Lyotropic Behaviors of a Phospholipid-based Lamella Liquid Crystalline Phase Hydrated by Propylene Glycol as a Polar Solvent: Correlation of DSPC *vs* PG Concentration

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The lyotropic behaviors to form the structure of distearoylphosphatidylcholine (DSPC)-based liquid crystal (LC) hydrated by only propylene glycol (PG) without water were examined by differential scanning calorimetry (DSC), X-ray diffractions (XRD), polarized microscope (PM) and transmission electron microscope (TEM). By increasing the amount of PG instead of water, it showed the phase transition to be gradually changed from anisotropic structures to other structures more close to isotropic ones and their appearance to be changed from solid-like states to liquid-like ones with more fluidity. Below 50% w/w PG, the mixtures of DSPC and PG resulted in no direct observation of LC structure through PM because they were very close to solid-states. From 55% w/w to 90% w/w of PG, the dense lamella crystalline structures were observed through PM, and their thickness and area decreased as the content of PG increased. Measured by DSC with heating process, the main phase transition from α-lamella phase to isotropic phase appeared from 52.89 °C to 47.41 °C to show linearly decreasing behaviors because PG affects the hydrophobic region of DSPC-based lamella phase. The repeating distance of the lamella phase and the interlayer distance between bilayers were calculated with XRDs and the average number of bilayers related to the thickness in LC structure was approximately estimated by combining with TEM results. The WAXS and DSC measurements showed that all of PG molecules contributed to swelling both the lipid layer in the edge region of lamella phase close to phosphate groups and the interlayer between bilayers below 90% w/w of PG. The phase and thermal behaviors were found to depend on the amount of PG used by means of dissolving DSPC as a phospholipid and rearranging its structure. Instead of water, the inducement of PG as a polar solvent in solid-lamella phase is discussed in terms of the swelling effect of PG for DSPC-based lamella membrane.

Key Words : Distearoylphosphatidylcholine, Propylene glycol, Lamella phase, Repeating distance, Interlayer distance

Introduction

Topical application of active substances has been widely investigated in recent years but their instability in water as well as the poor permeability of most active substances through the skin limits the use of topical drug delivery. Transdermal administration is limited by the stratum corneum (SC), the outer layer of skin, considered as the main barrier to percutaneous absorption of drugs. The SC is composed of keratin-filled dead cells which are entirely surrounded by crystalline lamellar lipid regions having a very dense structure.[1] When most of active substances applied onto the skin diffuse along the crystalline lamella lipid layer in the intercellular region, this dense structure obstructs the diffusion of active substances, reduces the efficiency of drug delivery into skin, and decreases the desirable effects such as whitening, anti-wrinkle, or moisturizing etc.[2] Increasing drug diffusivity or drug partitioning into the membrane using penetration enhancers is an option for improving permeation through the skin. And designing a similar structure to the SC is another way to realize an efficient drug delivery. In the view of above reasons, phospholipids are a potential group of penetration enhancers and have been administered as safe.[3] Being composed of natural body constituents and being biodegradable, phospholipids have been very useful in the cosmetic and pharmaceutical industry as unique natural and biocompatible emulsifiers.[4,5] Also, drug penetration can be more increased in the case of some combination of phospholipids and low molecular weight of polar solvents which efficiently decrease the barrier resistance of the stratum corneum (SC).[6,7] Mainly in pharmaceutical fields, numerous studies have evaluated the percutaneous penetration of some active substances such as testosterone, haloperidol, flurbiprofen etc using various penetrating enhancers such as propylene glycol (PG), polyethylene glycol (PEG), their derivatives, ethyl alcohol, glycerol monooleate, and so on.[8-11] Especially, among some enhancers having low molecular weight, polar materials such as PG, PEG and glycerin work for the formation of LC structure with phospholipids similar to the crystalline lamella lipid layer in the intercellular region, and they may affect less to the oxidization of some important active substances such as retinoids, ubiquinones, tocopherols than water when a phospholipids-based LC is formed in the use

of them by the replacement of water. However, most of studies done on the behaviors of LC phase mainly using phospholipids have been carried out using the mixture of phospholipids and water and limiting to the behaviors of spherical MLVs (Multilamella vesicle). In fact, there are no detailed studies of the interaction between phospholipids and glycols for protecting from the oxidation of unstable active substances such retinoids, tocopherols, ubiquinones etc, which are unstable at the moment of contacting with water molecules, as well as promoting their percutaneous penetration. In this paper, we chose distearoylphosphatidylcholin (DSPC) among many phospholipids and propylene glycol (PG) among many penetrating enhancers and have performed the correlational studies on the mixtures of DSPC as a main lipid and PG which is used as a solvent instead of water, using DSC, small angle X-ray scattering (SAXS), and wide angle X-ray scattering (WAXS). The aim of this work is to better understand the thermal and structural behaviors of non-aqueous LC depending on the amount of the solvent (PG) and apply them to cosmetics and pharmaceutics afterward in order not only to more stably encapsulate active substances mentioned above, but also to increase the efficiency of their drug delivery into skin.

Materials and Methods

Materials. DSPC as a phospholipid was purchased from Lipoid (Germany), PG was purchased from Merck (Germany). These materials were used without any pre-treatment.

Methods

Sample preparation: At first, DSPC as a main lipid was added to PG as a solvent under a moderate agitation, was heated to 80 °C and was continuously agitated until being completely melted. This melted mixture was cooled to 60 °C with a moderate cooling speed, and then was very slowly cooled to 35 °C and stored below 10 °C for 1 week before being analyzed.

DSC analysis: Thermal analysis was performed with a TA instrument (TA4100 model) from 10 °C to 70 °C at heating rate of 1 °C/min after being cooled to a lower temperature. Sample quantities were about 10 mg, which was sealed in an aluminum sample cell. This analysis was done under a nitrogen gas and was observed the phase transition temperature (T_c) and enthalpy change (ΔH) at the temperature to confirm the formation of LC structure and its thermal transition during heating process.

XRD analysis: XRD spectra were taken with XDS 2000 model (SCINTAG INC., USA). During this experiment the temperature of the samples deviated by maximum 1 °C from the adjusted temperature (24 °C). XRD experiments were carried out with Ni-filtered CuKα-ray (λ = 1.54 Å) using photo detection. SAXS data were recorded using a position sensitive proportional counter with a camera length of 350 mm and associated electronics (multichannel analyzer, etc. SCINTAG INC., USA). The scattering intensity was measured as a function of scattering vector, q. The scattering vector is defined as $q = (4\pi/\lambda)\sin\theta$, where 2θ is the scattering angle and λ is the X-ray wavelength (1.54 Å). The lamella repeat distance, D, was calculated as an average from the first and second order of diffraction according to $D = 2\pi/q_1$ for the first order of diffraction peak and $D = 4\pi/q_2$ for the second order of diffraction peak by following the same way as J. Zbytovska *et al.*[12] WAXS patterns were recorded by a flat-plate film cassette loaded with a high-sensitive X-ray film (Fuji Medical X-ray Film) with a camera length of 66.0 mm. Samples were sealed in a thin-walled glass capillary tube (outer diameter 1.0 mm) and mounted in a thermostable holder whose stability was ± 0.2 °C.

EM (Electron microscope) and PM analysis: EM analysis was examined with a transmission electron microscope (TEM, Hitachi H-7600). At that time, we introduced RuO_4 as a post-fixation agent to preserve these lipids in LC structure by means of the similar method as S.Y Hou *et al.* and B.A.I van den Bergh *et al.*[13,14] At first, a sample was slightly applied on the inner region of a tube. And then, for RuO_4 post-fixation, the specimens were post-fixed in 0.25% RuO_4 (EMS), the 0.1 M cacodylate buffer for 45 min at room temperature in the dark. All post-fixed tissues were rinsed in 0.1 M cacodylate buffer for 10 min, dehydrated in a graded ethanol series, respectively, and embedded in epon-epoxy resin. Ultra sections (Leica UCT) were cut, double stained with uranyl acetate and lead citrate, and then examined with TEM. PM study was performed with an Olympus BX-51 polarized microscope.

Results and Discussion

Lyotropic interaction of PG as a polar solvent with DSPC. SAXS has been widely used to characterize the structure of lipid amphiphile and lamella liquid crystal in polar media.[15-18] In polar solvents such as water, PG, PEG etc., many lipids are known to range themselves in lamella structures being composed of multibilayer with a repeat distance of few nanometers, thus, giving rise to Bragg diffraction.

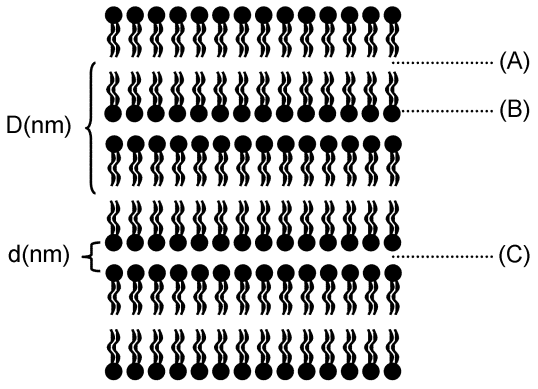

Figure 1. Schematic representation of lamella crystalline phases based on the DSPC when complexed with a polar solvent. Lipid headgroups are represented by blue circles attached to two hydrocarbons. The distance, D, is the ordinate for Fig. 2. The distance, d, is the ordinate for Fig. 4.

Figure 1 shows the scheme of this lamella structure and the location sites where each solvent can exist in the lamella phase whether they are hydrophobic or hydrophilic. In Figure 1, the repeat distance (D) corresponds to the thickness of lipid bilayer plus the interlayer thickness mainly being composed of polar solvents. This is illustrated in Figure 2 for DSPC at a fixed temperature from SAXS, depending on the amount of PG. Below the transition temperature (T_c), pure PC-based MLV in excess of water show two peaks, which represent the first and second order of diffraction of a lamella phase.[12] Theoretically, the bilayer distance of DSPC can be calculated as the following in the case of non-tilted bilayer; (18 × 1.3 Å) × 2 = 46.8 Å which '18' means the number of hydrocarbons, '1.3 Å' means the distance of methylene group (-CH_2), and the last '2' means the bilayer having two layers perpendicular together. But, experimentally measuring with SAXS, the SAXS patterns show three separated peaks at q = 0.130, 0.254, 0.397 $Å^{-1}$ and the repeat distance(D) can be calculated as 48.44 Å slightly higher than the theoretical value by averaging D values for each q value. As seen in Figure 2, the incorporation of PG into pure DSPC membrane made the SAXS patterns show three separated peaks similarly to DSPC, and the repeat distance(D) appeared to be gradually longer than the experimental D value of DSPC. As the amount of PG increases from 25.0% w/w to 55.0% w/w, the peaks slightly shifted to the left side having smaller q($Å^{-1}$), the repeat distance(D) increased from 50.55 Å to 52.89 Å, and the intensities of the first-order diffraction significantly decreased in comparison to pure DSPC. However, at 70% w/w of PG, the D value appeared to be more highly increased than the above values as 55.49 Å at q = 0.113, 0.225, 0.341 $Å^{-1}$ and the intensity of the first-order diffraction also decreased. At 80% w/w of PG, the D value was slightly more increased to 56.25 Å at q = 0.111, 0.220, 0.342 $Å^{-1}$ to reach the maximum point, and there was no increase in D value even if the PG content increases more. However, the intensities of the first-order diffraction gradually decreased depending on the PG content. The calculated repeat distances (D) are plotted in Figure 3 as a function of the ratio PG/DSPC. With the increase of PG content, D values almost linearly increase and get to be level-off above the maximum point of 80% w/w of PG as shown in Figure 3, but the increasing levels are smaller than in the case of water. It suggests that PG molecules penetrate into the polar region (C) of DSPC membrane, swell the lamella structure, and extend the lamella repeat distance (D). According to G. Klose *et al.*,[19] when water molecules were incorporated into egg phosphatidylcholin membrane, the D value from SAXS almost linearly increased to some amount of water about from 50 Å to 63 Å and the increase of D value became level-off over this amount of water. Also, by T. de Vringer *et al.*,[20] the inclusion of water into PGM (Polyglycerylmyristate) made these D values significantly increased from 70 Å to 89 Å. However, differently from the incorporation of water molecules, the increasing level for D value appeared to be relatively small as several Å and the reason might be understood that the inclusion of PG into DSPC-based lamella phase makes a non-polar group (-$CH_2CH_2CH_2$-) of PG located between (B) and (A) regions of the lamella phase (Fig. 1) to be closer to (B) region, work as a relatively good solvent for the hydrocarbon chains of DSPC due to much smaller dielectric constant (ε = 32.1) in comparison to water molecule (ε = 80), and decrease the rigidity of stearyl group to slightly overlap the hydrocarbon chains together and be intertwined. As shown in Figure 2, the weakening behaviors in the intensity of the first-order reflection can be explained by the above mechanism. Therefore, it could be understood that PG is affecting the hydrophobic region between (B) and (A) regions of DSPC-based lamella phase differently from water which almost don't affect the hydrophobic region of lamella phase.

The high intensity peaks obtained from WAXS patterns

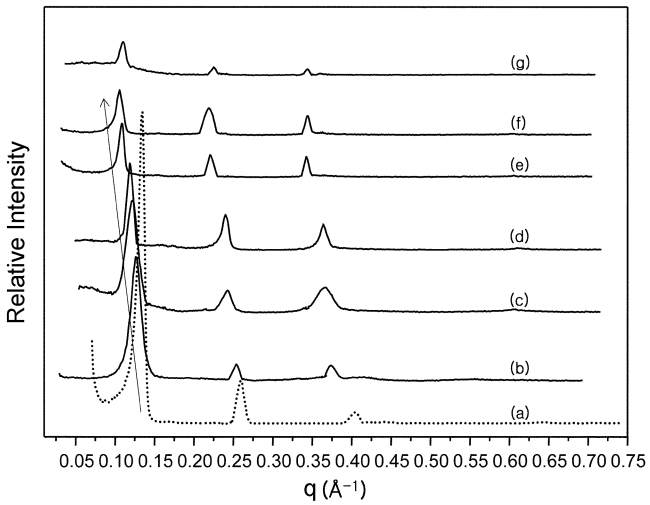

Figure 2. SAXS patterns of lamella crystalline phases based on DSPC depending on the amount of PG at 24 °C ± 1 °C below overall transition temperature (T_c): a) 0% w/w PG (DSPC), B) 25.0% w/w PG, C) 40.0% w/w PG, D) 55.0% w/w PG, E) 70.0% w/w PG, F) 80.0% w/w PG, G) 90.0% w/w PG.

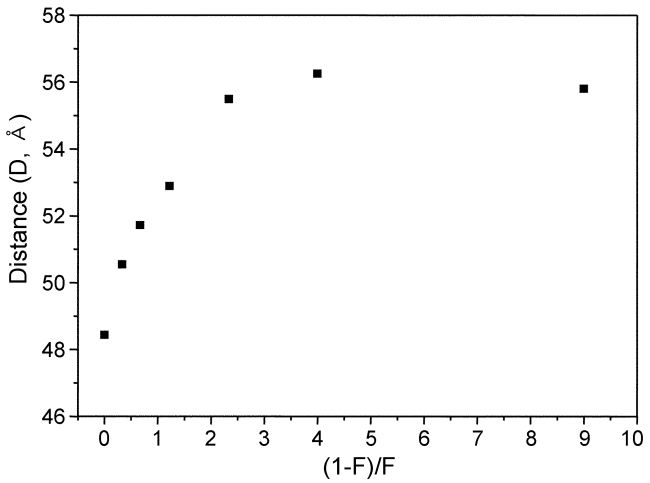

Figure 3. Variances of the repeat distance(D) in the mixtures of DSPC and PG at 24 °C *vs*. the ratio PG/DSPC, F is the weight fraction of DSPC.

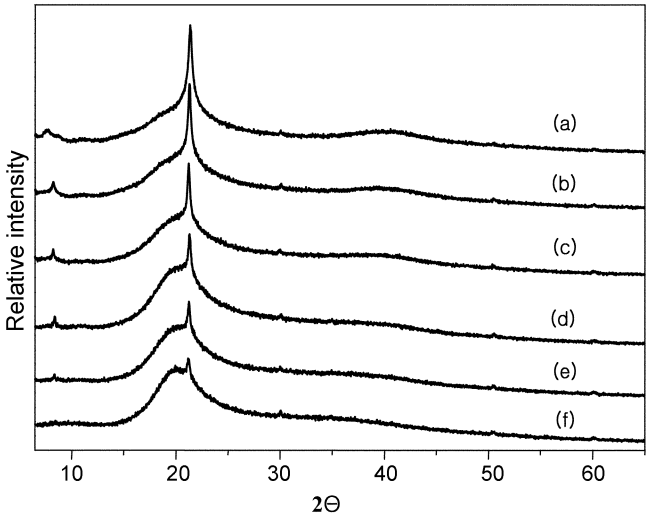

Figure 4. WAXS patterns of lamella crystalline phases based on DSPC depending on the amount of PG at 24 °C ± 1 °C below overall transition temperatures (T_c): a) 25.0% w/w PG, b) 40.0% w/w PG, c) 55.0% w/w PG, d) 70.0% w/w PG, e) 80.0% w/w PG, f) 90.0% w/w PG.

correspond to the electron dense phosphate fragments in DSPC head groups. Similarly to SAXS results (Fig. 2), WAXS results are illustrated in Fig. 4 for DSPC at a fixed temperature below each melting point, depending on the increase of PG contents. The interlayer distance (d) between bilayers is possible to be calculated from the intensity maxima as a main peak by following equation (1) below: d = interlayer distance, λ = wave length of X-ray (1.54 Å), 2θ = Bragg's angle

$$D = \lambda/(2\sin\theta) \quad (1)$$

From these results, the mean distance of interlayers (C) between the densely packed hydrophobic regions could be calculated. It showed WAXS patterns for the complexation of DSPC with PG and proved the function of PG as a solvent strongly affecting to the polar region (C) of the lamella phase, as well as the hydrophobic region (B). For all of samples, as the PG content increases, some diffused or broad peaks beside the main peak appeared and the intensities gradually increased. These diffused or broad peaks arise from the electron density contrast between the bilayer and the solvent such as PG.[21] It showed one sharp peak and another diffused peak representing for the miscibility between DSPC as a main lipid and PG. as a solvent corresponding to the limited solubility of the phospholipid in the concentration of the solvent to form not-separated interlayer.[22] The small peaks appearing in smaller diffraction angle ($2\theta = 8.0^o \pm 0.3$, d = 11.04 Å ± 0.4) show the formation of PG-rich lamella phase swollen by higher amount of PG and it indicates the formation of immiscible phase at this content of PG. The immiscibility of the lamella phase will lead to a phase being mostly composed of DSPC and another formed by DSPC and PG. Increasing the content of PG, the intensity of these peaks gradually decreased, and also the interlayer distances at this region gradually decreased from 11.04 Å to 10.54 Å and these peaks disappeared over 80% w/w PG. The incorporation of PG into the interlayer of DSPC membrane slightly weakened the intensity of the main diffraction peak to be almost completely diffused at 90% w/w PG. The interlayer distance (d) of the lamella phase swollen by PG is possible to be calculated from the main diffraction peak and overall data represent the 4.16 Å ± 0.02 at about $2\theta = 21.3 \pm 0.1$ by above equation (1). No further dependence between the interlayer distance and the PG content in DSPC membrane was found. But, there were diffused peaks beside the main peak representing the interlayer distance (d) of lamella phase and they were also significantly affected by increasing PG. These diffused or broad peaks at almost d = 4.82 Å ± 0.16, $2\theta = 18.4^o \pm 0.6$ are presumed to form the complex between DSPC head groups and PG to have low ionic strength. Below 55.0% w/w PG, the sharp peak at the intensity maxima and the diffused peak were not completely differentiated because the intensity of this diffused peak was too weak due to low level of complexation, but, more increasing PG content to 70% w/w, the intensity and area of this diffused peak was continuous to increase and form almost one diffused peak at 90% w/w PG. It should suggest that PG molecules gradually interact with hydrophilic interlayers in the lamella phase to form a complex together, that is, the interlayers between lipid bilayers are also continuous to be swollen by PG and increase another phase consisting of hydrophilic complexes between head groups of DSPC and PG molecules with the more increased interlayer distance. This can be the reason why the inclusion of PG into DSPC-based lamella phase makes a polar group (-OH) of PG located in (C) region (Fig. 1) which is called as the interlayer region between lipid bilayers and these polar groups of PG penetrate into space between lipid bilayers gradually to increase the electrostatic repulsive force between the polar head groups of DSPC, form another phase having longer interlayer distances, and expand this domain. However, these polar groups of PG have the lower ionic bonding strength than water, and so the peaks appeared to be diffused or broad and the interlayer distance (d) also increased relatively a little, differently to water.

Thermotropic interaction of PG as a polar solvent with DSPC. The DSC analysis of complexed mixture of DSPC with PG has been performed, and the results are presented in Figure 5. The main transition temperature (T_c) of pure DSPC from the α-lamella state to the isotropic state closely related to the melting temperature (T_m) of diacyl group of DSPC have been known to about 58 °C.[23] As seen in Figure 5, the main transition peaks appeared about from 53 °C to 47 °C with enthalpy changes due to the phase transition from the α-lamella state to the isotropic state in DSPC-based lamella structure. Extrapolating the data of phase transition temperature to x-axis as x = 0, T_c at this point as 57.6 °C get to be almost in the agreement of the T_c of pure DSPC. Below 55% w/w PG, there were relatively sharp and strong endothermal peaks corresponding to that the DSPC-based lamella structure swollen by small amount of PG were transformed to the isotropic phase. In this region, T_c gradually decreased from

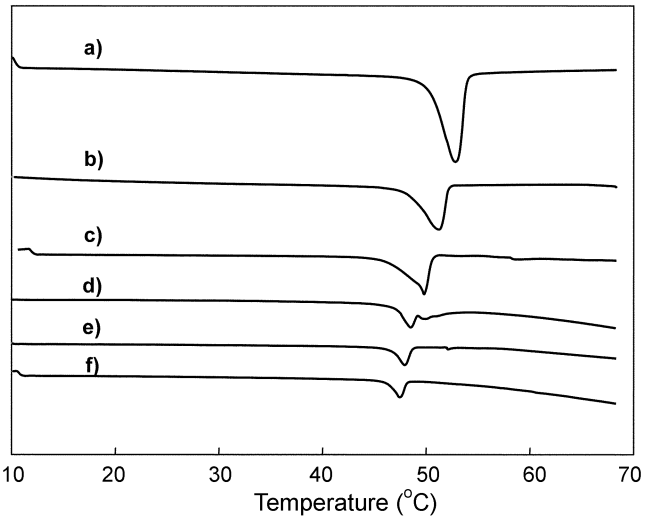

Figure 5. DSC curves of lamella crystalline phases based on DSPC depending on the increase of PG content during heating process from 10 °C to 70 °C: a) 25.0% w/w PG: 52.89 °C, 56.78 J/g, b) 40.0% w/w PG: 51.26 °C, 45.74 J/g, c) 55.0% w/w PG: 49.81 °C, 36.26 J/g, d) 70.0% w/w PG: 48.47 °C, 19.47 J/g, e) 80.0% w/w PG: 47.93 °C, 10.31 J/g, f) 90.0% w/w PG: 47.41 °C, 8.695 J/g.

52.89 °C to 49.81 °C and the enthalpy change (ΔH) also decreased from 56.78 J/g to 36.26 J/g. Likely to SAXS and WAXS results, in this region, PG molecules penetrate into the DSPC membrane to form little swollen lamella structure and the thermal behaviors are more close to DSPC itself. But, over 60% w/w PG, the enthalpy change (ΔH) started to be dramatically decreased and T_c gradually decreased. Especially, the enthalpy change (ΔH) decreased from 36.26 J/g to 19.47 J/g as almost half level at 70% w/w PG and also to 10.31 J/g at 80% w/w PG, respectively. At 90% w/w PG, the enthalpy change (ΔH) only slightly more decreased to 8.695 J/g and T_c was also shown to be 47.41 °C similar to 80% w/w. In Figure 6, it is illustrated how the phase transition temperature (T_c) of these lamella structures and their enthalpy change (ΔH) at each T_c are changed by the variance of PG content as a polar solvent. As seen in Figure 6, the increase of PG content makes the transition pattern of DSPC follow the linear behavior with a negative slope until 80% w/w PG. This should suggest that all of PG molecules added until 80% w/w participate in swelling the DSPC-based lamella structure and attribute to the formation of complexes with DSPC and these results are in good agreement with WAXS results. Also, as seen in Figure 6, the increase of PG content makes the pattern of their enthalpy change follow the almost linear behavior with a negative slope until 80.0% w/w PG. This should also support the above result related to the variance of T_c and prove that all of PG molecules added until 80.0% w/w take a part in the swelling process of LC. However, over 80% w/w PG, the slow down of above tendency which ΔH decreased may result from the reduction of its effect which attributes to the swelling process of the lamella structure. So actually, in the view of the results obtained from thermal transition behaviors, the maximum weight content of PG to swell the DSPC-based lamella

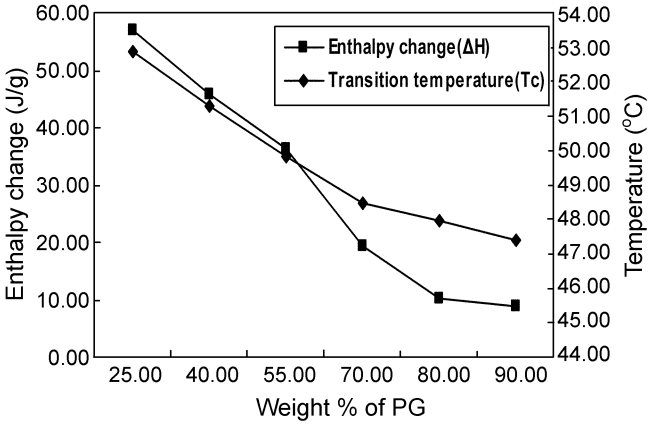

Figure 6. Variances of phase transition temperature (T_c) and enthalpy change (ΔH) at each T_c, depending on PG content.

structure might be supposed to be 80% w/w.

Visualization for the structure of DSPC-based lamella crystal swollen by PG from PM and TEM. With 1,000 magnifications, the PM results showed that all of samples to be monitored had a dense and regular lamella structure (Fig. 7). This showed an optical anisotropy behavior to form lamella structures. But, below 50% w/w PG, it was impossible to observe the lamella structures with PM because the lamella structure is too dense to be transmitted by light. As seen in Figure 7, increasing the PC content, DSPC-based lamella structure became less dense and their areas to form lamella structure significantly were reduced. Particularly, until the maximum content (80% w/w) of PG where all of PG molecules added contribute to the swelling process of lamella structure in Figure 6, their densities and areas were relatively well maintained in spite of broadening these structures due to the effect of PG as a relatively good solvent for the hydrophobic region between (B) and (A). As seen in Figure 7(d), over 80% w/w PG, the lamella density and area

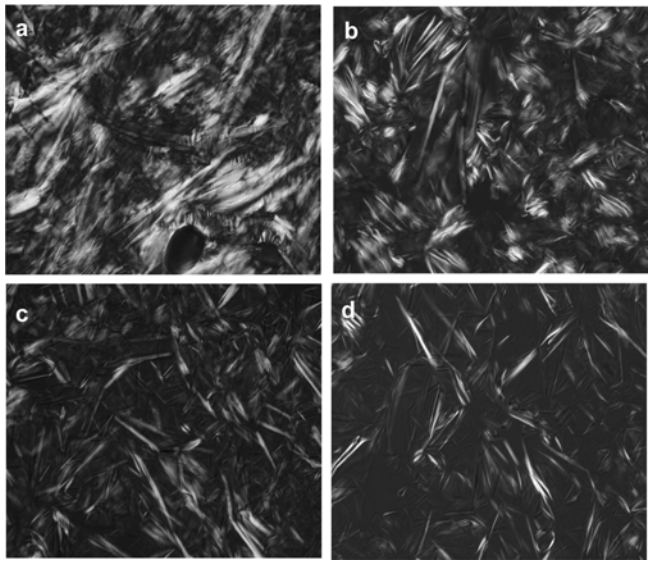

Figure 7. PM photographs of a) 55.0% w/w PG, b) 70.0% w/w PG, c) 80.0% w/w PG, d) 90.0% w/w PG.

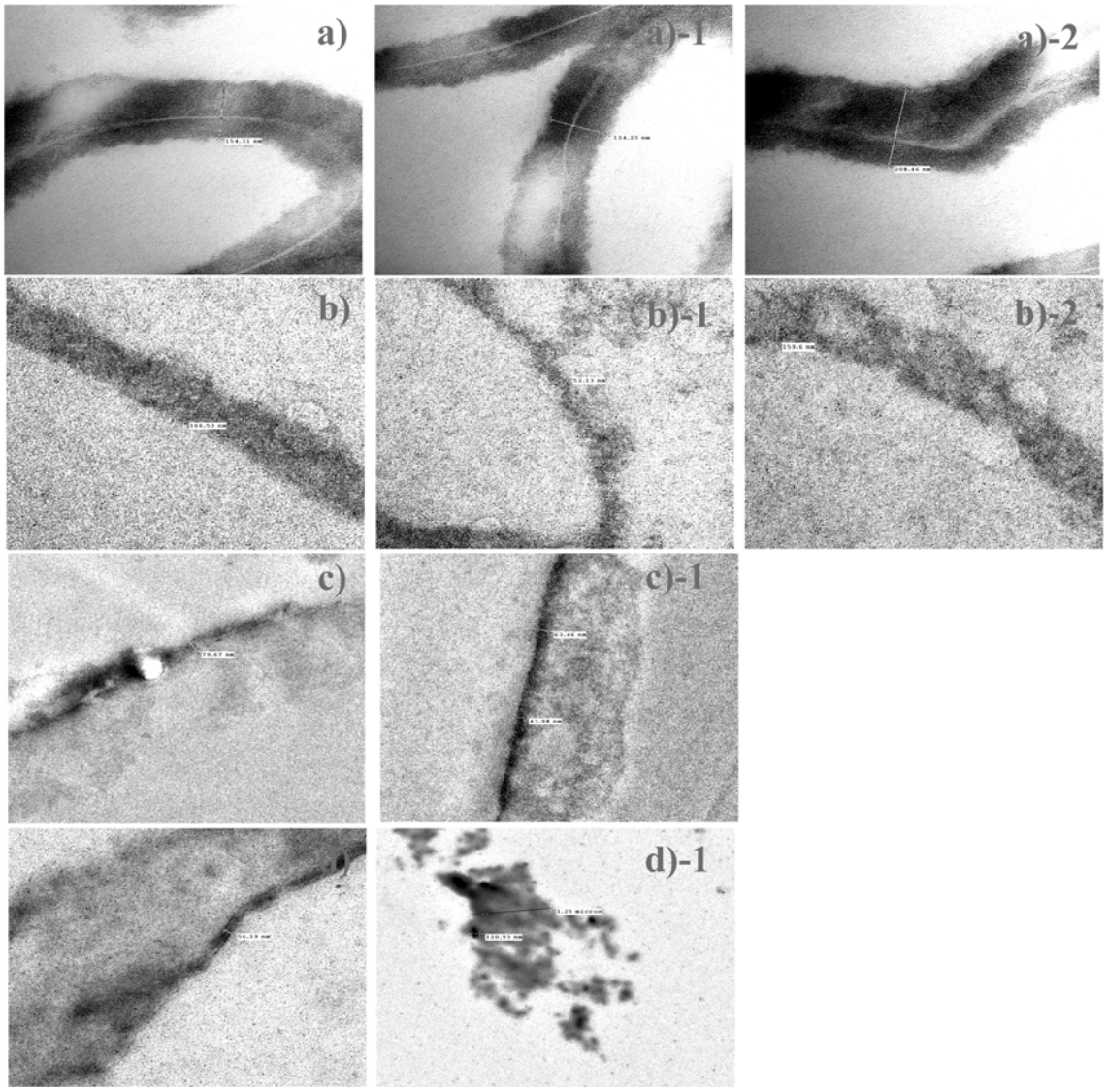

Figure 8. TEM photographs of DSPC-based lamella structure; at 25.0% w/w PG (30,000(x) Magnification), a): 154.31 nm, a)-1: 124.23 nm, a)-2: 208.46 nm, at 55.0% w/w PG (20,000(x) Magnification), b): 166.53 nm, b)-1: 52.13 nm, b)-2: 159.6 nm, at 80.0% w/w PG (10,000(x) Magnification), c)-1: 79.67 nm, c)-2: 43.98 nm, at 90.0% w/w PG, d): 56.19 nm (10,000(x) Magnification), d)-1: 1.25 μm (4,000(x) Magnification)

remarkably decreased in comparison to results below 80% w/w PG and this is also in the good agreement with the level-off point (Fig. 6) about the decrease of ΔH which suggest the maximum limit of PG molecules to attribute to the swelling process of the lamella structure.

In Figure 8, it showed TEM photographs of DSPC-based lamella phase, depending upon the PG content. In the case of small content of PG (25.0% w/w), it was shown that the lamella thicknesses were ranged from 124.23 nm to 208.46 nm and the structures were well-packed and dense lamella structure in Figure 8(a), (a)-1, (a)-2. Considering the lamella distance (D) obtained from SAXS pattern, the number of bilayers can be approximately estimated in the range of 24.57 to 41.24 layers. As seen in Figure 8, the lamella thickness diminished proportionally to the increased amount of PG and these results were in agreement with PM results and DSC results. In Figure 8(b), (b)-1, (b)-2, the lamella thickness can be estimated in the range of 9.86 to 31.49 layers at 55.0% w/w of PG with the same calculation as above. In Figure 8(c), (c)-1, the lamella thickness can be estimated in the range of 7.82 to 14.16 layers at 80.0% w/w of PG. However, to prepare samples for TEM analysis, we used 0.25% RuO_4 (EMS), 0.1 M cacodylate buffer as a post-fixation agent in order to preserve the arrangement of these lipids in the lamella phase and proceeded to some de-hydration to remove other solvents. Therefore, if the lamella structure were not well-packed and arranged, a dense lamella structure won't be observed and will be separated to form an irregular phase having a larger size as seen in Figure 8((d)-1).

Conclusions

In a series of investigations, researches on the lamella

structures have been presented in the mixture of phosphatidylcholins as biocompatible lipids and water. Elucidation of these structures could be achieved by a combination of experimental techniques. Particularly, the combinations of DSC, WAXS, and SAXS yield valuable information because these techniques provide quantitative results, and also, some microscopic techniques were used to confirm the lamella structures and match the quantitative results. PG molecules play a very important role in the formation of the lamella structure, while the behaviors of DSPC were very different in the presence of PG in comparison to using water. Mixtures of DSPC to 90.0% w/w PG can be regarded as opaque gels with a lamella structure. Increasing PG content, the lamella repeat distance(D) from SAXS patterns got to be gradually longer, but the increasing level was several Å, much smaller than the use of water. This can be the reason why non-polar group ($-CH_2CH_2CH_2-$) of PG works as a relatively good solvent for the hydrocarbon chains of DSPC due to lower dielectric constant than water and decrease their rigidity to be intertwined together. Combining TEM with SAXS results, the lamella thickness and number of bilayers in the lamella phase was approximately estimated and was shown to be gradually reduced by the incorporation of PG. Those were also confirmed with PM photographs. From WAXS patterns, the interlayer distance (d) was calculated and no further dependence between d value and PG content was found at the intensity maxima, but the diffused peaks which represent the complex formation between DSPC head groups and PG molecules appeared beside the main peak and their intensities were gradually larger with the increase of PG content below 90% w/w. Therefore, all of PG molecules can be considered to contribute to the swelling process of DSPC membrane. Also, from DSC results, the transition temperature (T_c) and enthalpy change (ΔH) linearly decreased with the increase of PG content below 90% w/w, even though the hydrophilic group of DSPC interact with the hydroxyl group (-OH) of PG. in the polar region (C) of the lamella phase. Summarizing the above results, PG molecules also contribute to the swelling process of DSPC membrane until being relatively higher content below 90% w/w, but the behaviors were very different from water molecules in the view of affecting to the non-polar region of DSPC, as well as the polar one. In order to elevate the accomplishment of this non-aqueous lamella system, it was needed to incorporate other materials to reinforce the lamella thickness, regularity, and density enough to avoid active materials such as retinoids, ubiquinones, and tocopherols etc. to contact to water and to make the structure and composition more similar to human stratum corneum (SC) to help transdermal drug delivery in the application of cosmetics and pharmaceutics. Therefore, incorporating above other materials such as ceramides, cholesterol, and higher alcohols etc., the lyotropic and phase behaviors will be discussed in a forthcoming paper.

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