Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 352 (2008) 248–255
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Characteristics of niosomes prepared by supercritical carbon dioxide ($scCO_2$) fluid

Aranya Manosroi [a,b,*], Romchat Chutoprapat [b], Masahiko Abe [c], Jiradej Manosroi [a,b]

[a] *Pharmaceutical-Cosmetic Raw Materials & Natural Products Research and Development Center (PCRNC), Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*
[b] *Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand*
[c] *Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Chiba, Japan*

Received 27 July 2007; received in revised form 9 October 2007; accepted 10 October 2007
Available online 22 October 2007

Abstract

Characteristics of niosomes prepared by a novel supercritical carbon dioxide fluid ($scCO_2$) technique have been investigated. Niosomes were composed of Tween61/cholesterol at 1:0, 3:1, 1:1, 1:3 and 0:1 molar ratios and entrapped with D-(+)-glucose by the $scCO_2$ method without and with ethanol at 5, 10 and 15 % (w/w) as a co-solvent, and the conventional chloroform film method with sonication. Tween61/cholesterol at 1:1 molar ratio niosomes prepared by all methods exhibited the best physical stability. Niosomes by the $scCO_2$ method with 10 % (w/w) ethanol gave higher trapping efficiency ($12.22 \pm 0.26\%$) than those by the conventional chloroform film method with sonication ($10.85 \pm 0.24\%$) and the $scCO_2$ method without ethanol ($8.40 \pm 1.60\%$). Niosomes by the $scCO_2$ method with and without ethanol were large unilamellar structure under TEM with the average sizes of 271.9 ± 159.6 and 202.5 ± 136.7 nm, respectively, whereas those by the conventional chloroform film method with sonication were multilamellar and unilamellar structure with the average size of 58.4 ± 74.6 nm. However, the dispersibility of niosomes by the conventional chloroform film method with sonication was better than that by the $scCO_2$ either with or without ethanol, because of smaller particle size. This present study has demonstrated the trapping efficiency enhancement of water-soluble compounds in niosomes by the $scCO_2$ method with 10 % (w/w) of ethanol.

Keywords: Niosomes; Supercritical carbon dioxide; Conventional method; Water-soluble compounds; Trapping efficiency

1. Introduction

Bilayer vesicles, such as liposomes and niosomes, are widely known as efficient and sophisticate delivery systems for drugs, vitamins and cosmetics. Spherical vesicles consisting of amphiphatic lipid for liposomes and non-ionic surfactants for niosomes are arranged in one or more concentric bilayers. Both vesicles can entrap water-soluble substances in the inner aqueous phase and oil soluble substances in the vesicular membrane. Niosomes, non-ionic surfactant vesicles, are now widely studied as an alternative to liposomes, because they alleviate the disadvantages associated with liposomes (Florence and Baillie, 1989; Beugin et al., 1998) such as chemical instability, variable purity of phospholipids and high cost (Vora et al., 1998). Niosomes can be prepared by the same procedure as of liposomes e.g. conventional chloroform film method (Bangham et al., 1965), reverse phase evaporation (Szoka and Papahadjopoulos, 1978) and ethanol injection (Batzri and Korn, 1973). Most methods require large amount of organic solvent that toxic to human and environments and have multisteps. Some preparation methods even without using organic solvents such as heating method (Mozafari et al., 2007) and polyol dilution method (Kikuchi et al., 1994), there are problems of using high temperature that is not suitable for heat labile substances. In pharmaceutical industries, techniques for preparing bilayer vesicles have been highlighted in decreasing toxicity while improving stability and

Abbreviations: $scCO_2$, supercritical carbon dioxide.

* Corresponding author at: Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand. Tel.: +66 53 894806/944338; fax: +66 53 894169.
E-mail address: pmpti005@chiangmai.ac.th (A. Manosroi).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.10.013

solubility of pharmaceutical materials. Recently, supercritical fluids have been used as an alternative one step preparation of several bilayer vesicles at low temperature. Supercritical fluid is defined as a substance above its critical temperature (Tc) and critical pressure (Pc). At the critical point, supercritical fluids have the properties of density as liquid and low viscosity with better flow property as gas. Carbon dioxide is a widely used gas to produce supercritical fluid because of its low critical temperature (Tc = 31.1 °C) and pressure (Pc = 73.8 bar). It has high solvating power property in the near critical point. Similar to non-polar solvent, it can be adjusted by changing pressure or temperature. Supercritical carbon dioxide ($scCO_2$) can be used to substitute organic solvent to form bilayer vesicles with the advantages of being environmental friendly, non-toxic, non-inflammable and inexpensive (Bunker et al., 1997; Heo et al., 2000; Cooper, 2000). The modest operating conditions of $scCO_2$ are attractive for trapping water-soluble and thermal-labile compounds in bilayer vesicles. However, the solvating power of carbon dioxide near critical point has some limitation for polar materials and most lipophilic drugs (DeSimone et al., 1992; Gupta and Shim, 2007). Thus, the term of "CO_2-philic" is often used instead (DeSimone et al., 1992). The additional of ethanol has been used as a co-solvent to enhance the trapping efficiency of several water-soluble compounds in the bilayer vesicles (Otake et al., 2001). Manosroi et al. (2003) have reported that niosomes composed of Tween61 and cholesterol (20 mM) at 1:1 molar ratio prepared by the conventional chloroform film method with sonication gave the trapping efficiency of glucose of only 5%. In this study, the preparation method based on $scCO_2$ will be used to improve trapping efficiency of water-soluble compounds in niosomes. The trapping efficiency of glucose in niosomes composed of Tween61 and cholesterol at various molar ratios by the $scCO_2$ method with and without ethanol as a co-solvent will be compared with the conventional chloroform film method with sonication. Other characteristics including particle size, morphology, microviscosity and dispersibility of the vesicles were also investigated.

2. Materials and methods

2.1. Materials

Tween61 (polyoxyethylene sorbitan monostearate, Sigma Chemical Co., St Louis, USA) and cholesterol, Dulbecco's PBS (Wako Pure Chemical Industrial Ltd., Osaka, Japan) were used. D-(+)-Glucose was purchased from Wako Pure Chemical Industrial Ltd. Ethanol was from Sigma Chemical Co. Glucose-CII-test kits were purchased from Wako Pure Chemical Industrial Ltd., Osaka, Japan. Cellophane tube was from Vinkase Companies, Inc.

2.2. Apparatus

The $scCO_2$ apparatus for niosome preparation consisted of two parts which were the volume view cell that has two glass windows on both sides and the high-pressure pump for feeding CO_2 gas. The temperature inside the volume view cell was measured by a Pt resistance thermometer. The pressure was measured with a strain gauge. The mixtures in volume view cell were continuously mixed by a magnetic stirring tip (Otake et al., 2001, 2006a, b; Imura et al., 2003; Ri et al., 2005).

2.3. Methods

2.3.1. Niosomes prepared by the conventional chloroform film method with sonication

The molar ratios of Tween61 and cholesterol were at 1:0, 3:1, 1:1, 1:3 and 0:1 with the concentration fixed to 20 mM. Briefly, Tween61 and cholesterol were dissolved in chloroform in a test tube. The solvent was removed by blowing nitrogen gas into the test tubes and the residual solvent was dried overnight at room temperature (37 °C) in a vacuum to give a thin film. An amount of 15 ml of 0.2 M of D-(+)-glucose in Dulbecco's PBS was added to the film and the mixture was warmed at 60 °C for 5 min. The test tube was shaken vigorously by a vortex mixer to yield multilamellar vesicles (MLV). The vesicles were sonicated for 3 min by a bath sonicator (Branson B220, Smithkline company) at 125-W energy output. Each sample at each time was put in the same location in the bath sonicator.

2.3.2. Niosomes prepared by the $scCO_2$ method without ethanol

The conditions for niosome preparation by the $scCO_2$ method were performed according to the work previously described (Otake et al., 2001; Manosroi et al., 2003; Imura et al., 2003). The total amount of 20 mM in each batch of Tween61 mixed with cholesterol together with 0.2 mol/l D-(+)-glucose in Dulbecco's PBS (15 ml) was added into the view cell. The volume of the view cell was ca 50 cm^3. The temperature in the cell was raised to 60 °C and the CO_2 gas was introduced into the view cell. The pressure and temperature in the view cell were maintained at 200 bar and 60 ± 1 °C, respectively. During the pressurization step, the temperature remained constant by a temperature controller equipped with a Pt resistant thermometer. After 30 min with magnetic stirring until equilibrium, the pressure was released and niosomal dispersions were obtained. The effect of composition of Tween61/cholesterol at 1:0, 3:1, 1:1, 1:3, 0:1 molar ratio on the physical stability and trapping efficiency were investigated.

2.3.3. Niosomes prepared by the $scCO_2$ method with ethanol

The niosomal composition which gave the best dispersibility and highest glucose trapping efficiency from the conventional chloroform film method with sonication was selected to prepare by the $scCO_2$ method with ethanol up to 15 % (w/w) as a co-solvent. Tween61, cholesterol, D-(+)-glucose in Dulbecco's PBS and ethanol were added into the view cell with the same procedure as described in Section 2.3.2.

2.3.4. Trapping efficiency of glucose in niosomes

The niosomal dispersions were dialyzed against water using a cellophane tube (Vinkase Companies, Inc.) to remove the unentrapped glucose. The temperature was kept at 0 °C during

the dialysis. Niosomes inside the tube were collected and their membranes were disrupted by ethanol. The amount of glucose was determined by the mutarotase GOD method (Coxon and Schaffer, 1971; Miwa and Okuda, 1974) and a spectrophotometer using Glucose-CII test kits. The trapping efficiency of glucose in niosomes was calculated according to following equation.

$$\%\ \text{Trapping efficiency} = \frac{\text{glucose contents in niosomes} \times 100}{\text{total glucose contents in the niosomal dispersion}}$$

2.3.5. Physical properties of niosomes

2.3.5.1. Particle size. The particle size of niosomes was measured by dynamic light scattering (DLS) apparatus (NICOMP 380 ZLS, Particle Sizing Systems, Santa Barbara, CA). The dispersions were diluted to about 100 times with Dulbecco's PBS. The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90° and wavelength at 535 nm.

2.3.5.2. Morphology. The dispersion of niosomes was rapidly frozen in liquid propane using cryo-preparation apparatus (Leica EM CPC, Leica Co., Vienna, Austria). The frozen sample was fractured in freeze-replica-making apparatus (FR-7000A, Hitashi Science Co., Tokyo, Japan) at −150 °C. The fracture surface was replicated by evaporating platinum at an angle of 45 °C and followed by carbon to strengthen the replica. It was placed on a 150 mesh copper grid after washing with acetone and water. The vesicles were observed under a transmission electron microscope (JEM-1200EX, JEOL Co.).

2.3.5.3. Transition temperature analysis of niosomes. Thermal analysis of the niosomal dispersion was determined by differential scanning calorimeter (DSC 8230, Rigaku Co., Tokyo, Japan). The Dulbecco's PBS was used as a reference. Twenty microliters of the niosomal dispersion was placed in the aluminium pan sample vessel and the vessel was then carefully sealed. The measurement condition was at 1 K/min for scanning rate, 30–70 °C for the scanning range and 0.1 mcal/s for the sensitivity.

2.3.5.4. Microviscosity of the niosomal membrane. DPH (1,6 diphenyl-1,3,5-hexatriene) in tetrahydrofuran (THF) was used as a fluorescent probe by a fluorometer. The molar ratio of niosomes to DPH was 300:1. One millimolar of DPH in THF was added to 10 ml of the niosomal dispersion. The mixture was incubated for 24 h at 37 ± 1 °C. The microviscosity of niosomal membrane was determined by fluorescence polarization (P) which was calculated according to the following equation previously described (Prendergast et al., 1981; Roy et al., 2005).

$$P = \frac{(I\text{p} - GI\text{v})}{(I\text{p} + GI\text{v})}$$

where Ip and Iv were the fluorescence intensity of the emitted light polarized parallel and vertical to the exciting light, respectively, and G was the grating correction factor. The fluorescence intensities Ip and Iv were measured at various temperatures by a spectrofluorophotometer. The excitation and emission wavelength were 350 and 450 nm, respectively.

3. Results and discussion

3.1. Comparison of trapping efficiency of glucose in niosomes

The trapping efficiency of glucose in niosomes depended on the molar ratios of Tween61 and cholesterol and the amounts of ethanol. Fig. 1 showed the effects of ethanol contents and the molar ratios of Tween61/cholesterol on trapping efficiency of glucose in niosomes. With the absence of cholesterol, the niosomal vesicle could not be formed from 20 mM solution of Tween61 by the conventional chloroform film method with sonication and the $scCO_2$ method. This has agreed with our previous study (Manosroi et al., 2003). The packing properties of the lipid (cholesterol) and the surfactants depend on the hydrophilic–lipophilic balance (HLB), the gel–liquid transition temperature (Tc) of the surfactants and the critical packing parameter (CPP) value which can be calculated from v/a_0l_c (where a_0 is a hydrophilic surface area, v is the volume of the hydrocarbon chain and l_c is the length of the hydrocarbon chain). The surfactants can form the bilayer vesicles of the condition at $1/2 < v/a_0l_c < 1$. Similar to liposomal structures, the addition of cholesterol (a rigid steroid molecule) to the surfactant was required to form a stable non-ionic surfactant-based vesicle (Van Hal et al., 1996; Otake et al., 2006a, b). Cholesterol can stabilize the bilayer structure by eliminating the phase transition temperature (Tc) peak of the vesicles, thereby strengthening the bilayer structures. The suitable molar ratio of Tween61/cholesterol to form niosomes was determined by the physical stability (dispersibility) and trapping efficiency of a water-soluble substance in vesicles. Tween61/cholesterol at 1:1 molar ratio prepared by the conventional chloroform film method with sonication and the $scCO_2$ method without ethanol demonstrated higher trapping efficiency of glucose than other molar ratios (Fig. 1).

Tween61/cholesterol at 1:1 molar ratio was selected to prepare niosomes by the $scCO_2$ method with ethanol. The trapping

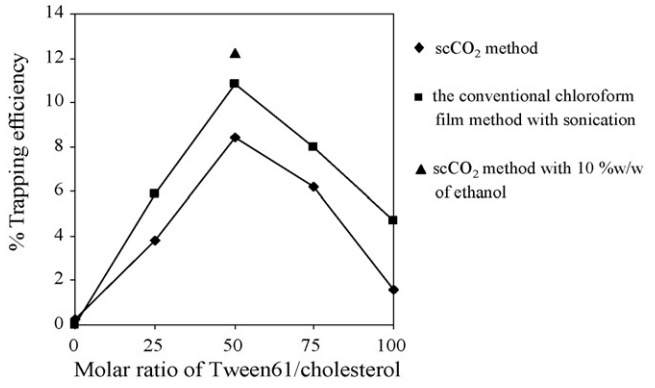

Fig. 1. Effects of molar ratios of Tween61/cholesterol prepared by $scCO_2$ without ethanol, the conventional chloroform film method with sonication and the $scCO_2$ with 10% (w/w) of ethanol on trapping efficiency of glucose in niosomes.

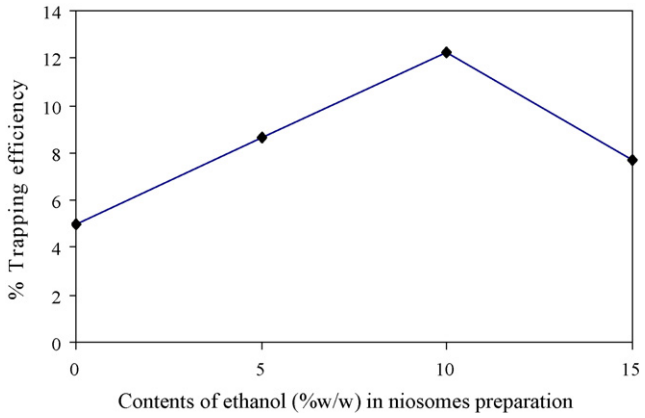

Fig. 2. Effects of ethanol contents on trapping efficiency of glucose in niosomes (Tween61/cholesterol 1:1 molar ratio) prepared by the $scCO_2$ method (60 °C, 200 bar).

efficiency of D-(+)-glucose in niosomes prepared by $scCO_2$ with ethanol had gradually increased and shown maximum value at 10% (w/w) of ethanol (Fig. 2) thus the niosomes prepared by $scCO_2$ with 10% (w/w) was performed for entrapping efficiency determination compared with the conventional chloroform film method with sonication and the $scCO_2$ without ethanol. The trapping efficiency of D-(+)-glucose in Tween61/cholesterol (1:1) niosomes prepared by the $scCO_2$ method with 10% (w/w) ethanol are 12.22 ± 0.26% statistically higher than that prepared by the conventional chloroform film method with sonication and the $scCO_2$ method without ethanol (Table 1). From visual observation in the view cell, the mixture of Tween61 and cholesterol had low solubility in $scCO_2$ fluid. Addition of ethanol, as a co-solvent, has increased the solubility of the mixture. However, high ethanol contents can disrupt the vesicles. The proper amount of ethanol at 10 % (w/w) for the niosomes composing of 20 mM of Tween61/cholesterol at 1:1 molar ratio gave the highest trapping efficiency of glucose.

3.2. Particle size and niosomal morphology

In general, the efficiency of substances entrapped in bilayer vesicles depends on vesicular lamellarity, particle size and size distribution. Table 2 showed the particle size of niosomes prepared by the conventional chloroform film method with sonication, the $scCO_2$ method without ethanol and with 10 % (w/w) ethanol observed by dynamic light scattering apparatus. The size distribution of the vesicles prepared by all methods exhibited a broad distribution. The results reveal that niosomes prepared by the $scCO_2$ method with 10 % (w/w) of ethanol and without ethanol were larger in size than those prepared by the conventional chloroform film method with sonication. This may be due to the different mechanisms of vesicle formation owing to the different preparation techniques. Imura et al. have reported that the mechanism of vesicle formation by the $scCO_2$ method was similar to the reverse phase evaporation (REV) method which yielded the large unilamellar vesicles (LUVs) with sizes ranging from 0.1 to 1 μm (Imura et al., 2003; Lasic, 1988). The smaller vesicles were obtained from the conventional chloroform film method with sonication because of size reduction by a ultrasonication probe. Although the particle size of the conventional niosomes is smaller than that of $scCO_2$-treated niosomes, the $scCO_2$-treated niosomes were in the nanosize range of about 200 nm, which were still advantageous for transdermal delivery. More importantly, the entrapment efficiency of the hydrophilic drug (glucose) in the $scCO_2$-treated niosomes was significantly higher than the conventional niosomes. However, it is possible to prepare niosomes with smaller size distribution. Ultrasonication (Chattopadhyay and Gupta, 2002) or polycarbonate extrusion method (Patty and Frisken, 2003) can be used together with the $scCO_2$ process in order to obtain a smaller size distribution of niosomes.

Table 1
Effects of preparation method on trapping efficiency of glucose in niosomes (Tween61/cholesterol at 1:1 molar ratio)

Method	Trapping efficiency (%)[a]
Conventional chloroform film method with sonication	10.85 ± 0.24
$scCO_2$ method without ethanol	8.4 ± 1.6
$scCO_2$ method with 10% (w/w) of ethanol	12.22 ± 0.26

[a] Mean ± S.D. of the percentage of trapping efficiency of glucose in niosomes prepared by the conventional chloroform film method with sonication, $scCO_2$ method without ethanol and with 10 % (w/w) of ethanol indicating significantly difference ($p < 0.05$, ANOVA with LSD test).

Table 2
Comparison of vesicular size ($\bar{X} \pm$ S.D.) of niosomes (Tween61/cholesterol at 1:1 molar ratio) prepared by the conventional chloroform film method with sonication, $scCO_2$ method without and with 10 % (w/w) of ethanol

Method	Vesicular size($\bar{X} \pm$ S.D.) (nm)
The conventional chloroform film method with sonication	58.4 ± 74.6
The $scCO_2$ without ethanol	202.5 ± 136.7
The $scCO_2$ with 10% (w/w) of ethanol	271.9 ± 159.6

The morphology of niosomes prepared by the three methods was observed by FF-TEM (Figs. 3 and 4). Niosomes by the conventional chloroform film method with sonication were both unilamellar and multilamellar structures (Fig. 3), while those by the $scCO_2$ method with or without ethanol were LUVs (Fig. 4) which were the appropriate structure for encapsulation of hydrophilic molecules. The results of vesicular sizes and morphology of niosomes appeared to support the higher trapping efficiency of glucose in niosomes prepared by the $scCO_2$ with ethanol.

3.3. Thermal analysis

Differential scanning calorimetry (DSC) was performed to investigate gel–liquid transition temperature of niosomes. The phase transition temperature was widely known as the temperature that induces a changing of lipid physical state from gel phase, closely packed molecule, to liquid crystalline phase, loosely packed molecule and fluid. The non-hydrated non-ionic

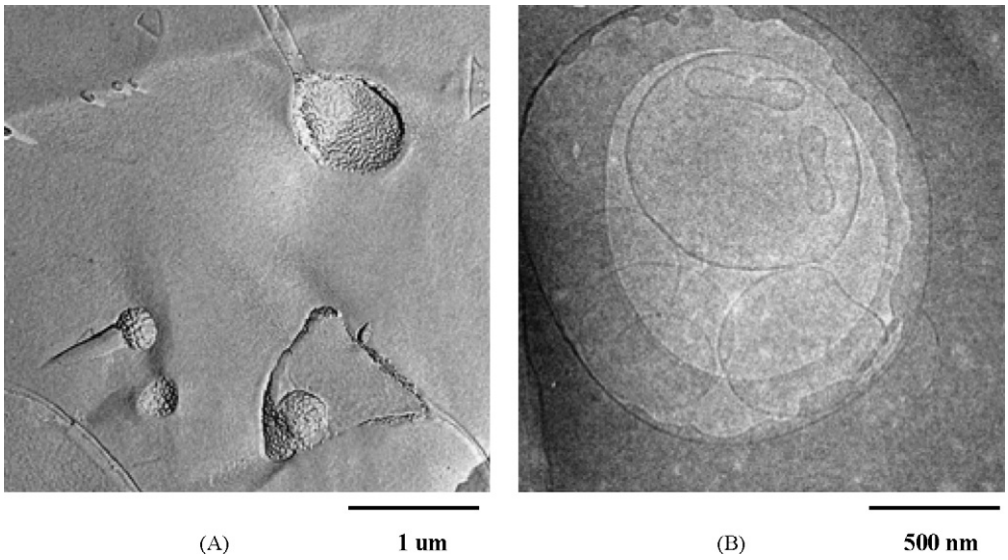

Fig. 3. (A) Freeze fracture TEM and (B) cryo TEM images of niosomes (20 mM Tween61/cholesterol 1:1 molar ratio) prepared by the conventional chloroform film method with sonication.

surfactants with stearyl chain (C18), such as Tween61, Span60 and Brij 72 show phase transition temperature (Tc) at 40.6, 45 and 40, respectively and can form the vesicles without cholesterol (Manosroi et al., 2003). For the hydrated surfactants and in the vesicular forms, it has been found that polyoxyethylene monostearyl ether ($C_{18}EO_6$) niosomes prepared by the supercritical reverse evaporation (scRPE) method showed a broad and small peak while those prepared by the conventional choloroform film method with sonication gave a sharp and large peak (Ri et al., 2005). Infact, large unilamellar liposomes show a broad and small peak and the mulitilamellar vesicles (MLVs) give a sharp and large peak (Takaichi et al., 1992). However, the hydrated Tween61 mixed or not mixed with cholesterol have never been determined. In our experiment, cholesterol has been incorporated in Tween61 at the molar ratios of Tween61/cholesterol at 1:0, 3:1, 1:1, 1:3 and 0:1 and the thermal analysis has been performed. Fig. 5. shows DSC curve of niosomes composing of Tween61/cholesterol (20 mM) at 1:0, 3:1, 1:1, 1:3 and 0:1 molar ratio prepared by $scCO_2$ method without ethanol. As known, cholesterol can stabilized the vesicular membrane. Similar to liposomes (Albertini et al., 1990; Lian and Ho, 2001), cholesterol incorporated niosome, Tc of Tween61 niosomes disappear, and do not shift to higher temperature when mixed with cholesterol. The Tc of the hydrated Tween61 without cholesterol in the form of vesicles showed too small peak that cannot be clearly seen. This may be due to not only the effects of the large unilamellar vesicular structure, but also the more rigidity of the polar head group of Tween61 when hydrated and interacted with the water molecule by hydrogen bonding as well.

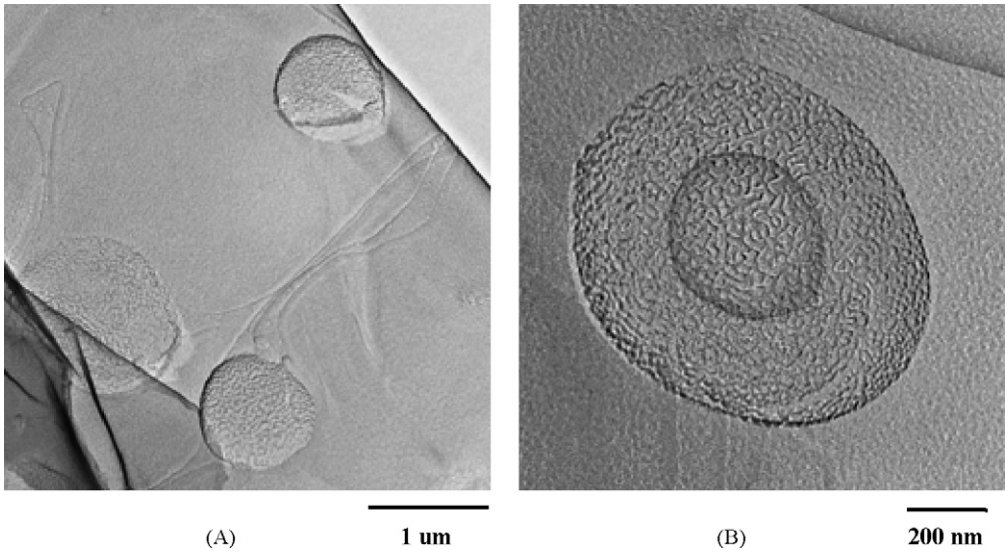

Fig. 4. Freeze fracture TEM images of niosomes (20 mM Tween61/cholesterol 1:1 molar ratio) prepared by (A) the $scCO_2$ method without ethanol and (B) the $scCO_2$ method with 10 % (w/w) of ethanol.

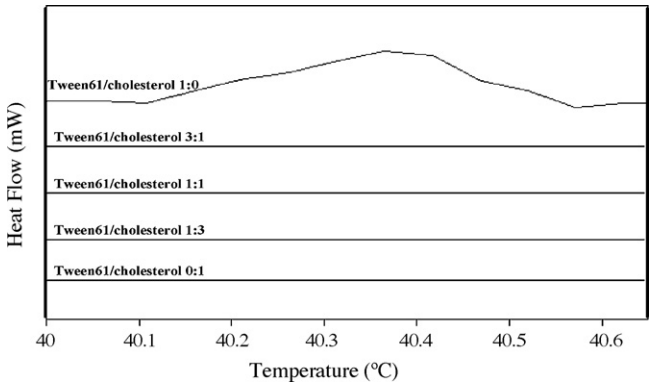

Fig. 5. DSC curve of niosomes composing of Tween61/cholesterol (20 mM) at 1:0, 3:1, 1:1, 1:3, 0:1 molar ratio prepared by $scCO_2$ without ethanol.

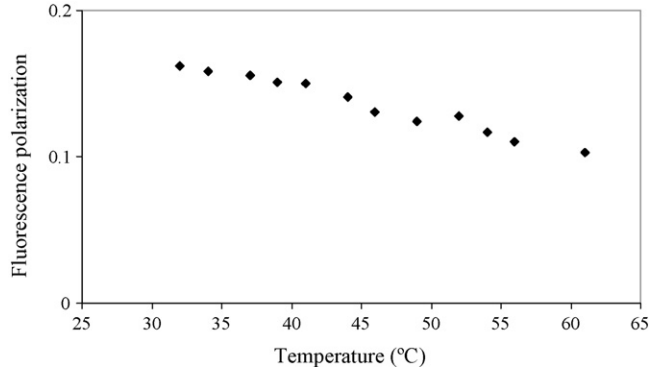

Fig. 6. The relationship between temperatures and fluorescence polarization of niosomes (20 mM Tween61/cholesterol 1:1 molar ratio) prepared by the $scCO_2$ with 10 % (w/w) ethanol.

3.4. Microviscosity of niosomal membranes

As known, the microviscosity which is directly correlated to the fluorescence polarization indicates the information of packing structure of the vesicular membrane. In Fig. 6, the fluorescence polarization of DPH in the niosomal membranes prepared by $scCO_2$ method without ethanol gradually decreased and became constant with increasing temperature. This temperature dependence of microviscosity by fluorometry of 1:1 Tween61/cholesterol niosomes as seen in Fig. 6 is quite similar to the phase transition behavior of niosomal membrane observed by DSC as seen in Fig. 5. The microviscosity measurement of Tween61/cholesterol (at 1:1 molar ratio) bilayer vesicles prepared by the conventional chloroform film method with sonication has been previously presented (Manosroi et al., 2003). The membrane microviscosity of niosomes slightly decreased with the increased temperature which is similar to our niosomes prepared by $scCO_2$ method without ethanol.

3.5. Dispersibility of niosomes

The physical appearances of Tween61/cholesterol (20 mM) niosomes at 1:0, 3:1, 1:1, 1:3, 0:1 molar ratios entrapped with D-(+)-glucose prepared by the conventional chloroform film method with sonication and the $scCO_2$ method were determined by virtual observation (Table 3, Fig. 7.). Tween61 alone (without cholesterol) can not form stable vesicles. The addition of cholesterol in niosomal composition increased trapping efficiency (Fig. 1). Niosomes which gave the best dispersibility and the highest trapping efficiency were those from the mixture of Tween61 and cholesterol at 1:1 molar ratio. Niosomes composing of Tween61/cholesterol at 1:1 molar ratio by the $scCO_2$ method without ethanol had lower physical stability and less trapping efficiency than those by the conventional chloroform film method with sonication. However, with ethanol at 10 % (w/w), niosomes appeared to be more physical stable than those without ethanol, and with 5 and 15 % (w/w) ethanol (Table 3, Fig. 7). After 1 and 3 days at 25 °C, the niosomal dispersions by the $scCO_2$ method without and with ethanol, respectively separated into two phases, due to the aggregation and fusion of the niosomes. Hence, niosomal dispersions by the conventional chloroform film method with sonication showed better dispersibility than those by the $scCO_2$ method either with

Table 3
Physical appearances of niosomes (Tween61/cholesterol at 1:0, 3:1, 1:1, 1:3 and 0:1 molar ratio) entrapped with D-(+)-glucose prepared by the conventional chloroform film method with sonication and the $scCO_2$ method without and with 5, 10 and 15% (w/w) of ethanol at initial and after 1, 3 and 7 days at 25 °C by visual observation

Preparation method	Tween61/cholesterol molar ratio	Sedimentation[a]			
		Initial	1 day	3 days	7 days
The conventional chloroform film method with sonication	1:0	–	–	–	–
	3:1	–	–	–	–
	1:1	–	–	–	–
	1:3	–	–	•	•
	0:1	•	•	•	•
$scCO_2$ method without ethanol	1:0	•	•	•	•
	3:1	–	•	•	•
	1:1	–	–	•	•
	1:3	–	•	•	•
	0:1	–	•	•	•
$scCO_2$ method with 5% (w/w) of ethanol	1:1	–	–	•	•
$scCO_2$ method with 10% (w/w) of ethanol	1:1	–	–	–	•
$scCO_2$ method with 15% (w/w) of ethanol	1:1	–	–	•	•

[a] *Note*: •, represents sedimentation; –, represents no sedimentation.

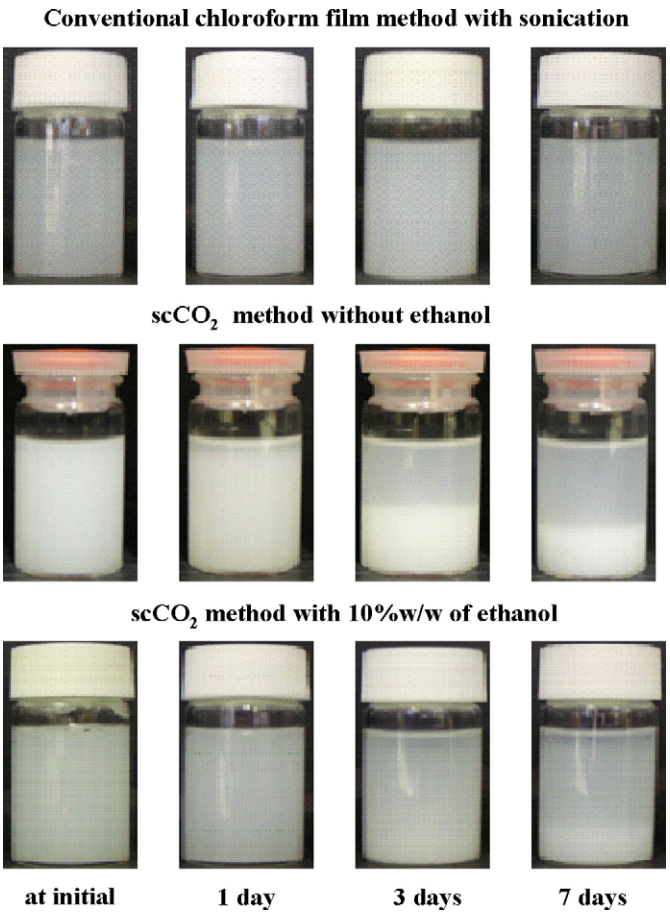

Fig. 7. Physical appearances of Tween61/cholesterol (20 mM) niosomes at 1:1 molar ratio entrapped with D-(+)-glucose prepared by the conventional chloroform film method with sonication, $scCO_2$ method without and with 10 % (w/w) of ethanol at initial and after 1, 3 and 7 days at 25 °C.

or without ethanol. The sonication of the niosomal dispersion reduced the size of the vesicles, thereby increasing dispersibility of the vesicular dispersion. Although the particle size of $scCO_2$-treated niosomes was larger than conventional niosomes, but their size existed in nano-range with statistically higher trapping efficiency of hydrophilic drug that can be developed and applied for transdermal delivery system. For the practically use as the carrier, the dispersion stability of $scCO_2$-treated niosomes will be improved with smaller particle sizes using ultrasonication (Chattopadhyay and Gupta, 2002) and narrower size distribution using polycarbonate extrusion method (Patty and Frisken, 2003; Bosworth et al., 1982) combined with $scCO_2$ method with ethanol.

4. Conclusion

This study has aimed to increase the trapping efficiency of the water-soluble compound in niosomes, non-ionic surfactant bilayer vesicles, by a novel $scCO_2$ technique. We have found that the $scCO_2$ method can be used to prepare niosomes composing of Tween61 and cholesterol at 1:1 molar ratio. The trapping efficiency of glucose, a water-soluble compound in niosomes was enhanced by the $scCO_2$ method with the maximum entrapment with ethanol at 10 % (w/w). However, without ethanol, the $scCO_2$ method appeared not to be a superior method for niosomal preparation since the solubility of the surfactant–lipid mixture in $scCO_2$ was low. This is similar to liposome preparation by this method. Thus, ethanol is required to be a co-solvent for Tween61 and cholesterol mixture to obtain a homogeneous niosomal dispersion. Tween61/cholesterol niosomes prepared by the $scCO_2$ method with and without ethanol were in large unilamellar niosomes and broad size distribution with the average diameter of 271.9 ± 159.6 and 202.5 ± 136.7 nm, respectively, whereas those prepared by the conventional chloroform film method with sonication were both in multilamellar and unilamellar structure with the average diameter of 58.4 ± 74.6 nm. Niosomes prepared by the conventional chloroform film method with sonication which had smaller particle size gave better dispersibility than those prepared by the $scCO_2$ either with or without ethanol. The clearly physical state, gel–liquid crystalline phase, of the niosomes was not observed by both the thermal analysis and the microviscosity assay because of the intercalation of cholesterol between the non-ionic surfactants. This study has demonstrated the enhancement of the trapping efficiency of water-soluble compounds in niosomes by the $scCO_2$ method with 10 % (w/w) of ethanol as a co-solvent. However, the broad vesicular size distribution was obtained. The combination of $scCO_2$ method with sonication or polycarbonate extrusion method to get a smaller size distribution is suggested.

Acknowledgements

This work was supported by the Thailand Research Fund (TRF) under the RGJ-PhD program in Thailand, Pharmaceutical-Cosmetic Raw Materials & Natural Products Research and Development Center (PCRNC), Institute for Science and technology (IST), Chiang Mai University, Thailand, Tokyo University of Science (TUS) in Japan, and Nanoscience and Nanotechnology Research Center Project, Faculty of Sciences, Chiang Mai University, Thailand.

References

Albertini, G., Bossi, G., Dubini, B., Ponzi Bossi, M.G., Rustichelli, F., 1990. Structural modifications in the liquid crystalline L_α phase of disteroyl phosphatidyl–choline liposomes induced by variable cholesterol concentration. Mol. Cryst. Liq Cryst. 179, 383–404.

Bangham, A.D., Standish, M.M., Walkins, J.C., 1965. Diffusion of univalent ions across the lamellar of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Batzri, S., Korn, E.D., 1973. Single bilayer liposomes prepared without sonication. Biochim. Biophys. Acta 289, 1015–1019.

Beugin, S., Edwards, K., Karlsson, G., Ollivon, M., Lesieur, S., 1998. New sterically stabilized vesicles based on nonionic surfactant, cholesterol and poly(ethylene glycol)–cholesterol conjugates. Biophys. J. 74, 3198–3210.

Bosworth, M.E., Anthony Hunt, C., Pratt, D., 1982. Liposome dialysis for improve size distributions. J. Pharm. Sci. 71, 806–812.

Bunker, C.E., Rollins, H.W., Gord, J.R., Sun, Y.P., 1997. Efficiency photodimerization reaction of anthracene in supercritical carbon dioxide. J. Org. Chem. 62, 7324–7329.

Chattopadhyay, P., Gupta, R.B., 2002. Protein nanoparticles formation by supercritical antisolvent with enhanced mass transfer. AIChE J. 48, 235–244.

Cooper, A.I., 2000. Synthesis and processing of polymers using supercritical carbon dioxide. J. Mater. Chem. 10, 207–234.

Coxon, B., Schaffer, R., 1971. Characterization and quantitative analysis of D-glucose for use in clinical analysis. Anal. Chem. 43, 1565.

DeSimone, J.M., Guan, Z., Elsbernd, C.S., 1992. Synthesis of fluoropolymers in supercritical carbon dioxide. Science 257, 945–947.

Florence, A.T., Baillie, A.J., 1989. Non-ionic surfactant vesicles, alternatives to liposomes in drug delivery? In: Prescott, L.F., Nimmo, W.S. (Eds.), Novel Drug Delivery and its Therapeutic Application. John Wiley and Sons, New York, pp. 281–296.

Gupta, R.B., Shim, J.J., 2007. Solubility in Supercritical Carbon dioxide. CRC Press, Boca Raton, pp. 1–960.

Heo, J.H., Kim, S.Y., Kim, H.S., Yoo, K.P., 2000. Enzymatic preparation of a carbohydrate ester of medium-chain fatty. Biotechnol. Lett. 22, 995–998.

Imura, T., Goto, T., Otake, K., Yoda, S., Takebayashi, Y., Yokoyama, S., Takebayashi, H., Sakai, H., Yuasa, M., Abe, M., 2003. Control of physicochemical properties of liposomes using a supercritical reverse phase evaporation method. Langmuir 19, 2021–2025.

Kikuchi, H., Yamauchi, H., Hirota, S., 1994. A polyol dilution method for mass production of liposome. J. Liposome Res. 4, 71–91.

Lasic, D.D., 1988. The mechanism of vesicle formation. Biochem. J. 256, 1–11.

Lian, T., Ho, R.J.Y., 2001. Trends and developments in liposome drug delivery systems. J. Pharm. Sci. 90, 667–680.

Manosroi, A., Wongtrakul, P., Manosroi, J., Sakai, H., Sugawara, F., Yuasa, M., Abe, M., 2003. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloids Surf. B 30, 129–138.

Miwa, I., Okuda, J., 1974. An improved mutarotase assay using beta-D-glucose oxidase and an oxygen electrode. J. Biochem. (Tokyo) 75, 1177.

Mozafari, M.R., Reed, C.J., Rostron, C., 2007. Cytotoxicity evaluation of anionic nanoliposomes and nanolipoplexes prepared by the heating method without employing volatile solvents and detergents. Die Pharmazie 62, 205–209.

Otake, K., Goto, T., Imura, T., Furiya, T., Yoda, S., Takebayashi, Y., Sakai, H., Abe, M., 2006a. Preparation of liposomes using an improved supercritical reverse phase evaporation. Langmuir 22, 2543–2550.

Otake, K., Imura, T., Sakai, H., Abe, M., 2001. Development of a new preparation method of liposomes using supercritical carbon dioxide. Langmuir 17, 3898–3901.

Otake, K., Shimomura, T., Goto, T., Imura, T., Furiya, T., Yoda, S., Takebayashi, Y., Sakai, H., Abe, M., 2006b. One-step preparation of chitosan-coated cationic liposomes by an improved supercritical reverse-phase evaporation method. Langmuir 22, 4054–4059.

Patty, P.J., Frisken, B.J., 2003. The pressure-dependence of the size of extruded vesicles. Biophys. J. 85, 996–1004.

Prendergast, F.G., Haugland, R.P., Callahan, P.J., 1981. 1-[4-(Trimethylamino) pheny1]-6-phenylhexa-1,3,5-triene: synthesis, fluorescence properties, and use as a fluorescence probe of lipid bilayers. Biochemistry 20, 7333–7338.

Ri, K., Yamaguchi, S., Wongtrakul, P., Hashimoto, S., Otake, K., Ohkubo, T., Sakai, H., Abe, M., 2005. Preparation and characterization of nonionic surfactant vesicles using supercritical carbon dioxide. Mater. Technol. 23, 340–347.

Roy, S., Mohanty, A., Dey, J., 2005. Microviscosity of bilayer membranes of some *N*-acylamine acid surfactants determined by fluorescence probe method. Chem. Phys. Lett. 414, 23–27.

Szoka F.Jr., Papahadjopoulos, D., 1978. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc. Natl. Acad. Sci. U.S.A. 75, 4194–4198.

Takaichi, Y., Kittaka, S., Kodama, M., 1992. Calorimetric studies of phase transition of sonicated vesicles of dimyristoylphosphatidylcholine and their stabilities. Netsu Sokutei 19, 103–112.

Van Hal, D.A., Bouwstra, J.A., Van Rensen, A., Jeremiasse, E., De Vringer, T., Junginger, H.E., 1996. Preparation and characterization of nonionic surfactant vesicles. J. Colloid Interface Sci. 178, 263–273.

Vora, B., Khopade, A.J., Jain, N.K., 1998. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Control Rel. 54, 149–165.