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INTRODUCTION

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. Since the first generation of elastic liposomes (Transfersome®) was introduced by Cevc and Blume[1] that transfersome can be used for skin delivery of various hydrophilic and lipophilic drugs into deep skin region. The new categories of liposome with high elasticity such as ethosomes[2], flexosomes[3] and invasomes[4] have been developed. Several studies suggested that the permeability of drug in liposomes and their analogues depends on their physicochemical characteristics (e.g., particle size, size distribution, zeta potential, elasticity, entrapment efficiency, etc.) and these characteristics were directly affected by lipid composition. In the development of a transdermal drug delivery systems, it is important to design the optimized pharmaceutical formulations having appropriate skin permeation. The aim of this study was to optimize and charactarize the potential use of deformable liposomes (DF) formulation for transdermal drug delivery of MX. For this objective, a nonlinear response-surface method incorporating thin-plate spline interpolation (RSM-S) was employed. Using RSM-S, complicated relationships between causal factors and response variables can be easily understood, and a stable simultaneous optimal formulation is obtained.

MATERIALS AND METHODS

The 10 formulations of MX-loaded deformable liposomes (DF) composed of a controlled amount of phosphatidylcholine (PC) and meloxicam (MX), and various amounts of cholesterol (Chol) as membrane stabilizer and cetylpyridinium chloride (CPC) as penetration enhancer were prepared. The concentration of PC and MX were fixed at 0.773 and 0.077% (w/v), respectively. The concentrations of Chol and CPC were varied from 10 to 40% (mole ratio) according to the formulation obtained from the two-factor spherical second-order composite experimental design (Table 1). The concentration of Chol (X_1) and CPC (X_2) were selected as causal factors. DF were prepared by the sonication method. Briefly, the lipid mixtures of PC, Chol, CPC and MX were dissolved in chloroform/methanol (2:1 v/v). The solvent was evaporated under nitrogen gas stream. The lipid film was placed in a desiccator for 6 h to remove the remaining solvent. The dried lipid film was hydrated with acetate buffer solution (pH 5.5). Liposomes were subsequently sonicated for two cycles of 15 min using a bath-type sonicator. Particle size, zeta potential, elasticity, entrapment efficiency and skin permeation parameters of DF formulations were investigated and used as the response variables to obtain optimal formulation. The optimal formulation estimated by RSM-S was also determined by the experiment and compared to conventional liposome (CL) for skin permeability.

Table 1 Composite spherical experimental design for two factors and model formulation

Formulation	1	2	3	4	5	6	7	8	9	10
X_1	-1	-1	1	1	$-\sqrt{2}$	$\sqrt{2}$	0	0	0	0
Chol (%)	14.4	14.4	35.6	35.6	10	40	25	25	25	25
X_2	-1	1	-1	1	0	0	$-\sqrt{2}$	$\sqrt{2}$	0	0
CPC (%)	14.4	35.6	14.4	35.6	25	25	10	40	25	25

RESULTS

Identification of the response surface The 10 formulations of DF were prepared and formulated. The concentration of Chol and CPC were selected as causal factors. The physicochemical characteristics of DF (particle size, size distribution, zeta potential, elasticity, entrapment efficiency and skin permeation parameters) were selected as response variables. The response surfaces estimated by RSM-S show the relationship between causal factors response variables.

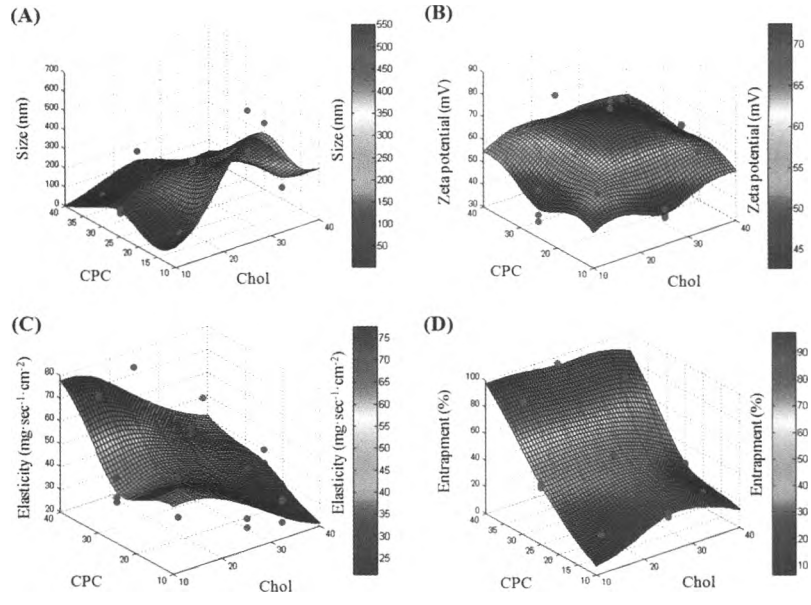

Fig. 1 The response surface of the model formulation of (A) particle size, (B) zeta potential. (C) elasticity and (D) entrapment efficiency.

Fig. 1 shows the response surfaces of particle size, zeta potential, elasticity and entrapment efficiency determined by RSM-S. The response surface represented the effect of Chol and CPC in MX-loaded DF on their physicochemical characteristics. The response surfaces suggested that an increase of Chol resulted in a significant increase in size, a decrease in elasticity and a slight increase in entrapment efficiency, while an increase in CPC resulted in a significant decrease in size, an increase in zeta potential, an increase in elasticity and an increase in entrapment efficiency.

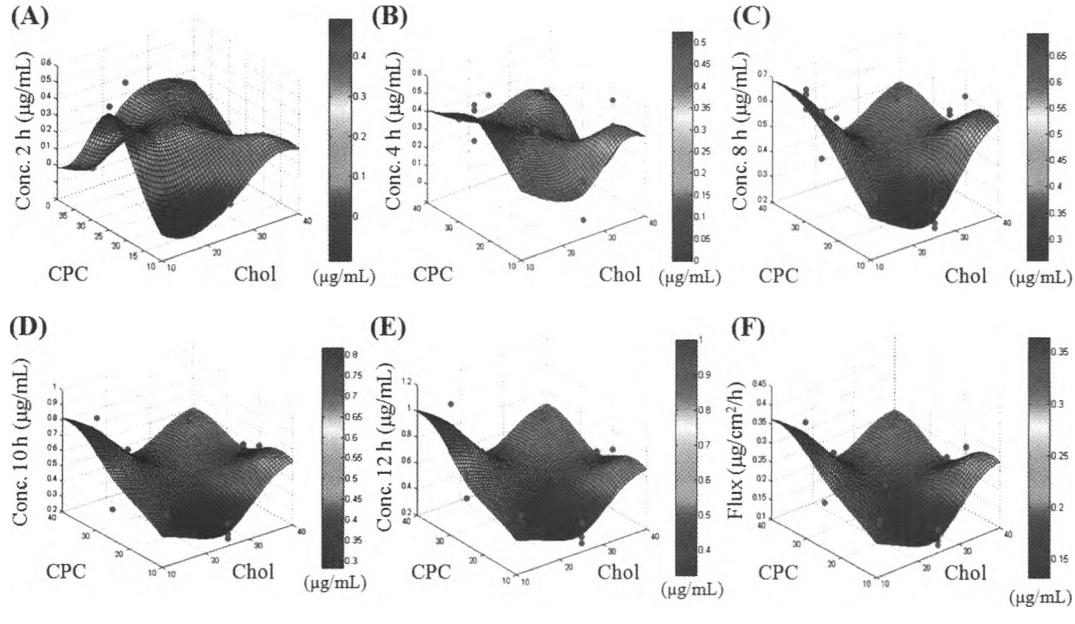

Fig. 2 The response surface of the concentration of MX permeated the skin at *t* h of (A) 2 h. (B) 4 h, (C) 8h, (D) 10 h, (E) 12 h and (F) flux.

Fig. 2 shows the response surfaces of the concentration of MX-permeated skin at 2, 4, 8, 10 and 12 h and the steady state flux determined by RSM-S. The effect of incorporating Chol and CPC on the skin permeation of MX-loaded DF was evaluated using an *in vitro* skin-permeation study. The similar patterns of response surface suggested that the concentration of MX-permeated skin and flux increased as the concentrations of Chol and CPC increased. This result suggested that RSM-S successfully estimated the relationship between the causal factors and response variables attributed to the DF.

Table 2
Predicted and experimental response variables for the optimal formulation

Response	Concentration of MX permeated the skin at *t* h (μg/mL)					*Flux*
	2 h	4 h	8 h	10 h	12 h	(μg/cm^2/h)
Predicted	0.36	0.50	0.64	0.68	0.79	0.31
Experimental	0.40±0.13	0.50±0.14	0.68±0.15	0.73±0.11	0.82±0.11	0.31±0.06

Optimization of DF formulation The formulation of MX-loaded DF was optimized based on the original data set using RSM-S. The search directions for the response variables were set to produce a high concentration of MX-permeated skin at 2, 4, 8, 10 and 12 h and also a high flux. X_1 = 10.5 and X_2 = 29.0 (% mole ratio) were estimated as the optimal formulation. The optimal formulation estimated by RSM-S was formulated by the experiment. The following variables estimated RSM-S and the experiment values are shown in Table 2 and Fig. 3A. Moerover, the skin permeability of optimal formulation and conventional liposomes (CL) was also compared as shows in Fig. 3B for confirming the potential of DF optimized by RSM-S.

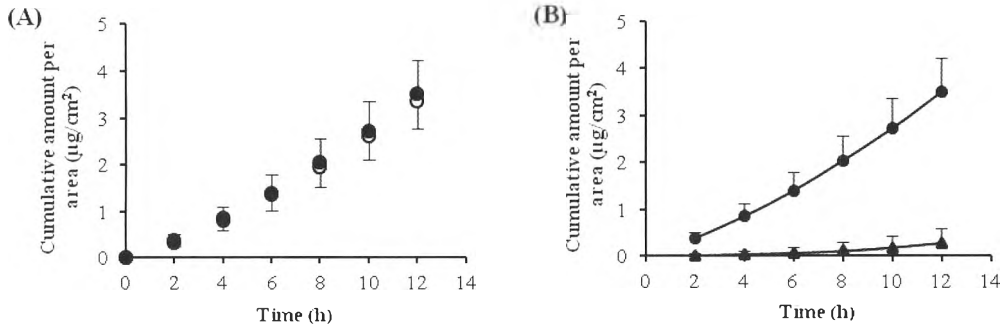

Fig. 3 (A) The skin-permeation profile of MX from the optimal formulation: (●) experimental values; (○) predicted values. (B) The skin permeation profile of MX from the experimrntal optimal formulation (●) and conventional liposomes (▲). Each experimental value is a mean ± S.D. (n = 3-4).

DISCUSSION

Several studies of liposomes and their analogues for skin permeation studied under different conditions (*in vitro*, *in vivo*, *ex vivo*), different skin model (human, animal), different type of drug (hydrophilic, lipophilic) and different lipid composition (type, amount); therefore, the obtained results cannot be compared and used to fully understand the behavior. According to this problem, the effect of lipid compositions on the physicochemical characteristics and skin permeability of MX in DF was studied by reliable statistical techniques. The response surface of the physicochemical characteristics suggested that lipid compositions (Chol and CPC) were closely related to physicochemical characteristics e.g., particle size, zeta potential, elasticity and entrapment efficiency of DF.

The presence of Chol in DF, the particle size increased when the Chol in DF was low concentration (10–25% mole ratio). It has been reported that 11 mol% Chol can increase the net repulsion force and reduce the van der Waals attraction force between PC bilayers[5], whereas the particle size decreased as the Chol in DF was high concentration (30–40% mole ratio) which might be attributed to the decrease in surface energy with increase in hydrophobicity[6]. The presence of Chol had a slight effect on zeta potential[5]. Consistency with the previous study[7], as Chol results in an increased packing density of PC molecules, which led to decreased elasticity of the PC bilayers. In addition, the incorporation of Chol had a slight effect on increasing entrapment efficiency when the Chol in DF was low concentration (10–25% mole ratio), whereas the DF containing high concentration of Chol (30–40% mole ratio) resulted in a slight decrease or no significant increase in entrapment efficiency. The effect of the amount of Chol on particle size and entrapment efficiency have been consistented with a previous study[8], which reported that when high Chol concentration (30–50%) is incorporated in the liposome formulation, the hydrophobicity in the interfacial region of the lipid bilayer can increase, and this factor could influence entrapment efficiency within the bilayer. However, the obtained results indicated that in the case of this hydrophobic drug (meloxicam; MX), a minor increase in entrapment efficiency occurred in the formulations containing low Chol concentration and a minor decrease in entrapment efficiency containing high Chol concentration may be the result of two contradictory factors. On the one hand, increase in hydrophobicity of the bilayer with increasing Chol concentration (at low Chol) may efficiently entrap the MX within the lipid bilayer. On the other hand, high Chol concentration may compete with MX for packing space within the bilayer, thereby excluding MX as the amphiphiles assemble into liposomes.

These results indicated that the incorporation of Chol and the amount of Chol affects the particle size, zeta potential, elasticity and entrapment efficiency of the DF.

The presence of CPC in DF, as CPC was increased, particle size decreased. It has been reported that the size of vesicles loaded with a positively charged drug was smaller for anionic vesicles because of neutralization of their negative charge[9]. Correspondingly, the vesicles with our negatively charged drug, MX, were also smaller when incorporated in a positively charged (cationic) surfactant because of neutralization. The incorporation of CPC (a cationic surfactant) resulted in a significant increase in positive charges on the DF. The DF containing CPC also had a significant effect in increasing the elasticity because of intrinsic properties of surfactant. CPC has a high radius of curvature that may destabilize lipid bilayers of the DF and increases deformability of the bilayers[7]. Moreover, the CPC within the DF also makes a significant effect in increasing entrapment efficiency because the beneficial role of surfactant within lipid bilayers is well recognized as leading to solubility enhancement of MX in the vesicle bilayer. These results indicated that the incorporation of CPC and the amount of CPC also affects the particle size, zeta potential, elasticity and entrapment efficiency of the DF.

The lipid composition (Chol and CPC) was the important factor affecting the physicochemical characteristics and also affected the skin permeability of MX-loaded DF. The optimal formulation was confirmed the accuracy and reliability of the optimal formulation estimated using RSM-S. by experiment. The physicochemical characteristics and *in vitro* skin permeation of the experimental optimal formulations were also formulated and determined. The lipid composition of the optimal formulation was PC:Chol:CPC = 10:1.05:2.90 mole ratio. The concentration of MX-permeated skin at 2–12 h and the flux values predicted by the RSM-S were very close to the experimental values (Table 2 and Fig. 3A), suggesting that RSM-S successfully estimated the optimal formulation of MX-loaded DF. Moreover, the comparative study of optimal formulation estimated by RSM-S and CL (Fig. 3b) suggested that optimal formulation had a significant higher skin permeability than CL. These results indicated that optimal formulation estimated by RSM-S was suitable for transdermal delivery carriers of MX.

CONCLUSION

Considering the concentration of MX-permeated skin and steady state flux value of the optimal formulation estimated by RSM-S, as compared to experimental optimal formulation and conventional liposomes, indicating that our study was successful in showing the feasibility of transdermal delivery carriers of MX using deformable liposomes.

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