C and 25±1 °C for 30 days. The physicochemical stability of MX loaded liposome formulation was evaluated. The vesicle size and zeta potential were determined using Zetasizer Nano ZS instrument. The MX content was evaluated by measuring MX remaining in the liposome formulation by HPLC at day 1, 7, 15, 30 and 120. The physicochemical stability of the formulation after freshly preparation (at day1) was used as control and MX content at day 1 was also normalized to 100%.

HPLC analysis The MX concentration in all samples was analyzed by HPLC. All samples were stored at 4 °C until analysis. The HPLC system consists of a SIL-20A autosampler, LC-20AT liquid chromatograph and SPD-20AUV detector (Shimadzu Corporation, Kyoto, Japan). The analytical column was YMC-Pack ODS-A (150 mm × 4.6 mm i.d., S-5, YMC Co. Ltd, Kyoto, Japan), and the mobile phase was composed of acetate buffer solution (pH 4.6)/methanol (50:50, v/v). The flow rate was set at 0.8 ml/min, and the wavelength used was 272 nm. The calibration curve for MX was in the range of 1-50 µg/mL with a correlation coefficient of 0.999. The percent recovery was found from 99.85-100.30%, and relative standard deviation for both intra-day and inter-day was less than 2%.

Data analysis The data are reported as the means ± standard error (S.E.) (n=3-6), and statistical analysis of the data was carried out using one-way ANOVA followed by student's *t*-test. A *p*-value of less than 0.05 was considered to be significant.

Table 1 The composition of different liposome formulation

Formulation	Liposome component (%W/V)					ABS pH 5.5 qs to
	PC	Chol	C16	A16	MX	
cLP	0.77	0.04	0.10	-	0.07	100 mL
nLP	0.77	0.04	-	-	0.07	100 mL
aLP	0.77	0.04	-	0.10	0.07	100 mL

RESULTS AND DISCUSSION

Physicochemical characteristics of liposome The physicochemical characteristics i.e., vesicle size, zeta potential, elasticity and MX content of liposome formulations were shown in Table 2. The addition of cationic and anionic surfactant resulted in significant difference in physicochemical characteristics of MX loaded liposome formulation. The vesicle size of cLP was significantly smaller than nLP. While, the vesicle size of aLP was significantly larger than nLP. As, the effect of neutralization between anionic drug (MX) and cationic liposome (cLP) could reduce the repulsive forces between the liposome bilayer, these might decrease the vesicle size of liposomes. In contrast, the synergistic effect between anionic drug (MX) and anionic liposome (aLP) might result in a large vesicle size [12]. The size distribution of all liposome formulation was not significant difference. The zeta potential of cLP, nLP and aLP was positive, neutral and negative charge, respectively, depended on intrinsic properties of their surfactant charge and total net charge of liposome composition. Under the experimental condition of pH 5.5, the isoelectric point (PI) of PC (PI = 6) was higher than pH. On the other hand, the PI of MX (PI = 2.6) was lower than the pH. Thus, PC and MX carried the net positive charge and negative charge, respectively. The liposome

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formulation consisted of neutral charge material e.g., phosphatidylcholine and cholesterol, positive charge material e.g., cetylpyridinium chloride and negative charge material e.g., sodium hexadecyl sulfate and meloxicam. Therefore, the total net charge of the liposome composition may affect the net charge of liposome.

The elasticity of cLP and aLP was significantly higher than nLP. Because, the incorporation of single-chain surfactant (edge activator) i.e., cetylpyridinium chloride (C16) and sodium hexadecyl sulfate (A16) could increase the elasticity of liposome bilayer [13]. The cationic and anionic surfactants which have a high radius of curvature could increase deformability of the liposome bilayer. However, these results indicated that the same carbon chain length with different polar head group of surfactant resulted in significant difference in elasticity value. Moreover, the MX content of cLP and aLP was significantly higher than nLP. These results indicated that the addition of C16 and A16 surfactant might increase the solubility of MX in liposome bilayer. Our results were consistency with the previous study [10] that the drug content of liposome with sodium stearate (anionic surfactant) was increased.

Table 2 The physicochemical characteristics of liposome formulation after freshly preparation

Formulation	Size (nm)	Zeta potential (mV)	Elasticity ($mg \cdot sec^{-1} \cdot cm^{-2}$)	MX content (%)
cLP	90.60 ± 9.20	+48.30 ± 0.67	88.7 ± 0.98	68.06 ± 0.84
nLP	108.80 ± 10.60	1.30 ± 1.01	11.6 ± 1.64	26.36 ± 0.26
aLP	164.30 ± 3.20	-60.80 ± 0.51	19.2 ± 1.68	54.11 ± 0.33

Physicochemical stability of liposome Figure 1 shows the effect of cationic and anionic surfactant on the (A) vesicle size, (B) zeta potential and (C) MX remaining of liposome formulation from day 1 to day 120 at 4 °C and 25 °C. The results indicated that no sedimentation was observed in any formulation after fresh preparation. No sedimentation was found in any formulation, after storage at 4 °C for 30 days. The vesicle size was not significantly different from the initial liposome formulation at day 1 (control). However, the sedimentation was found in the some formulation (i.e., aLP and cLP), after storage at 4 °C for 120 days. The vesicle size on day 120 had a trend to increase, and the size of liposome was larger than control, however most of formulations was smaller than 200 nm (except; aLP). After storage at 25 °C for 30 days, no sedimentation was found in any formulations till day 30, and the vesicle size had a trend to increase after the storage age of 15 days, however all formulations was still smaller than 200 nm. In contrast, after storage at 25 °C for 120 days, sedimentation was found in all formulations. The vesicle size on day 120 was significantly different from the initial formulation which was around 200-400 nm. In addition, the size distribution of all liposome formulation was not significant difference.

The zeta potential of liposome formulation was depended on the net charge of their liposome composition. After storage at 4 °C for 30 days, the zeta potential was slightly different from the initial formulation; and some formulations had a trend to increase while some formulations had a trend to decrease depended on their composition. However, after storage at 4 °C for 120 days, the zeta potential was significantly different from the initial formulation (especially; aLP). After storage at 25 °C for 30 days, the result was similar to those found in 4 °C for 30 days. However, after storage at 25 °C for 120 days, the zeta potential was significantly different from the initial formulation, and the zeta potential was markedly increased and higher than the initial formulation. The MX remaining in the formulation after freshly preparation at day 1 was normalized to 100%. After storage at 4 °C for 30 days, the MX remaining was slightly decreased but still higher than 90% of the initial formulation. However, after storage at 4 °C for 120 days, the MX remaining of almost formulations decreased around 70-80% from the initial formulation, while some of them (i.e., aLP) decreased around 90% from the initial formulation. After storage at 25°C for 30 days, the MX remaining of almost formulations was slightly decreased but still higher than 90% and 80% of the initial formulation at day 15 and day 30, respectively. However, after storage at 25°C for 120 days, the MX remaining of all formulations decreased around 80-90% from the initial formulation. Especially, the MX remaining in the formulation of aLP was lower than 10% of the initial formulation.

In short term stability (on day 1 to day 30), the results suggested that the physicochemical stability in different formulation factor was not significantly different. However, in long term stability (on day1 to day120), the results indicated that the liposome composition i.e., A16 was a major component affecting the physicochemical instability of aLP formulation. Moreover, the addition of Chol in liposome formulation might be a main factor affecting the physicochemical stability of all formulations. Because,

Chol caused an increase rigidity and packing density of PC molecules, thus Chol lead to increase stability of liposome formulation [13]. These results indicated the stability of cLP was better than nLP and aLP. The good physicochemical stability of our liposome was at 4 °C (at least) for 30 days and was also stable at 25 °C (at least) for 15 days. The physicochemical stability of liposome formulation was slightly or not significantly different between the experimental temperature of 4 °C and 25 °C for 30 days, while the physicochemical stability of liposome formulation was significantly different between the storage age of day 1 and day 120. Therefore, the storage age was the major factor and the temperature was the minor factor affecting the physicochemical stability of vesicle formulation. The recommended storage condition for the vesicle formulation was 4 °C for 30 days and/or 25 °C for 15 days.

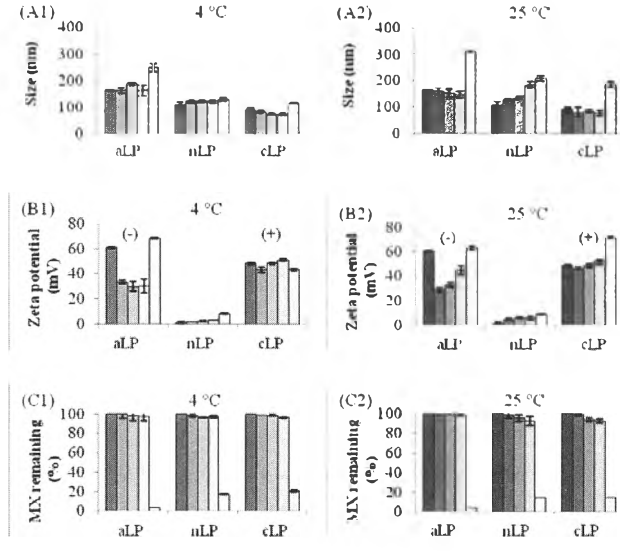

Figure 1 The effect of cationic and anionic surfactant on the (A) vesicle size, (B) zeta potential and (C) MX remaining of liposome formulation from day 1 to day 120 at 4 °C and 25 °C.

CONCLUSION

In this study, three types of liposome formulations were prepared for skin delivery of meloxicam. The physicochemical characteristics of liposome were depended on types of liposome. The liposome composition affected directly on the physicochemical characteristics of liposome formulation and their stability. Therefore, the physicochemical characteristics and the stability of liposome formulation was the major factor affecting the skin permeability of liposome formulations. These finding provided the useful information for optimizing the novel liposome formulation for enhancing skin delivery of meloxicam, especially liposomes containing cationic surfactant. The optimal formulation with good stability is used for further transdermal delivery carrier of meloxicam.

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