Effect of Hydrogenated Lecithin on Cytotoxicity of Liposome

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Received September 2, 2008, Accepted December 29, 2008

In this study, a hydrogenated lecithin-containing liposome was prepared using a sonicator and microfluidizer at high shear. The surface tension, particle size, turbidity, and transition temperature (T_c) of the liposome were investigated and compared with the commercially available standard surfactant Tween-60. The cytotoxicities were characterized using the MTT method. The surface tension of hydrogenated lecithin was found to be higher than that of Tween-60. The particle size prepared at above T_c was smaller than that prepared below T_c. The results of the cytotoxicity experiment indicated that Lecinol 10 exhibits the highest IC_{50} value, which shows its high safety in this study.

Key Words: Lecithin, Liposome, Particle size, Turbidity, Cytotoxicity

Introduction

As overall interest in product safety for the human body has recently been heightened, new surface active agents made of non-toxic and safe natural substances are being developed. Lecithin is a natural substance found in cell membranes and is manufactured in the liver from dierary choline. It is mostly a mixture of glycolipids, triglycerides, and phospholipids.[1,2] Lecithin has for a long time been much used as a pharmacological agent and a food supplement, created by separating and refining the egg yolk and soy bean. Lecithin has low solubility in water. In aqueous solution its phospholipids can form liposomes, bilayer sheets, micelles, or lamellar structures, depending on hydration and temperature. Nevertheless, injection lecithin contains many unsaturated fatty acids and its application has been difficult in cosmetics, for which long-term stability of unsaturated fatty acids is required. Hydrogenated lecithin, a type of phospholid, and an element in the body, has been used most widely.[3,4]

Recent advances in synthetic technologies may turn unsaturated fatty acids into saturated fatty acids. Production of hydrogenated lecithin could be applied to cosmetics where long-term stability is required. Hydrogenated lecithin has almost no solubility in water or in general emulsifiers, so an aqueous solution cannot be produced due to low hydrophilic–lipophilic balance (HLB).[5,6] The critical packing parameter is the ratio of the tail volume to the volume projected by the optimal head group area. Since the geometric structure of the lecithin molecule, namely the critical packing parameter, is 1/2-1 in phosphatidylcholine, and is the principal ingredient of hydrogenated lecithin.[7] It is a known fact that lecithin vesicles could be formed. Thus, the anticipation was that the only method of producing aqueous hydrogenated lecithin was to provide high shear in the system, which could yield vesicles and nanoemulsions. Hence, it was predicted in this study that sonicator and Microfluidizer apparatuses that could provide high shear in the system could be utilized to produce the vesicles from hydrogenated lecithin.

In this study, a hydrogenated lecithin-containing liposome was prepared using a sonicator and Microfluidizer at high shear, and the surface tension, particle size, turbidity, transition temperature (T_c) and cytotoxicity of the liposome were characterized using a surface tensiometer, a Submicron Particle Sizer, a turbidimeter, a differential scanning calorimeter (DSC), and the MTT method.

Experimental

Materials. The phospholipid used in preparation of the liposome was L-α-phosphatidylcholine (lecithin, C_nPC; n = 12, 14, 16, 18), kindly provided by Sigma. Hydrogenated lecithin was commercially available Lecinol S10 (Nikkol Co., PC content of 39%), DS-HPC 50S (Doosan Co., PC content of 50%), and Lipoid S75-3 (Lipoid Co., PC content of 75%). Phospholipid compositions of Lecinol S10 were 39% phosphatidylcholine (PC), 38% phosphatidylethanolamine (PE), 17% phosphatidylinositol (PI), and 6% phosphatidicacid (PA). Fatty acid compositions of Lecinol S10, DS-HPC 50S, and Lipoid S75-3 were 20.1% palmitic acid, 69.9% stearic acid, and 10.0% oleic acid. Polysorbate-20~80 (Tween-20~80), dipalmitoyl phosphatidycholine (DPPC), phenyltrimethicone (silicone oil), and 3-(4,5-dimethylthiazol-2-yl)-2,5-dophenyl tetrazolium bromide (MTT) were purchased from Sigma. V79-4 cell used as cell was supplied by American Type Culture Collection. Water used was distilled water (resistivity: 18.0 MΩ·cm, pH: 6.7) prepared with the Milli Q Plus system. The chemical structures of hydrogenated lecithin, Tween-60, and silicone oil are shown in Figure 1.

Preparation of liposome. The liposome was prepared using a sonicator and Microfluidizer, for preparation of a small and large quantity, respectively, of liposome.

(1) Preparation of liposome using sonicator: 0.04 g PC and 40 g water were placed in a 100 mL beaker. The mixtures were mixed with a magnetic stirrer for 1 h, and then sonicated

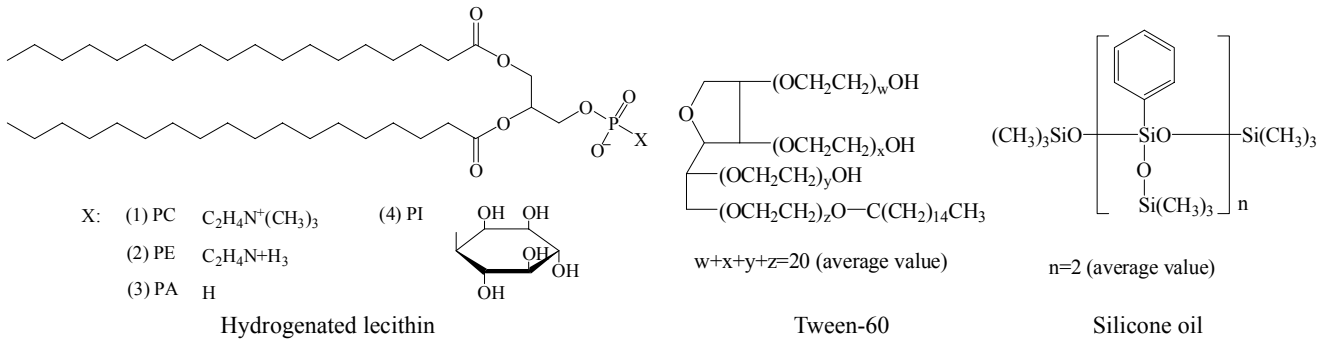

Figure 1. Chemical structures of the materials used.

using sonicator (Heat Systems Ultrosonics Co., Model W-380). The liposome was obtained by cooling the mixtures and filtering with a microfilter (0.8 μm).

(2) Preparation of liposome using Microfluidizer: A designed amount of hydrogenated lecithin and water was placed in a 2000 mL beaker. The mixtures were then mixed with mechanical stirrer for 1 h. The liposome was prepared using a Microfluidizer with variations of temperature, number of shear applications, pressure, and PC content.

Surface tension measurement. The surface tension of liposome with variation of concentration of lecithin and standard surfactant was measured using a surface tensiometer (K12) at 25 °C according to the ring method.

Turbidity measurement. The turbidities of samples prepared using sonicator and Microfluidizer were determined with a turbidimeter (Model NDH-300A, Japan).

Particle size measurement. The particle size of the samples was determined using a Submicron Particle Sizer (Nicomp Co., Model 370) and Mastersizer (Malvern Co.).

Transition temperature measurement. The transition temperature of the samples was investigated with a differential scanning calorimeter (Hart Scientific Co., Model 4207) at a heating rate of 1 °C/min in a nitrogen atmosphere.

MTT measurement. Materials were dissolved in serum-free media (DMEM, GIBCO), and the media was controlled at pH 7.1 to 7.5. Cell was cultivated on a 96-well plate in a T-75 flask, and the media was added to the 96-well plate. After 24 h, upper solution was removed. 100 μL MTT stock solution (5 mg of MTT/ml of distilled water) was transferred into the 96-well plate. The mixture was incubated for 4 h, and upper solution was removed by centrifugation. The formazan precipitate was dissolved in 120 μL of isopropanol. The optical density was measured at 570 nm using a spectrophotometer (Beckman DU 7500).

Results and Discussion

Preparation of liposome. It is important that the particles form a small and uniform size in the preparation of the hydrogenated lecithin-based liposome. According to size and shape of liposome, liposome can be divided into small unilamella vesicles (SUV), large unilamella vesicles (LUV), multilamella vesicles (MLV), giant unilamella vesicles (GUV), and small multilamella vesicles (SMV). The size ranges of SUV, LUV, GUV, SMV, and MLV are 100 nm and below, 100-1000 nm, 1000 nm and upward, 50-500 nm, and 100-5000 nm, respectively. HLB is an important property of lecithin. HLB of lecithin is about 7-9, and the solubility of lecithin in water is 10^{-10}. Hydrogenated lecithin was prepared by changing the unsaturated fatty acid in lecithin into a saturated fatty acid. Thus, the HLB and solubility of hydrogenated lecithin were lower than those of lecithin. To uniformly disperse the hydrogenated lecithin required high shear.[8]

From the chemical structure of hydrogenated lecithin in Figure 1, it is apparent that lecithin has a large head (phosphatic group part) and long tail (fatty acid part), and thus can wet or be hydrated in water. But, separation should be happen after several minutes. That is, lecithin is difficult to disperse naturally in water. Dispersion of lecithin uniformly in water requires definite shear. Thus, hydrogenated lecithin-containing liposome was prepared using a sonicator and Microfluidizer at high shear.

Surface tension. Surface tension is defined as the force along a line of unit length, where the force is parallel to the surface but perpendicular to the line. Surface tension of Lecinol S10 and Tween-60 was measured with the ring method. The results, shown in Figure 2, indicate that the surface tension sharply decreases with increasing concentration of surfactants up to 0.1 wt% and then surface tension decreases slowly above this concentration. The surface tension of the standard surfactant Tween-60 was lower than that of Lecinol S10 at the same concentration of surfactant. This is probably because Lecinol S10 has low HLB and solubility in water and thus decreasing hydrophile, resulting in an increase in surface tension.[9]

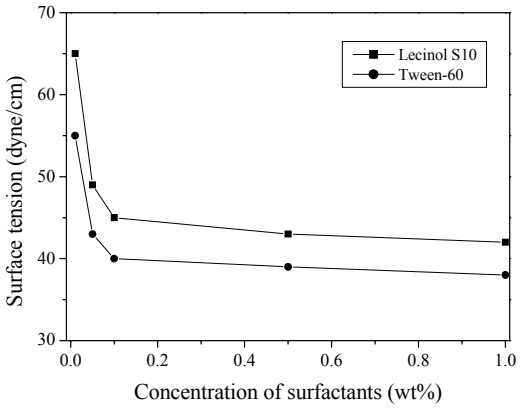

Figure 2. Surface tension of Lecinol S10 and Tween-60 measured by ring method.

Table 1. Cytotoxicity measured by using the MTT method

Commercial name	Chemical name	HLB	Cytotoxicity (IC50)
SLS	Sodium lauryl sulfate	40.0	0.0025
Tween-20	POE(20) Sorbian monolaurate	16.7	0.03
Tween-40	POE(20) Sorbian monomyristate	15.6	0.04
Tween-60	POE(20) Sorbian monostearate	14.9	0.06
Tween-80	POE(20) Sorbian monooleate	15.0	0.5
Lecinol S10	Mixture of PC, PE, PI, and PA	About 9	> 1

Mean diameter and turbidity. The liposome containing lecithin was characterized by means of particle diameter and turbidity measurements. The diameter of particles was measured via dynamic light scattering, and the mean diameter was calculated using the Einstein-Stokes equation, as follows:[10]

$$r = \frac{kT}{6\pi D\eta} \quad (1)$$

where r is the diameter of particles, η the viscosity of the medium, k Boltzmann's constant, D Brownian diffusivity, and T absolute temperature.

The turbidity (τ) was calculated using the following equation.[11]

$$\frac{I}{I_0} = \exp(-\tau l) \quad (2)$$

where I_0 is the intensity of incident light, I the intensity of transmission light, and l the distance.

Figure 3 shows the mean diameter and turbidity as a function of pressure. As shown in Figure 3, the mean diameter and turbidity at room temperature and 70 °C decreased with increasing pressure. The mean diameter and turbidity at room temperature were higher than those at 70 °C.

Figure 4 shows the mean diameter and turbidity as a function of passes. The mean diameter and turbidity decreased with increasing passes. This result indicated that the hydrogenated lecithin forms small multilamella vesicles at high shear. The mean diameter and turbidity decreased with increasing temperature at the same number of passes, as shown in Figure 4.

Figure 5 shows the mean diameter as a function of PC content and shear. The mean diameter decreased with increasing time and PC content. These results can be due to the critical packing parameters of PC and PE, which are 1/2-1 and 1, respectively.[12-14]

Figure 6 shows the mean diameter and turbidity as a function of fatty acid in PC. The mean diameter increased with increasing chain length. These results can be due to the large

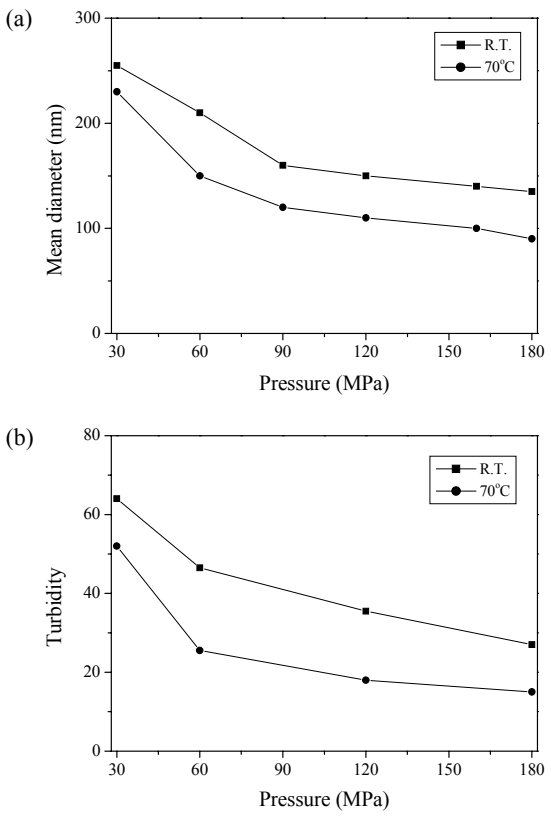

Figure 3. Effect of the microfluidizer on mean particle size (a) and turbidity (b) of liposome containing 1% Lecinol S10.

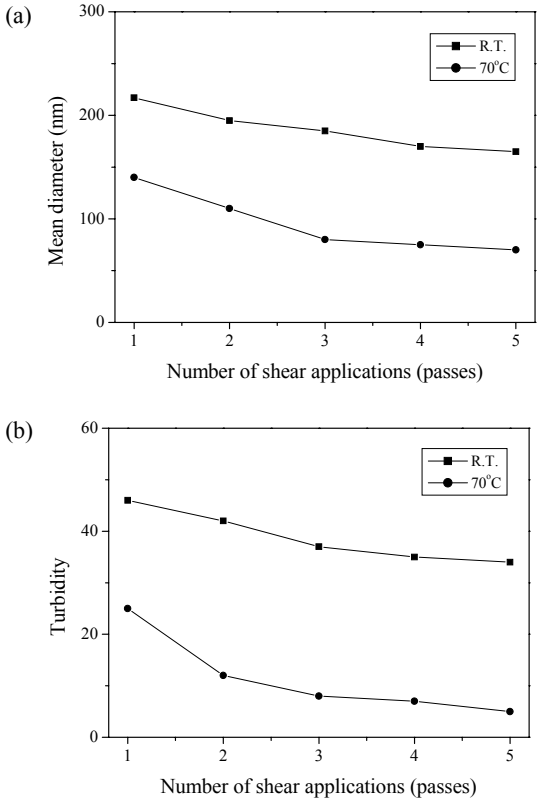

Figure 4. Effect of shear application on the mean diameter (a) and turbidity (b) of droplets in liposome containing 1 wt% hydrogenated lecithin.

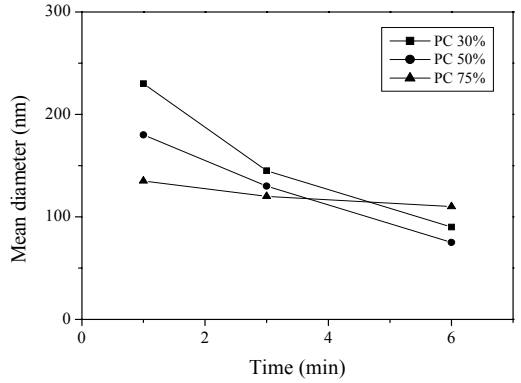

Figure 5. Change in the diameter of the hydrogenated lecithin (Sonicator power 125W; temperature 60-70°C; hydrogenated lecithin concentration 1 wt%; diameter was determined at 25°C by dynamic light scattering method).

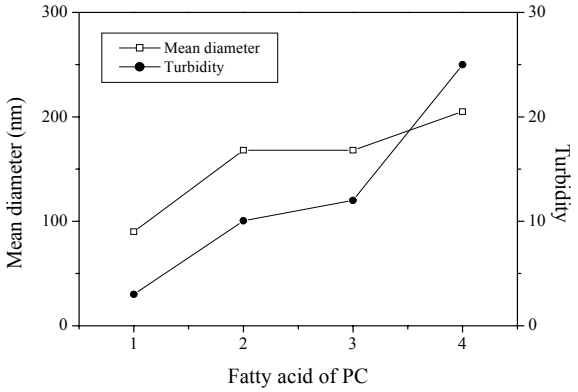

Figure 6. Particle size and turbidity measurement of liposome containing 0.1 wt% $(C_n)_2$PC with sonicator. (1: $(C_{12})_2$PC; 2: $(C_{14})_2$PC; 3: $(C_{16})_2$PC; 4: $(C_{18})_2$PC)

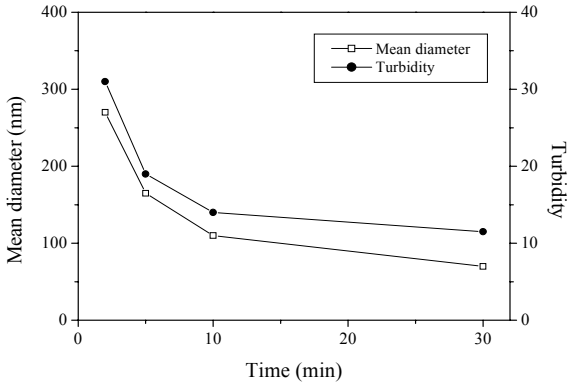

Figure 7. Particle size and turbidity measurement of liposome containing 0.1 wt% $(C_n)_2$PC with sonicator.

chain length in PC increasing mean diameter and thus increasing turbidity of the system.

Figure 7 shows particle size and turbidity as a function of shear time when 0.1 wt% $(C_{18})_2$PC was used. The mean diameter and turbidity sharply decreased with increasing shear time up to 10 min, and then decreased slowly after this time. The decreased particle size was due to the critical packing parameter of PC.

Transition temperature. The transition temperature (T_c) affects the stability of phospholipid and also affects leaking phenomenon of materials in vesicles or stability of phospholipid. The T_c was measured using DSC. The results indicated that the T_c values of 1 wt% Lecinol S10 and DPPC were 65 °C and 41.5 °C, respectively. The T_c of 1 wt% Lecinol S10 was higher than that of 1 wt% DPPC, which was due to the high content of distearoyl in Lecinol S10.[4] As shown in Figure 3, the particle size prepared at above T_c was smaller than that prepared at room temperature (below T_c). This result indicated that the particle size can be controlled when T_c value was given.

Cytotoxicity. Cytotoxicity is the quality of being toxic to cells. The cytotoxicities were measured using the MTT method, and the results are shown in Table 1. The IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. It is the half maximal (50%) inhibitory concentration of a substance.[15] The MTT results indicated that Lecinol S10 shows the highest IC_{50} and lowest HLB values. The basic elements of Lecinol S10 are similar to the body's constitution and thus Lecinol S10 is not causes any side effects to the substance, resulting in shows highest IC_{50} and lowest HLB value. From this result, it was confirmed in this study that Lecinol S10 has high safety.

Conclusions

A hydrogenated lecithin-containing liposome was prepared using a sonicator and Microfluidizer at high shear, and the properties of the liposome were characterized. Surface tension of hydrogenated lecithin was higher than that of the standard surfactant Tween-60. The particle sizes decreased with increasing PC content and shear, which was due to the critical packing parameter of PC. The cytotoxicity experimental results indicated that Lecinol S10 exhibits the highest IC_{50} value, which has high safety in this study. According to these results, it was confirmed that the lecithin-containing liposome has greater surface characteristics and cytotoxicity than those of the commercially available standard surfactant Tween-60.

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