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Stability and characterisation of phospholipid-based curcumin-encapsulated microemulsions

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ABSTRACT

The ternary phase diagram of a curcumin-encapsulated O/W microemulsion system using food-acceptable components, lecithin and Tween 80 as the surfactants and ethyl oleate as the oil phase, was constructed. The stability and characterisation of curcumin in microemulsion were investigated. The results indicated that a composition of curcumin microemulsion (DI water: surfactants (the mole ratio of lecithin/Tween 80 was 0.3): EO = 10:1.7:0.4 in wt ratio) was stable for 2 months with an average diameter of 71.8 ± 2.45 nm, as detected by UV–Vis spectra and diameter distributions. The microemulsion possesses an ability to be diluted with aqueous buffer without destroying its structure for 48 h. A skin permeation study for testing the penetration effect of various curcumin loading in the microemulsions with different particle diameters was also performed and discussed in this contribution.

1. Introduction

Curcumin (diferuloylmethane), the yellow pigment in turmeric and curry, is used extensively in the food and chemical industry as a colouring, flavouring and preservative agents. It has also been found to exhibit anti-oxidative (Sharma, 1976; Sharma, Stutzman, Kelloff, & Steele, 1994) and anti-inflammatory properties in clinical trials (Lin, Chen, Huang, & Lin-Shiau, 1997; Sharma, 1976; Sharma et al., 1994). During the past decades, many research reports indicated that curcumin can induce apoptosis and suppress the formation of procarcinogens through various mechanisms (Chauhan, 2002; Huang et al., 1994; Leu & Maa, 2002). Curcumin possesses not only chemopreventive but also anti-cancer activities (Huang et al., 1992, 1994; Kunchandy & Rao, 1990; Sharma et al., 1994). Curcumin has been considered by the National Cancer Institute (NCI) as the third generation of cancer chemopreventive agent in America and phase II clinical trials have been carried out in Germany (Tamimi, Lagiou, Adami, & Trichopoulos, 2002).

Curcumin with polyphenolic structure is water insoluble and scarcely dissolved in the organic phase. Previous studies addressing the absorption and metabolism of curcumin after oral administration showed its poor bioavailability in vivo (Ravindranath & Chandrasekhara, 1980, 1981, 1982). Curcumin, however, is unstable at neutral-basic pH values and in serum-free medium, and is degraded to vanillin, ferulic acid, feruloyl methane and trans-6-(4′-hydroxy-3′-methoxy-phenyl)-2,4-dioxo-5-hexenal (Huang et al., 1994).

Microemulsions have attracted much interest over the past years as potential drug delivery systems because of their transparency, ease of in preparation and long-term stability (Kreilgaard, 2002). Moreover, the radius of microemulsion is as small as less than 100 nm, which indicates that the nano-scale effect of microemulsion will enhance either the penetration into or absorption by cells. Microemulsions are self-assembled mixtures of water, oil and surfactants and have the advantages of being optically isotropic and thermodynamically stable. It is also known that using mixed surfactants or adding can reduce the surface tension between oil and water when preparing microemulsion. Much attention has recently been given to the utilisation of phospholipids in formulating pharmaceutically acceptable microemulsions (Lundberg, 1994; Magdassi & Siman-Tov, 1990). Choice of the surfactant is critical for the formulation of microemulsions. The hydrophilic–lipophilic balance (HLB) of surfactant may be adjusted by a short-chain alcohol, or adding either a non-ionic surfactant for the preparation of stable microemulsion.

Lecithin, which has two long hydrocarbon chains, is a major component of lipid bilayers of cell membranes and a natural, biological amphiphile. Furthermore, it is in many respects regarded as an ideal biological surfactant because it is biodegradable. It may be used for various purposes. Careful selection of additives can help adjust appropriately the hydrophilic–lipophilic balance (HLB). Recent study has indicated that phosphatidylcholine embedded microemulsion systems improved the transmembrane bioavailability in both rat skin and Caco2 cells (Spernath, Aserin,

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Sintov, & Garti, 2008; Spernath, Aserin, Ziserman, Danino, & Garti, 2007). The present research was carried out to study the solution properties of lecithin in relation to its potential use for nutraceutical and cosmeceutical applications.

In the field of nutraceuticals and functional foods, there has been growing interest in taking the advantages of microemulsion in solving the problems of solubility as well as stability of nutraceuticals and food additives in aqueous solution. The techniques and applications of nanoparticles in nutraceutical delivery system has been discussed and reviewed significantly over the past few years (Acosta, 2009; Flanagan & Singh, 2006). Recent studies in food microemulsions have focused on using food-grade, non-ionic surfactants derived from natural products (Garti, Yaghmur, Leser, Clement, & Watzke, 2001). The solubilisation of lycopene and lutein derivatives in aqueous media was successfully improved by Amar et al. using microemulsion technology (Amar, Aserin, & Garti, 2003; Sherman, 1968). Construction of lecithin-based microemulsions with a wide range of food-acceptable surfactant in food application has been studied (Flanagana, Kortegaarda, Pinderb, Rades, & Singha, 2005; Patel, Schmid, & Lawrence, 2006). However, no publication regarding the encapsulation of curcumin involving food-grade concerns has been appeared.

The stability of curcumin in microemulsion and solid lipid nanoparticles (SLNs) has been discussed (Tiyaboonchai, Tungpradit, & Plianbangchang, 2007). However, high dose of ethanol used in the formulation and submicron of particle diameter (about 450 nm) in SLNs implied a need for improvement. The main aim of this study was to find stable microemulsions containing curcumin encapsulated with food-acceptable ingredients, lecithin and polysorbate 80 (Tween 80), to enhance the bioavailability of curcumin. A skin permeation study was performed for testing the penetration effect of various curcumin loading doses with different particle diameters. Not only does microemulsion offer the space for curcumin carrier, it also prevents the degradation of curcumin.

2. Materials and methods

2.1. Chemicals

Lecithin (L-α-phosphatidylcholine, purity >60%), soybean oil (SO), Tween 80, ethyl oleate (EO) and isopropyl myristate (IPM) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Peppermint oil (PO) and curcumin powder of 70% purity were purchased from Fluka (Seelze, Germany). Deionised water (DI water) with conductivity above 17 ΩM cm was used for preparing microemulsions in this study.

2.2. Solubility of curcumin in different solvents

To test the solubility of curcumin at room temperature, five different solvents, including deionised water (D.I. water), ethyl oleate (EO), peppermint oil (PO), soybean oil (SO) and isopropyl myristate (IPM) were chosen in this study. One milligram of curcumin was added to a test tube containing 10 g of solvent using a vortex mixer (KMC-1300V, Vision Scientific Co., Kyunggi-do, Korea) to dissolve curcumin for approximately 10 min. If curcumin could dissolve in this solvent, the previous procedure was repeated until the mixture maintained a persistent cloudy appearance or visible grains of solid were found deposited at the bottom of the test tube after being mixed for 10 min. To determine the maximum solubility, curcumin was added to the solvent until no more dissolution occurred.

2.3. Preparation of microemulsion with encapsulated curcumin

The ternary phase diagram of curcumin microemulsion was constructed where the microemulsion region was identified by a clear and transparent appearance of the solution. The mixture, prepared by adding appropriate amounts of oil, lecithin and Tween 80 of different mole ratios as surfactants, and curcumin in a test tube, was kept at 50 °C and well mixed using a vortex mixer. Water was then added to the mixture in a bath sonicator at 50 °C until a transparent and isotropic microemulsion was obtained.

2.4. Measurement of diameters distributions of curcumin-encapsulated microemulsions

The diameter distributions of curcumin-encapsulated microemulsions in different formulations were measured by a Zetasizer NANO-S (Malvern Instruments Ltd., Worcestershire, UK), equipped with a 632.8-nm 4 mW Helium–Neon laser light source. Diameter distributions were calculated using autocorrelation data analysis by the NIBS® (non-invasive back scatter) technology built in for increased particle sizing sensitivity, thus making it possible to characterise proteins and polymers of less than 1 nm in diameter and with molecular weights as low as 1000 Da. For fitting, 70 bins distributed logarithmically between 0.4 and 10,000 nm were chosen. The collection times for the autocorrelation function were 1–3 min.

2.5. Stability of curcumin microemulsions

A stable composition of curcumin microemulsion (DI water: surfactants (lecithin/Tween 80 = 0.3): EO = 10:1.7:0.4 in wt ratio) was diluted consecutively from 30, 60, 120 and 240 folds of the stock solution. The stability of microemulsions was then measured by UV/Vis absorption. If the microemulsions are not stable, the curcumin encapsulated will be released instantly and the UV/Vis absorption of the sample will decrease. Several other stable micelle compositions with curcumin encapsulated were also tested for the time course experiments.

2.6. Encapsulation capacity of curcumin in microemulsions

Various amounts of curcumin (ranging from 5 to 60 mg) were added to a 10.63-g microemulsion formulated as described above. The resultant microemulsions were passed through a 0.45-μm filter to remove excess curcumin and then subjected to high pressure liquid chromatography (HPLC) for analyzing the loading capacity. The separation was performed on a Cosmosil 5C 18 MS column (5 μm, 25 cm × 4.6 mm I.D., Nacalai Tesque, Kyoto, Japan). The sample (20 μl) was eluted with the mobile phase composed of 0.1% H_3PO_4 (40%) and acetonitrile (60%). The flow rate and detection wavelength were set to be 1.0 ml/min and 420 nm, respectively. The standard curve of curcumin in 50% of ethanol ranging from 0.1 to 0.001 mg/ml was used for calculation.

2.7. Skin permeation of curcumin microemulsions

The permeability of curcumin through the BALB/c mouse skin was investigated using Franz diffusion cells with an effective diffusional area of 0.785 cm^2. The hair of mice was removed. The skins were excised and then clamped between the donor and the receptor chamber with 5.6 ml of cell volume. The receptor chamber was filled with 50% of ethanol to ensure sink conditions. The receptor chamber was thermostated at 37 °C and the solution was stirred continuously at 300 rpm. One millilitre of formulation was pipetted into each donor compartment and sealed with paraffin to prevent evaporation. At time interval of 2, 4 and 6 h, 500 μl of the receptor medium was taken for determining the permeated amount of curcumin using its unique absorbance at 420 nm. At the end of the experiment, the skin was removed from the Franz cell, cleaned with a gauze soaked in 50% of ethanol. The permeation area of skin was

then excised, soaked in 5 ml of ethanol and homogenised at 13,000 rpm. The resultant solution was filtered and the amount of curcumin remaining in the skin was measured by a GENESYS UV–Vis spectrometer (Thermo spectronic, Madison, WI) at 420 nm. The standard curve of curcumin in 50% of ethanol ranging from 10 to 1000 ng/ml was used for calculation.

3. Results and discussion

3.1. Encapsulation of curcumin into microemulsions

The solubility of curcumin at room temperature has been determined using four kinds of oil, PO, SO, IPM and EO, which are pharmaceutically acceptable ingredients for preparing microemulsion. The results showed that curcumin is insoluble in D.I. water and the solubilities of curcumin in SO, IPM, PO, and EO were 0.02, 0.03, 0.03 and 0.04 wt%, respectively.

A micromulsion containing one of the oils was prepared when the weight ratio of aqueous phase (deionised water): surfactants (Tween 80 + lecithin): oil phase ranged from 10:5:1 to 10:1:0.3 at the fixed lecithin to Tween 80 molar ratio of 0.3. In these regions of the formula, homogeneous and yellow transparent curcumin-encapsulated microemulsions were obtained using IPM and EO as the oil phase. On the contrary, the solutions were yellow milky turbid and did not form stable microemulsions when SO and PO were used as the oil phase. The characteristics of curcumin-encapsulated microemulsion with the four oils at the weight ratio of DI water/surfactant/oil of 10/2.6/1 was shown in Table 1. Moreover, the curcumin-encapsulated microemulsion became turbid after 1 week when IPM was used as the oil phase in the formulation, but the microemulsion remained transparent even after 2 weeks when EO was used. In view of this, EO was chosen as the oil phase for preparing a stable microemulsion in this study.

3.2. Stability of curcumin microemulsions

In this study, the microemulsion that remained yellow transparent and isotropic for at least 14 days at 37 °C after being sonicated for 20 min was defined as a stable microemulsion. The pseudo ternary phase diagram is presented in Fig. 1a. The phase behaviour of the systems composed of lecithin, Tween 80, DI water and curcumin soluble in EO was examined There were stable microemulsion regions which were in the inside area of these enclosed curves when the curcumin concentration was 2.8 mM at the lecithin/Tween 80 mole ratio of 0.15, 0.2, 0.3 or 0.6, respectively. The largest stable oil-in-water microemulsion region was obtained with the lecithin/Tween 80 molar ratio of 0.3. The range of oil-phase weight percent in formulation of microemulsion varied from 2.1 to 10.3 wt% when the lecithin/Tween 80 mole ratio was 0.3.

The four formulas of microemulsion, A, B, C and D, inside the enclosed curve of the lecithin/Tween 80 molar ratio of 0.3 in Fig. 1b were prepared for further studies. The weight ratio of DI water/surfactant/oil were 10.0/2.3/1.1, 10.0/2.0/0.9, 10.0/1.3/0.4 and 10.0/1.7/0.4 for formula A–D, respectively. The mean diameters of the four microemulsions were examined by diameter analysis after 0, 4, 7 and 14 days of storage in the dark at room temperature, as shown in Fig. 1c.

Although the colour of microemulsions remained transparent yellow in microemulsions A–D for 14 days, the mean diameters of microemulsion droplets were gradually increased at the 14th day in the formula of A, B and D. It was possible that the microemulsion droplets with more oil wt% (over 0.5%) or less surfactant/oil ratio (lower than 4:1) aggregated readily after 14 days. In micoemulsion C, the mean diameter of droplet almost maintained constants at 72.8 ± 2.76 nm. Fig. 2a and b present the diameter distribution and the UV–Vis absorption of the curcumin-encapsulated microemulsion C, as shown in Fig. 1b, at the 0th, 4th, 7th and 14th day at room temperature (25 °C). There were no significant differences in diameter distribution as well as the absorption curve of UV–Vis spectra among the microemulsions during 2-week study. The results demonstrated that microemulsion C was more stable than microemulsions A, B and D at 25 °C with no aggregation and curcumin was not released from the microemulsion after 2 weeks. In addition, the long-term stability of the four microemulsions was compared by measuring the particle diameters during 60 days. The result indicated that the microcmulsion C was very stable when kept at freezer (4 °C) for 2 months with averaged diameter of 71.8 ± 2.45 nm as showed in Fig. 2b, however, the particle diameters of compositions A, B and D were gradually increased over the period of time.

Table 1
The characteristic of curcumin-encapsulated microemulsion formation with different oil phase when the surfactant was lecithin/Tween 80 (mole ratio in 0.3), and aqueous phase (deionised water): surfactant (Tween 80 + lecithin): oil phase = 10:2.6:1 (wt%).

Oil phase	Characteristic	Status
Ethyl oleate (EO)	Homogeneous yellow transparent microemulsions	
Isopropyl myristate (IPM)	Homogeneous yellow transparent microemulsions	
Soybean oil (SO)	Homogeneous yellow milky turbid	
Peppermint oil (PO)	Homogeneous yellow milky turbid	

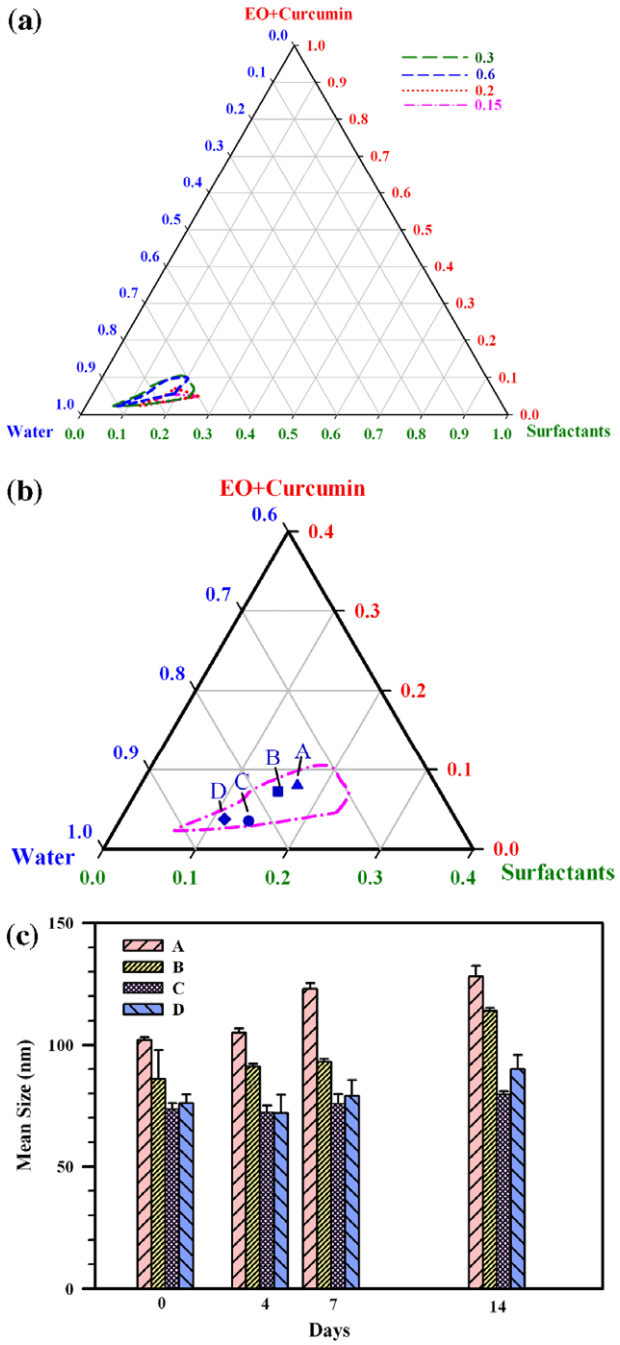

Fig. 1. The ternary phase diagram of curcumin microemulsions in a mixture of DI water, surfactants, and EO mixture. (a) The stable areas of curcumin microemulsions when using surfactant with the lecithin to Tween 80 molar ratio being increased from 0.15 to 0.6. (b) The molar ratio of lecithin to Tween 80 was 0.3. (c) The mean diameter of curcumin-encapsulated microemulsions in 2 weeks and the composition of the microemulsions was the same as that of microemulsions A–D of (b).

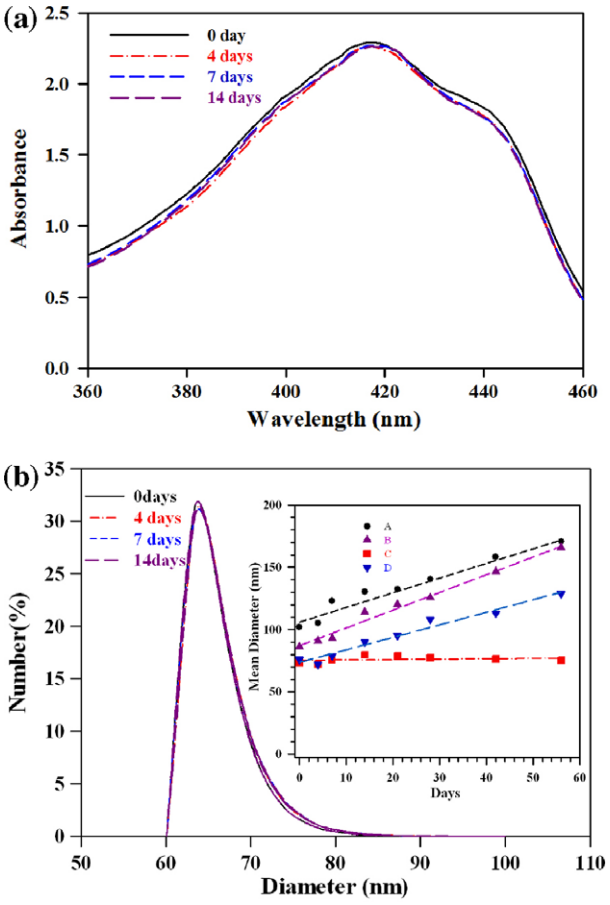

Fig. 2. (a) The UV–Vis absorption of the curcumin-encapsulated microemulsions after storage at room temperature (25 °C) for 2 weeks. (b) The diameter distribution of the curcumin-encapsulated microemulsions. The stable composition was microemulsion C as shown in Fig. 1b.

3.3. Stability of curcumin-encapsulated microemulsion after dilution

According to the above discussion, one stable curcumin-encapsulated microemulsion (i.e., point C in Fig. 1b) with the weight ratio of DI water/surfactant/oil being 10.0/1.7/0.4, was selected to test the stability of the microemulsion by dilution with an aqueous buffer of pH 7.4 at 30, 60, 120 and 240 folds of microemulsion volume, respectively. The stabilities of these curcumin-encapsulated microemulsions were determined by measuring their absorbance when stored in the dark at 25 °C for 0, 24 and 48 h as shown in Fig. 3. The results indicated that the absorbance at 420 nm decreased with increased storage time at each dilution fold. After 48 h of storage, the decreased rates of curcumin absorption were 7%, 14%, 28% and 29% at 30, 60, 120 and 240 folds of dilution, respectively. The results suggested that when the molar ratio of lecithin to Tween 80 was firmly settled at 0.3 in the interfacial layer to stabilise the structure, the integrity of most microemulsion droplets was maintained after dilution through 48 h. On the other hand, the folds of dilution volume were in direct proportion to the decreased rates of curcumin absorption from 30 to 120 folds of dilution volumes. However, the decreased rate was almost equal at 120 and 240 folds of dilution. It is possible that the decreased rate of curcumin reached a fixed value from 120 to 240 folds of dilution. These results indicated that the stability of the microemulsion system in our study is better than that in Ruth's study where the microemulsion turned turbid and phase separation was observed after dilution (Ruth, Attwood, Ktistis, & Taylor, 1995).

3.4. Determination of the loading capacity of curcumin

In order to determine the maximum loading capacity of curcumin in the microemulsion, a series of increasing amounts of curcumin were loaded to the previous formulations. The result indicated that as the amount of curcumin added increased, the encapsulated concentration increased in a dose-dependent manner. The calculated percentages of incorporation efficiencies were exceeded 90% (data not shown). The maximum capacity was obtained at 4.1 mg/ml and then dropped immediately. This phenomenon is

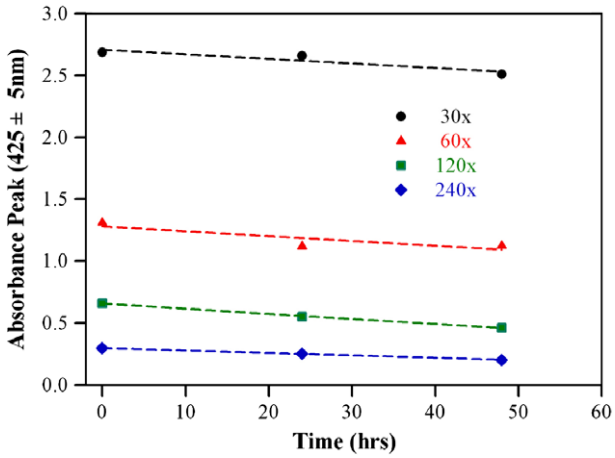

Fig. 3. The stability study of the dilution effect of the curcumin microemulsions in aqueous buffer.

probably due to the sudden rupture of the thermodynamically stable microemulsion.

3.5. In vitro skin permeation studies

Three microemulsion formulations prepared from loading curcumin of different doses (MEC1, MEC2, MEC3) were tested for their permeation into the mouse skin. Owing to the poor solubility of curcumin in water, 50% of ethanol was used as the donor medium to provide the sink condition. The amounts of curcumin permeated as well as retained in mouse skins were determined at – hour time intervals of permeation for the three formulations. As shown in Fig. 4a, time-dependent increases in permeated curcumin were observed in both MEC1 by 1.3, 3.9 and 8.4 and MEC2 by 2.1, 5.5 14.4 μg/cm^2 at 2, 4 and 6 h, respectively. On the contrary, MEC3 exhibited lower permeation activity and non-time-dependent manner compared with the results of MEC1 and MEC2. In Fig. 4b, MEC1 caused the cumulative amounts of curcumin by 0.9, 1.2 and 4.1 and MEC2 by 3.9, 5.9 and 8.8 μg/cm^2 at 2, 4 and 6 h, respectively. However, MEC3 retained low amount of curcumin in the mouse skin up to approximately 2 μg/cm^2 after 4 h. Although the loading dose of curcumin in MEC3 was higher than in MEC1 and MEC2, the enhancing effect by concentration gradient across the skin was not observed in MEC3. To further characterise the physical properties of these three formulations, the diameter distributions were determined and the result was shown in Fig. 4c. For MEC1 and MEC2, the diameters were distributed within the range of 80–120 nm. In the case of MEC3, an abrupt increase in diameter was observed with the mean diameter of 1 μm. It is concluded that though MEC3 entrapped the maximum curcumin, the thermodynamic stability of the microemulsion system was destroyed as indicated by the sudden increased particle diameter of MEC3.

Several studies on the mechanism of microemulsion have concluded that there are three key factors that may contribute to the enhancement of skin permeation, i.e., the mobility of the bioactive ingredient in the designed formulation, the concentration gradient and the particle diameter (Chen et al., 2004; Escribano, Calpena, Queralt, Obach, & Domenech, 2003; Peltola, Saarinen-Savolainen, Kiesvaara, Suhonen, & Urtti, 2003; Sintov & Shapiro, 2004). The choice of oil components, surfactant/co-surfactant and co-solvent in the formulation should influence the ease of releasing the ingredient across the barrier. A recent study indicated that the enhancement of anti-inflammation activity in mouse ear inflammation is correlated with the particle diameters of curcumin nanoemulsions (Wang et al., 2008). In our study, the result indicated that the increased concentration gradients in MEC2 and the smaller particle

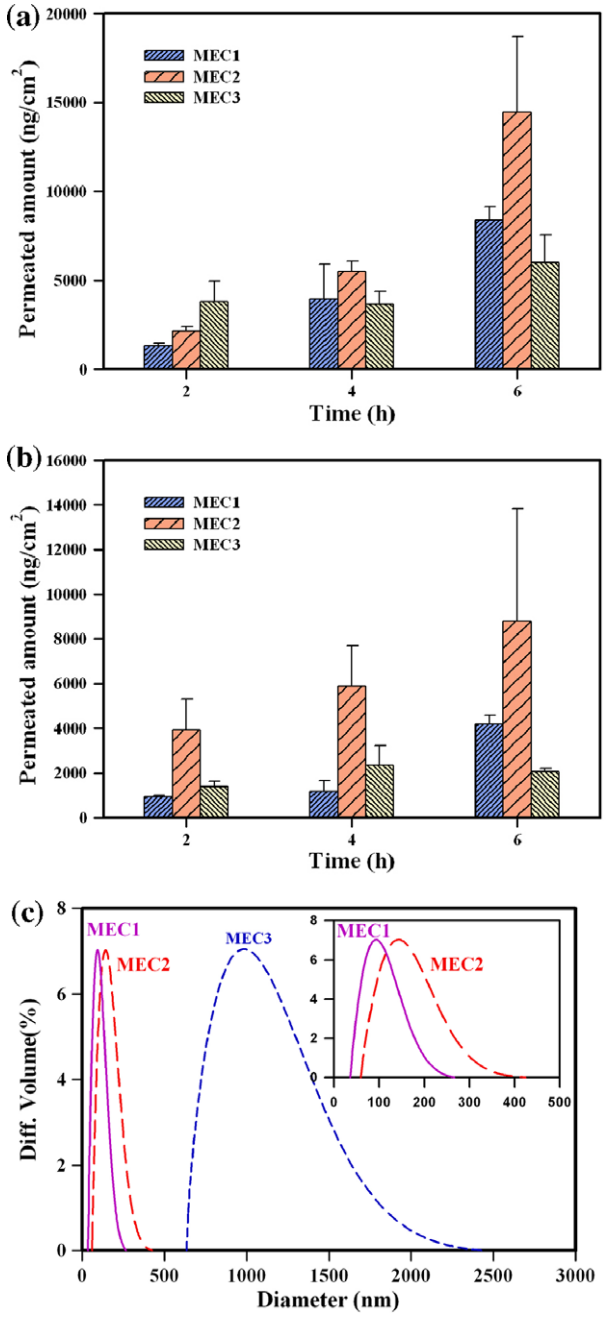

Fig. 4. The effect of the loading dose of curcumin in microemulsion on mouse skin permeation. (a) The time-course profile of curcumin permeation through the skin. (b) The time-course profile of curcumin remaining in the skin. Each data represents an average of 3–6 determinations. MEC1-3 were prepared by loading 10, 30 and 50 mg of curcuminoids to 10 ml of microemulsion solution, respectively. (c) The diameter distributions of the microemulsions of the three formulations MEC1, MEC2 and MEC3.

diameters, as in the two cases of MEC 1 and MEC2, resulted in skin permeation enhancement, in comparison with MEC3. Further study on the controlled release of this established microemulsion system is underway.

In conclusion, this study demonstrated the preparation of a curcumin-encapsulated oil-in-water microemulsion. The results show that the maximum amount of oil solubilised in the microemulsion system was 10.3 wt% when the lecithin/Tween 80 molar ratio was 0.3. The stability and characteristics of curcumin in microemulsion were examined. The findings indicated that the encapsulation of curcumin in microemulsion was not only preventing the degrada-

tion process of curcumin but also increasing the concentration of curcumin in aqueous solution. The microemulsion in particular possesses an ability to be diluted without destroying its structure. In the in vitro skin permeation study, both the dose–response and time-dependent studies of the encapsulated curcumin formula showed that MEC2 was the most suitable formulation with reduced particle diameter and maximum permeation capability. In particular, the formulation has the advantage of non-alcoholic co-surfactant. These properties make it a potentially suitable dosage form for delivery in nutraceuticals and functional food area.

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