Preparation and Characterization of a New Lipid Nano-Emulsion Containing Two Cosurfactants, Sodium Palmitate for Droplet Size Reduction and Sucrose Palmitate for Stability Enhancement

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Received January 22, 2008; accepted May 7, 2008; published online May 12, 2008

A new lipid nano-emulsion (LNE) was prepared from soybean oil and phosphatidylcholine (PC) employing two cosurfactants, sodium palmitate (PA) for reduced droplet size and sucrose palmitate (SP) for stability enhancement. The mean droplet size of LNEs prepared at a PA/PC (w/w) ratio of larger than 1/10 was found to be *ca.* 50 nm by dynamic light scattering and atomic force microscopy. However, during the 12-month storage, the PA/PC (1/10)-LNE showed an increase in mean droplet size and broadening of the droplet size distribution due to coalescence of the LNE particles. In a saline solution, the coalescence proceeded very rapidly, *i.e.*, the mean droplet size increased to more than 150 nm within 0.5 h. To suppress the coalescence of LNE particles, four sucrose fatty acid esters of different chain lengths were examined as candidate cosurfactants. The results showed that PA/SP/PC (1/4/10)-LNE could maintain a mean droplet size around 50 nm for 12 months. In a saline solution, the mean droplet size could be maintained within 100 nm even after 24 h. Slight formation of flocculation in the LNEs depending on the storage period was suggested by measurement of the ^{31}P nuclear magnetic resonance line width of the LNEs.

Key words lipid nano-emulsion; sodium palmitate; sucrose palmitate; droplet size; stability

It has been reported that the accumulation and retention of some macromolecules, *e.g.*, some kinds of lipid particles or polymeric drugs, are extensively enhanced in tumor tissues as compared with those in normal tissues.[1—3] This phenomenon is now well-known as the EPR (enhanced permeability and retention) effect in solid tumors. By contrast, most low-molecular-weight drugs such as anticancer agents cannot accumulate in tumor tissues at higher concentrations than in plasma[4] and are distributed by passive diffusion to various tissues and organs. Therefore, the cytotoxicity of these drugs frequently results in serious side effects in normal tissues.

Commercially available lipid emulsions (LEs) that are widely used as parenteral nutrition are mostly soybean oil-in-water formulations emulsified with phosphatidylcholine (lecithin; PC). They are also employed as drug carriers in drug delivery systems (DDS) and improve the therapeutic index of drugs by increasing the drugs' efficacy or reducing their toxicity. Examples include LE pharmaceuticals with prostaglandin E_1, dexamethasone palmitate (corticosteroid), and flurbiprofen axetil (nonsteroidal sedative), all approved for clinical use. However, the droplet size of these commercial LEs, generally ranging from 200—300 nm, is too large to be used for tumor targeting; it has been recognized that only drug carriers less than 100 nm in diameter can pass through the discontinuous capillary endothelium of tumors,[5] while particles with the droplet size of 100 nm or larger often do not penetrate the tumor interstitium.[6,7] Thus, LEs less than 100 nm in droplet size, denoted as lipid nano-emulsions (LNE), are expected to have high selectivity to tumor tissues,[8,9] even though their targeting is passive. However, it is difficult to prepare stable LNEs with the same components as traditional LEs. The addition of suitable cosurfactant(s) in LE preparation may facilitate the production of stable LNEs having a droplet size less than 100 nm.

An important problem in using LEs or LNEs as drug carriers is that they may be affected by inorganic ions in the body when LNEs are administrated in intravenous blood, *i.e.*, the emulsions are usually flocculated rapidly by ions counter to the surface charge of the LE particles.[10]

Another problem is the stability of LNEs during their storage. As LNEs are thermodynamically unstable, they tend to split into two distinct phases through a variety of physicochemical destabilizing processes known as flocculation, coalescence, creaming and phase inversion.[11,12]

In the present study we attempted to prepare a new stable LNE having a droplet size of 50 nm using two cosurfactants that are widely used as pharmaceutical additives, sodium palmitate (PA) and a sucrose fatty acid ester (SFAE). The aim of using PA was to reduce the diameter by providing a negative surface charge to LNE particles, and the aim of using an SFAE was to prevent the flocculation and coalescence of LNE particles induced by the most abundant cation in the blood, Na^+ ions. In recent years, SFAEs have received attention as emulsifiers that have a wide range of hydrophilic–lipophilic balance (HLB) values, from 1 to 16.[13] In addition, characterization of the prepared LNEs and evaluation of their stabilities in a saline (0.9 (w/v)% NaCl) solution and in long-term storage were performed using dynamic light scattering (DLS), atomic force microscopy (AFM), and ^{31}P and ^{23}Na nuclear magnetic resonance (NMR) spectroscopy, respectively.

DLS has been widely used as a convenient and useful tool to measure the mean droplet size and size distribution of LEs. On the other hand, AFM is an important technique to obtain images of individual colloidal particles as opposed to evaluating mean data, and to observe their particle morphology and surface features.[14] The use of ^{31}P-, and ^{23}Na-NMR spectroscopy will provide information on the dynamic features of these particles at the molecular or atomic level.

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Experimental

Reagents Soybean oil and glycerin were purchased from Kanto Chemical Co. (Tokyo, Japan). Egg yolk PC (COASOME NC-50) with the purity of more than 98% was supplied by NOF Co. (Tokyo, Japan) and was used without further purification. PA and SFAEs (RYOTO® sugar ester: sucrose laurate (SL) L-1695, sucrose myristate (SM) M-1695, sucrose palmitate (SP) P-1670, and sucrose stearate (SS) S-1670) were purchased from Sigma (MO, U.S.A.) and Mitsubishi-Kagaku Foods Co. (Tokyo, Japan), respectively. Sorbitan monooleate (Tween 80) and sucrose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Cholesterol was purchased from Tokyo Kasei Co. (Tokyo, Japan) and was used after recrystallization from ethyl acetate.

Preparation of LNE The formulations of LNEs are listed in Table 1. The amounts of soybean oil (SO) and phosphatidylcholine (PC) to be used in the LNE preparations, respectively, were given by a theoretical calculation based on the assumption that the lipid particles would be spheres having diameters of 50 nm.[15] To the mixture of SO, PC, and glycerol (2.2 (w/v)%) in 30 ml of deionized-distilled water, various amounts of PA and SFAE, cholesterol, Tween 80, or sucrose were added, and the mixture was emulsified by sonication with a probe-type sonicator (VC-501, Biomic) for 1 h at 55 °C in a thermostatic water bath (SB-35, Tokyo Rikakikai Co., Ltd., Japan). Sonication for 3 min was repeated at 3-min intervals. The obtained LNEs were then centrifuged at 2000×***g*** to eliminate sediment from the sonication tip. All of the prepared LNEs were stored in tight, light-resistant glass containers at room temperature under a nitrogen atmosphere.

Measurement of LNE Droplet Size Samples of the prepared LNEs were further diluted in deionized-distilled water containing 2.2 (w/v)% glycerol (1→1000) for the storage stability test. The mean diameter and droplet size distribution of the LNE particles was determined by DLS method using a submicron particle analyzer (Nicomp Model 380, Particle Sizing Systems, U.S.A.) with an He–Ne laser of 5 mW operated at 632.8 nm using a 90° angle between incident and scattered beams. Data were analyzed by Gaussian Analysis using Nicomp CW388 software with a viscosity of 0.933 and 0.693 cPoise, and a refractive index of 1.331 and 1.333 at 23 and 37 °C, respectively. The LNE droplet size was reported in terms of volume-weighted distribution.

Atomic Force Microscopy Imaging Prepared LNEs were further diluted in water (1→100) to obtain less sticky solutions. One-hundred microliters of the diluted sample was deposited in droplets onto a mica disk 1 cm in diameter. To remove excess water, the sample was kept in a desiccator overnight with a desiccant. AFM observations were performed with an atomic force microscope (Nano Scope IIIa, Digital Instruments, U.S.A.) using a standard Si_3N_4 probe of 123-μm length with an oscillation frequency of 350 kHz and a spring constant of 42 N/m. The AFM image was obtained with an intermittent contact (or tapping) mode with a scan speed of 1 Hz.

^{31}P-, and ^{23}Na-NMR Measurements To a 1-ml volumetric flask, we added 900 μl LNE and then 100 μl D_2O to the required volume. After shaking the flask constituents for a short time, all of the LNE sample solution was transferred to an NMR tube 5 mm in diameter. ^{31}P-, and ^{23}Na-NMR spectra were measured at 161.9 and 105.8 MHz, respectively, by a Varian (Palo Alto, CA, U.S.A.) ^{UNITY}INOVA 400NB spectrometer locked on D_2O (10%) added to the LNE samples. The reference signal for ^{31}P-NMR was the phosphate signal of PC in the LNE. The probe temperature was 21—23 °C. The numbers of free induction decay (FID) accumulations to improve the signal-to-noise ratio were 1000 for ^{31}P-NMR and 100 for ^{23}Na-NMR measurements, respectively.

Stability Test of LNE in a Saline Solution To a 499-ml saline solution (0.9 (w/v)% NaCl, 37 °C) in a 500-ml beaker placed in a thermostatic waterbath, 1 ml of LNE was added. The mixture was stirred at 500 rpm using a magnetic bar. A small portion of the mixture was withdrawn at suitable time intervals and transferred to a glass tube, and the droplet size of the LNE was analyzed by the DLS method as described above.

Table 1. Formulation of LNE

Soybean oil	1.50 g
PC	0.36 g
PA	Appropriate
SFAE	Appropriate
Glycerol	0.66 g
Water	Total 30 ml

Results and Discussion

Effect of PA on the Droplet Size The DLS results regarding the effect of PA on the droplet size of LNE are shown in Fig. 1. The mean diameter of the LNE prepared without PA was observed to be approximately 175 nm, though its expected value calculated from the amounts of SO and PC used was 50 nm.[15] However, the addition of PA reduced the LNE droplet size depending on the PA/PC ratio. At a PA/PC (w/w) ratio of larger than 1/10, the mean droplet size approached 50 nm. The typical size distribution of PA/PC (1/10)-LNE is depicted in Fig. 2. The size distribution curve in Fig. 2 confirmed that more than 90% of the PA/PC (1/10)-LNE particles had droplet sizes of smaller than 90 nm.

An AFM image of the PA/PC (1/10)-LNE was measured on a mica disk. The result in Fig. 3 shows that the LNE particles observed by AFM are spherical and have diameters mostly ranging from 40 to 80 nm. Thus, the AFM study supported the DLS results.

From the results of the DLS and AFM experiments, PA can be considered to work as a cosurfactant that facilitates the emulsification of PC and reduces the diameter of LNE particles. As the surfaces of lipid particles in the PA/PC-LNEs are negatively charged due to the COO^- of the PA molecules, the electrostatic repulsive force between the particles may serve to maintain their small diameters.

Lundberg has reported the preparation of a lipid emulsion having a mean droplet size of 50 nm using the cosurfactant polysorbate 80 in a w/w/w composition (castor oil : PC : polysorbate 80) of 1 : 0.40 : 0.12.[16] However, in our preparation, an LNE having a droplet size of 50 nm could be obtained by employing a smaller amount of PA (1 : 0.24 : 0.024) than that of polysorbate 80 in the Lundberg study.

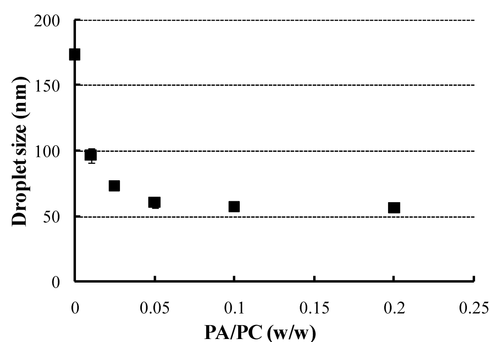

Fig. 1. Effect of PA Content on the Droplet Size of LNE Determined by the DLS Method (n=3)

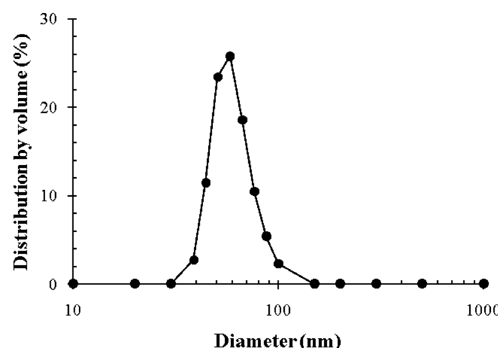

Fig. 2. Size Distribution of LNE Prepared at a PA/PC (w/w) Ratio of 1/10 Determined by the DLS Method

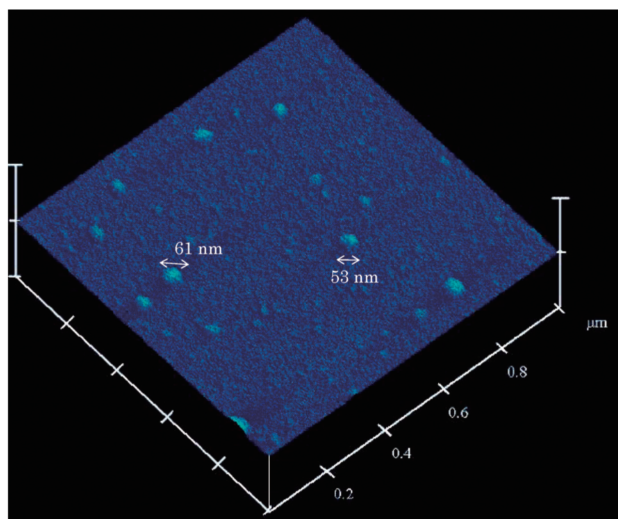

Fig. 3. AFM Image of LNE Prepared at a PA/PC (w/w) Ratio of 1/10 Adsorbed on a Mica Disk

Imaging was performed using the tapping mode.

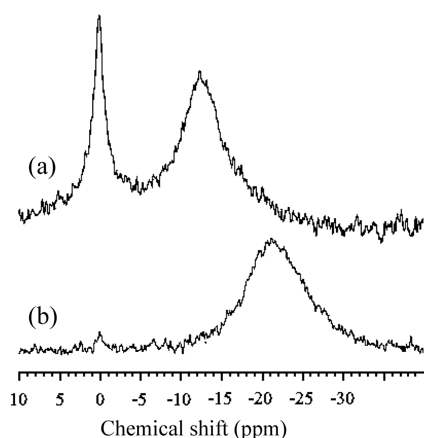

Fig. 4. ^{31}P-NMR Spectra of LNEs Prepared without (a) and with (b) PA at a PA/PC (w/w) Ratio of 1/10 Measured after the Addition of 4 mM Eu^{3+}

The reference (0 ppm) is the phosphorus signal of PC in the LNE in the absence of Eu^{3+}.

Confirmation of Liposome-Free LNE It has been reported that in the process of traditional LE preparation, liposomes are simultaneously produced.[17,18] To confirm the existence of liposomes, ^{31}P-NMR has been effectively used employing lanthanide shift reagents, Eu^{3+} and Pr^{3+}.[15,19—21] Figure 4 illustrates the ^{31}P-NMR spectra of our LNEs prepared without (a) and with (b) PA measured in the presence of a shift reagent (Eu^{3+}). In Fig. 4a, two signals are observed. The high-field signal was assigned to the phosphorus atoms of PC in the monolayer of LNE particles and in the outer layer of liposomes, which Eu^{3+} ions can access and cause the high-field shift accompanied by a little line broadening induced by shortening the relaxation time.[22] Another signal observed at the original position was assigned to the PC in the inner monolayer of the liposomes, which Eu^{3+} cannot access. Thus, Fig. 4a proves that the LNE prepared without PA contains liposomes.

On the other hand, the spectrum in Fig. 4b measured for the PA/PC (1/10)-LNE shows an upfield-shifted signal alone.

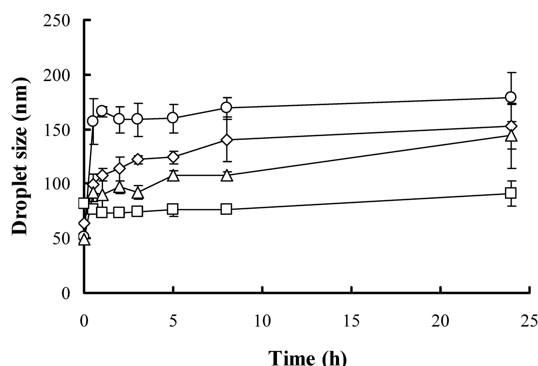

Fig. 5. Droplet Size Changes of PA/Cosurfactant/PC (1/2/10)-LNEs in Saline (0.9% NaCl) Solutions as a Function of Time at 37 °C (n=3)

Cosurfactant: (○) none, (△) SP, (◇) cholesterol, (□) Tween 80.

Therefore, Fig. 4b indicates that all of the PC molecules used in the LNE preparation formed monolayer surfaces on the LNE particles. The results confirm that liposome-free LNE having a mean diameter of *ca.* 50 nm can be prepared using PA as a cosurfactant at a PA/PC ratio of larger than 1/10.

Enhancement of the Stability of PA/PC (1/10)-LNE in Saline Solution by Using an Additional Cosurfactant, SFAE, Cholesterol, or Tween 80 Though the PA/PC (1/10)-LNE was confirmed to consist of particles with a mean diameter of 50 nm and to be liposome-free, it can be expected that when LNE is administrated intravenously, abundant Na^+ ions in the blood will reduce the electrostatic repulsive force between the particles, and thus the LNE will flocculate rapidly.[10] The results of our experiment in which PA/PC (1/10)-LNE was placed in a saline solution showed an increase in mean droplet size to more than 150 nm, even within 0.5 h, as seen in Fig. 5.

To suppress the coalescence of LNE particles induced by Na^+, three nonionic surfactants, SFAE (SP), cholesterol, and Tween 80,[12] were examined as candidates for an additional cosurfactant, and the results are shown in Fig. 5.

The addition of cholesterol (cholesterol/PC of 2/10, w/w) in the preparation of PA/PC (1/10)-LNE had little suppression effect on the increase of LNE droplet size as compared with that prepared without cholesterol; the mean droplet size of PA/cholesterol/PC (1/2/10)-LNE in a saline solution became larger than 100 nm within 0.5 h, 140 nm after 8 h, and 150 nm after 24 h, respectively.

On the other hand, the addition of SP (SP/PC of 2/10, w/w) effectively suppressed the increase in the droplet size; the mean droplet size of PA/SP/PC (1/2/10)-LNE was 90 nm (0.5 h), 100 nm (8 h), and 140 nm (24 h), respectively.

The mean droplet size of the PA/Tween 80/PC (1/2/10)-LNE showed no change even after 24 h. However, the mean droplet size of the PA/Tween 80/PC (1/2/10)-LNE just after preparation was approximately 70—100 nm. Moreover, since Tween formulas including Tween 80 have a narrow range of HLB values,[23,24] they are applicable in only a few areas of pharmaceutical preparation. Therefore, Tween 80 was not employed as a cosurfactant to stabilize the PA/PC (1/10)-LNE even though the PA/Tween 80/PC (1/2/10)-LNE was very stable in saline solution.

From these results, SP could be recommended as a useful cosurfactant to suppress the coalescence of LNE particles in-

duced by Na^{+}.

Effect of Fatty-Acid Chain Length of SFAEs on the Stability of PA/SFAE/PC-LNE in a Saline Solution As SP is an SFAE having a fatty-acid chain of 16 carbons, the effect of the chain length of SFAE on the enhancement of the LNE stability in saline solution was investigated. The results for four SFAEs having different chain lengths, *i.e.*, SL (C12), SM (C14), SP (C16), and SS (C18), respectively, are illustrated for SFAE/PC (w/w) ratio of 2/10 in Fig. 6a and for that of 4/10 in Fig. 6b. The results show that the increase in the mean droplet size of LNE in a saline solution after standing times of 0.5, 8, or 24 h was markedly suppressed depending on the fatty-acid chain length of the SFAE, up to 16 carbons; *i.e.*, SL (C12)<SM (C14)<SP (C16). The stability enhancement of these SFAEs also depended on the amount of SFAE.

However, SS, which has the longest chain length (18C) among the SFAEs used, showed different results as seen in Figs. 6a and b; *i.e.*, the stability of PA/SS/PC-LNE in a saline solution was not enhanced compared to that of PA/SP/PC-LNE. Furthermore, at the SS/PC of 4/10, SS had the poorest suppression effect on the 24-h droplet size increase, as shown in Fig. 6b. Though there is no clear evidence, the result that SP gave the best stability enhancement among these four SFAEs may relate to the fact that PA has the same chain length as SP.

When SP was used as a cosurfactant for stability enhancement in saline solution, it was unexpectedly observed that SP induced a further reduction of the mean droplet size; *i.e.*, the mean droplet size of 58.2 nm for an SP/PC (w/w) ratio of 0/10 was reduced to 44.7 nm for an SP/PC (w/w) ratio of 4/10. This result shows that SP works not only to strongly enhance LNE stability in a saline solution, but also to reduce the droplet size of the LNE.

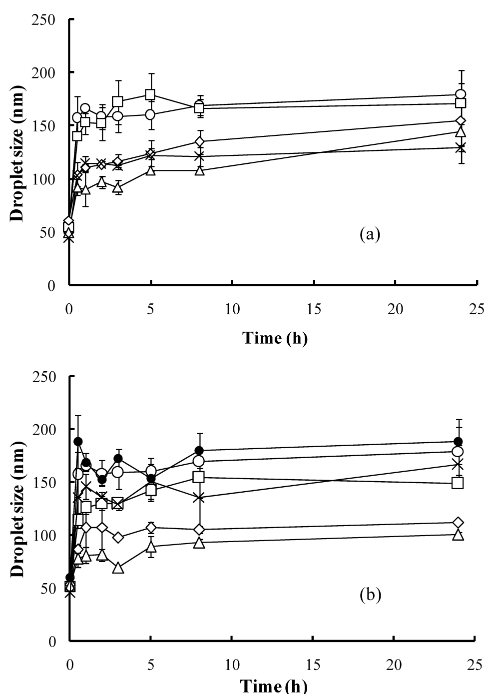

Fig. 6. Droplet Size Changes of PA/SFAE/PC (1/2/10)-LNE (a), and PA/SFAE/PC (1/4/10)-LNE and PA/Sucrose/PC (1/4/10)-LNE (b) in Saline (0.9% NaCl) Solutions as a Function of Time at 37 °C (n=3) Respectively

SFAE: (○) none, (□) SL, (◇) SM, (△) SP, (×) SS, and sucrose: (●).

Effect of Sucrose Sucrose, a moiety of SFAEs, has been frequently used as a lyoprotectant to prevent the size change of lipid particles during the processes of freeze-drying and rehydration[25,26]; therefore, the ability of sucrose itself for stability enhancement was examined. Figure 6b shows that the use of sucrose in the PA/PC (1/10)-LNE preparation did not effectively enhance the stability of PA/PC (1/10)-LNE particles in saline solution. This result shows that the added sucrose in the aqueous phase has little interaction with the surfaces of LNE particles; however, when sucrose is a moiety of SFAEs whose fatty-acid chains are inserted into PC layers of LNE particles, the sucrose moiety can exist near the surface of the LNE particles and thus prevent the approach of Na^{+} ions.

Storage Stability of LNE The long-term stability of the PA/SP/PC-LNE at room temperature under nitrogen gas was investigated by three different methods: DLS, ^{31}P-NMR, and ^{23}Na-NMR.

The mean droplet size and the size distribution, expressed by the standard deviation of the mean droplet size, were periodically measured for the three preparations (SP/PC: 0/10, 2/10, 4/10) over 12 months by the DLS method, and the results are listed in Table 2. The mean droplet size of the PA/PC (1/10)-LNE prepared without SP shows a significant difference between 0-month storage and 6- or 12-month storage. Also, the size distribution of the PA/PC (1/10)-LNE shows broadening within a few months.

Neither the mean droplet size nor the size distribution showed significant difference for the PA/SP/PC-LNEs. Thus, it is shown that SP prevents the coalescence of LNE particles and enhances the storage stability of the PA/SP/PC-LNE.

However, the DLS results may not reflect the flocculation of LNE particles, because the LNE sample solutions for the DLS measurement are diluted LNE (1→1000); thus, flocculation may be suppressed in diluted solutions. To examine the flocculation of LNE particles, another complementary method that does not require dilution of the LNE sample solution was needed.

^{31}P-NMR measurement of intact LNEs can offer information on the LNE droplet size increase due to LNE particle flocculation, since when the LNE particles do not coalescence but flocculate and thus their apparent droplet size increases, the molecular mobility of PC in LNE derived from the rotation of LNE particles will decrease and thus the line width of the ^{31}P-NMR signal of PC will be broader than that observed before the flocculation. The ^{31}P-NMR spectra of

Table 2. Effect of SP Content on the Mean Droplet Size and Size Distribution of PA/SP/PC-LNE in Long-Term Storage

SP/PC (w/w)	Mean droplet size±S.D.[a]		
	Month		
	0	6	12
0/10	58±26	61±29*	65±33**
2/10	51±24	50±24	51±26
4/10	45±20	44±23	47±23

The significance of the difference from the mean droplet size at 0 month is indicated by * $p<0.05$ or ** $p<0.01$. *a*) Size distribution is represented by a standard deviation (S.D.) of the Gaussian distribution curve obtained by DLS measurement for each PA/SP/PC-LNE.

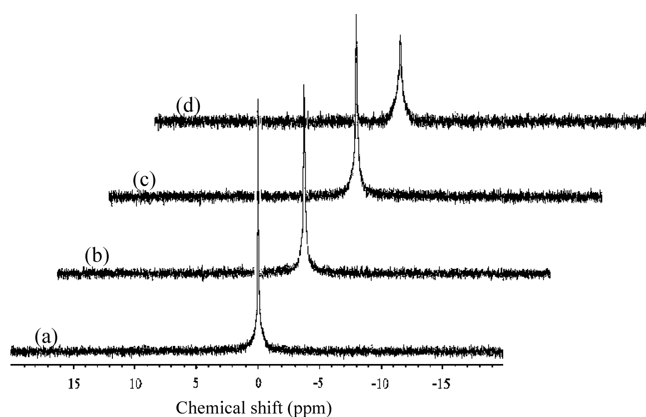

Fig. 7. ^{31}P-NMR Spectra of PA/PC (1/10)-LNE Just after the Preparation (a) and Stored for 2 (b), 3 (c) and 12 (d) Months

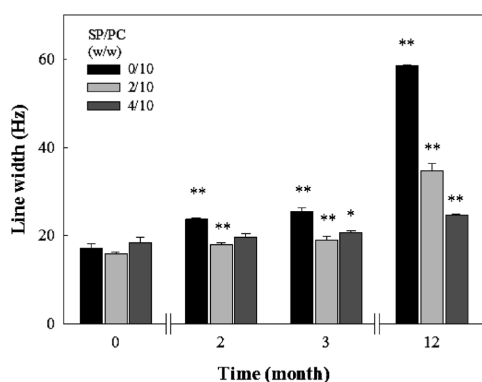

Fig. 8. The ^{31}P-NMR Signal Line Width (Hz) Changes of PC in PA/SP/PC (1/0/10—1/4/10)-LNEs during the Storage ($n=3$)

When the difference in the line width compared with that measured just after the preparation (0 month) is significant, the bar is indicated by $*p<0.05$ or $**p<0.01$.

PA/PC (1/10)-LNE measured periodically over 12 months are shown in Fig. 7. The ^{31}P-NMR signal of the PA/PC (1/10)-LNE did not show the chemical shift change during the 12-month storage, however, its line width were broadened drastically as seen in Fig. 7. The line-width change of PC in the PA/PC (1/10)-LNEs prepared with SP/PC (w/w) ratio of 0/10 to 4/10 is illustrated in Fig. 8. The line widths measured just after these LNEs were prepared do not show a significant difference; however, that of the PA/PC (1/10)-LNE prepared without SP shows approximately 1.5- and 3.4-fold increases after 3 and 12 months, respectively. According to the DLS results described above, the increase in the mean droplet size of the PA/PC (1/10)-LNE due to coalescence was rather small. Thus, the result of the ^{31}P-NMR measurement showing reduction of the molecular mobility of PC in the PA/PC (1/10)-LNE suggested the occurrence of flocculation in the PA/PC (1/10)-LNE during storage.

However, the line widths of both the PA/SP/PC (1/2/10)-LNE and PA/SP/PC (1/4/10)-LNE were smaller than that of the PA/PC (1/10)-LNE; in particular, the line width of the PA/SP/PC (1/4/10)-LNE shows only a 1.4-fold increase even after 12 months. This result indicated that SP enhances the storage stability by preventing flocculation as well as coalescence. Thus, from the viewpoint of the mobility of PC molecules in the LNEs, the ^{31}P-NMR measurement could offer information on flocculation that could not be ascertained by the DLS method.

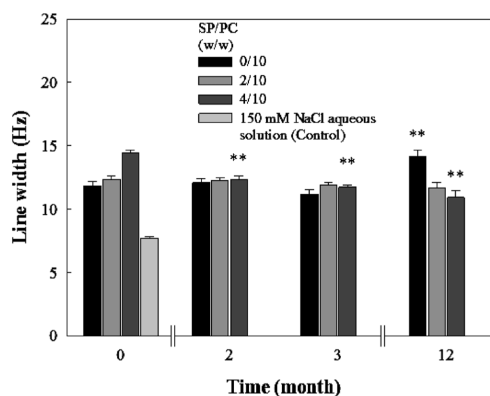

Fig. 9. The ^{23}Na-NMR Signal Line Width (Hz) Changes of Na^+ Ions in PA/SP/PC (1/0/10—1/4/10)-LNEs during the Storage ($n=3$)

When the difference in the line width compared with that measured just after the preparation (0 month) is significant, the bar is indicated by $**p<0.01$.

As described above, in saline solutions of PA/PC-LNEs, sodium cations induced coalescence of the LNE particles, while the secondary cosurfactant SP in the PA/SP/PC-LNEs effectively suppressed it. Since even PA/SP/PC-LNE themselves contain sodium cations derived from PA, the behavior of the sodium cations in these neat LNEs was monitored during a 12-month storage period by ^{23}Na-NMR observing the Na^+ signal line width. The results are shown in Fig. 9.

At every storage time, the line width of Na^+ ions of PA in the LNEs was broader than that of the 150 mM NaCl aqueous solution employed as a control. Thus, the mobility of Na^+ ions in the LNEs may be slightly restricted due to their interactions with negatively charged particle surfaces of the LNEs. As in the ^{31}P-NMR measurement, only the PA/PC (1/10)-LNE (without SP) showed a significant increase ($p<0.01$) in the line width of the Na^+ signal after 12 months of storage. Therefore, it was suggested that Na^+ ions might participate in the flocculation of the LNE during the storage.

From these three different methods, DLS, ^{31}P-NMR, and ^{23}Na-NMR, it has been confirmed that PA/SP/PC (1/4/10)-LNE can maintain a stable particle state for 12 months because the SP prevents both coalescence and flocculation of the LNE particles.

Conclusions

We prepared a stable LNE having a mean droplet diameter of 50 nm by employing two cosurfactants, PA and SP. These cosurfactants have different functions: PA reduces the diameter of LNE particles, and SP prevents the increase in droplet size induced by Na^+ ions and enhances storage stability.

The obtained PA/SP/PC (1/4/10)-LNE has the potential to be used as a tumor-targeting carrier. Its droplet size of less than 100 nm and the hydrophilic nature of the particle surfaces will also serve to prevent the uptake of the PA/SP/PC (1/4/10)-LNE by the reticulo-endothelial system, thereby ensuring prolonged circulation.[27)]

We further demonstrated that ^{31}P-, and ^{23}Na-NMR spectroscopy can conveniently offer useful information on the storage stability of LNEs, in addition to that obtained by the DLS method.

Acknowledgments This work was partially supported by the Academic

Frontier Project for Private Universities, and a Grant-in-Aid (No. 18790032) for Young Scientists (B) from The Ministry of Education, Culture, Sports, Science, and Technology of Japan. The authors would also like to thank their undergraduate students, Messrs. Kazunori Hayashida and Kohei Aoki for their experimental work.

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