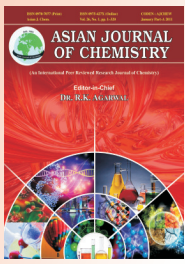
Asian Journal of Chemistry; Vol. 26, No. 14 (2014), 4149-4156

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16041



Studies of Lateral Interactions Between C18 Unsaturated Fatty Acid with Polyethoxylated Phospholipid Mixed Langmuir Monolayer at Air-Aqueous Interface for Liposome Formulation

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Received: 12 July 2013; *Accepted*: 3 December 2013; *Published online*: 5 July 2014; AJC-15431

Intermolecular interactions between C18 polyunsaturated fatty acid and polyethoxylated phospholipids have been studied by surface pressure-area isotherm. The effect of fatty acids' unsaturation degree and polyethoxylated phospholipids with different molecular weight namely, 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy-(polyethylene glycol)-2000] (DPPE-PEG2000) and 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-5000] (DPPE-PEG5000) on the stability of monolayer have been investigated. The obtained isotherms were analyzed quantitatively to evaluate the thermodynamic properties such as miscibility, compression modulus and stability of the mixed monolayer on phosphate buffer subphase. We found that miscibility for mixture of oleic acid and DPPE-PEG5000 is limited to higher mole fraction of DPPE-PEG5000. Whereas DPPE-PEG2000 is more compatible with oleic acid and DPPE-PEG5000 is more compatible with linoleic acid. These findings provide valuable information for the preparation of PEGylated fatty acid liposome and understanding on the interaction between fatty acid and polyethoxylated phospholipid in the lipid bilayer membrane as well as their membrane stability.

Keywords: Unsaturated fatty acid, Polyethoxylated phospholipid, Monolayer, Stability.

INTRODUCTION

Physical stability of the liposome in aqueous suspension is one of the major concerns in promoting their potential applications such as in drug delivery system and cosmetics. The stability of liposome in suspension is governed by the net forces of attraction and repulsion among the vesicles. This net force is affected by the matrix, compositions and the type of amphiphiles in liposomes.

In order to enhance the stability of liposome, polyethoxylated phospholipid is included in the preparation of liposome[1,2]. This is due to the steric repulsion force of the long polyethylene glycol (PEG) chain extended out from the surface of liposome and may prevent the possibility of aggregation. Furthermore, PEG grafted onto phosphatidylethanolamine has so far been reported as the best polyethylene glycolated lipid in prolonging the time of liposome in blood circulation[3-6]. Their efficiency was greater than that of ganglioside GM1[3].

There are several techniques have been used to study the effects of polyethoxylated phospholipids in liposomes. Micropipette technique is a common method to evaluate the mechanical stability of PEGylated bilayer in liposome through the study of their elasticity behavior[7]. A two dimensional structure of Langmuir monolayer at air-water interface apparently simulate half of the bilayer membrane is another widely used method to study the molecular interaction of a substance with biological membrane or synthetic liposomes. In the likely manner, the effect of PEG in lipid monolayer was carried out using neutron reflectometry by studying the density distribution of monolayer structure at the air-water interface[8,9].

Despite the great experimental potential of polyethoxylated phospholipids, their mixed behavior and intermolecular interactions with fatty acid at the air-aqueous interface using Langmuir monolayer technique is not yet well understood. To our best of knowledge, there are only limited investigations on the interaction between polyethoxylated phospholipid and phospholipids[10].

In this work, we will study the interfacial properties of binary mixture composing C18 polyunsaturated fatty acid and polyethoxylated phospholipids. The effect of double bond in the fatty acid hydrocarbon chain and molecular weight of the polyethoxylated phospholipids on the cooperative intermolecular interaction is highlighted. Information on the intermolecular forces can be qualitatively analyzed from the half of membrane bilayer that can be studied using Langmuir monolayer isotherm. In order to qualitatively illustrate the miscibility of fatty acid with polyethoxylated phospholipids mixed monolayer, excess area of the mixed monolayer is calculated.

The excess free energies of these mixtures are analyzed by applying surface thermodynamic analysis and the most stable composition of each mixed fatty acid/polyethoxylated phospholipids is also determined.

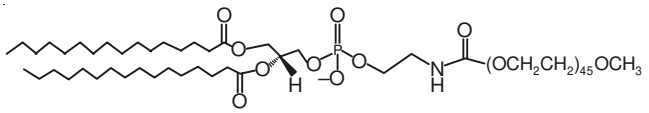
EXPERIMENTAL

Oleic acid (*cis*-9-octadecenoic acid, = 99 %) and linoleic acid (*cis*, *cis*-9, 12-octadecadienoic acid, = 99 %) were purchased from Fluka (Buchs, Switzerland). 1,2-Dipalmitoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DPPE-PEG2000) and 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanol-amine-*N*-[methoxy(polyethylene glycol)-5000] (DPPE-PEG5000) were from avanti polar lipids Inc. (Alabama, USA). Hydrochloric acid, sodium hydroxide 98 % and chloroform (distilled) of analytical grade were purchased from HmBG Chemicals. Monosodium dihydrogen phosphate 95 % and disodium hydrogen phosphate dehydrate 99.5 % were supplied by Systerm. All chemicals were used as received. Deionized water with 18.2 μS cm^{-1} was obtained from Barnstead NANO pure® Diamond™ ultrapure water system. Deionized water was further distilled and deaerated under nitrogen gas prior to use.

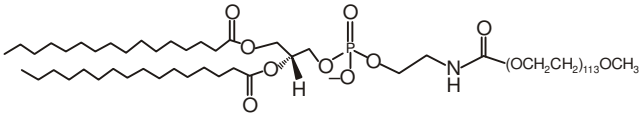
Measurement of langmuir film pressure: KSV 5000 Langmuir Blodgett balance (KSV Instrument Ltd., Helsinki, Finland) were used to record the surface pressure-area (P-A) monolayer isotherm. The TEFLON trough with dimension of 150 mm × 512 mm was placed on an anti-vibration bench and kept in a clear perspex chamber in order to isolate from the surrounding environment. The through was connected with a temperature controller by means of water circulator and two mechanically coupled Delrin® barriers for symmetric compression. Platinum Wilhelmy plate suspended from a microbalance was used to continuously monitor the surface pressure. The preparation and surface pressure-area measurements procedures were described elsewhere[11]. The instrument was calibrated using stearic acid on deionized water subphase prior to the samples measurements. The typical characteristics was observed that the de tion from zero surface pressure at area per molecule around 25 $Å^2$ $molecule^{-1}$. In addition, at surface pressure about 25 mN m^{-1}, a significant change of the slope was observed. The extrapolated area per molecule is found at in the range of 20 ± 1 $Å^2$ $molecule^{-1}$. The surface purity of the subphase was confirmed prior to the measurement by expanding and compressing the barriers, whereby surface pressure readings did not differ by more than ± 0.2 mN m^{-1}. Phosphate buffer pH 7 (50 mM) as a subphase was thermo equilibrated at 25 ± 0.5 °C. Stock solution of each substance was first dissolved in chloroform/ethanol (80:20 by volume). A series of C18 fatty acid and polyethoxylated phospholipid mixtures with various mole fractions (X) were prepared by appropriate dilution of stock solutions using chloroform/ethanol (80:20 by volume). A proper amount of sample was deposited drop by drop on the subphase by using a hamilton microsyringe. The solvent was allowed to evaporate and the sample to equilibrate for about 10 min. The experiment was then started at a constant compression rate of 10 mm min^{-1}. The experiment was repeated at least twice to obtain a reproducible surface pressure-area isotherm.

RESULTS AND DISCUSSION

In this study, the surface pressure-area isotherms were recorded during the compression of monolayer on 50 mM phosphate buffer pH 7 at 25 °C until a maximum value of surface pressure was obtained. The experiments were performed at pH 7 to enhance the possibility of pseudo-double chain surfactant formation between COOH and COO^- through hydrogen bonding that is similar to the molecular structure in bilayer membrane of liposome. The amount of fatty acid used was dominant while the amount of PEGylated lipids were limited to a small amount in order to resemble the same environment as that of in liposome. Both DPPE-PEG2000 and DPPE-PEG5000 have the same head group type and hydrocarbon chain length at the tails. However, they differ from each other by 1.5 times higher in the degree of polymerization at the ethoxylate group for DPPE-PEG5000 than DPPE-PEG2000 as shown in Fig. 1. Since the PEG group is relatively larger than the phosphate group, therefore PEG plays a more dominant role in determining the intermolecular interaction in a monolayer.



DPPE-PEG2000



DPPE-PEG2000

Fig. 1. Molecular structure of DPPE-PEG2000 and DPPE-PEG5000

The surface pressure-area isotherm of the monolayer of pure DPPE-PEG2000 and DPPE-PEG5000 at an air-aqueous interface are shown in Fig. 2.

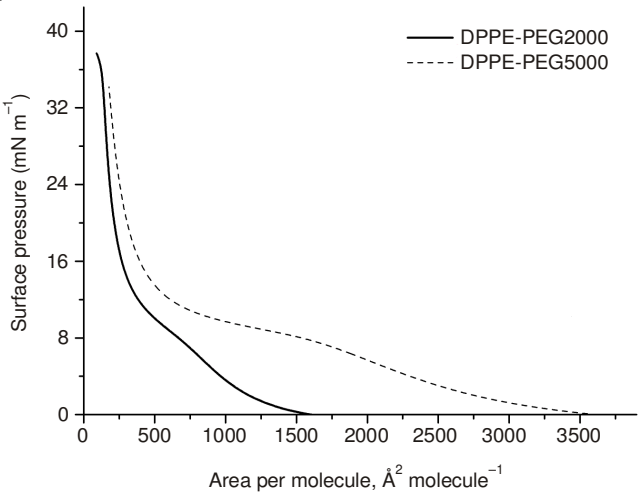


Fig. 2. Surface pressure-area isotherm for pure DPPE-PEG2000 and DPPE-PEG5000 at the air-aqueous interface at 25 °C

The molecular area at the departure of surface pressure from zero for DPPE-PEG5000 is greater than DPPE-PEG2000 as expected. This would be due to stronger intermolecular electrostatic repulsive interaction between DPPE-PEG5000 molecules than DPPE-PEG2000 molecules at low surface pressure. The strong electrostatic interaction arises from the negatively net force charge inherent with the long polyethoxylated chain. It is proposed that hydroxyl ion released from dissociation-association of water molecules can be adsorbed specifically at the interface covered with polyoxyethylene group *via* hydrogen bonding at the ether oxygen[12]. Hence, this contributes negatively charge on the non-ionic PEG moiety. Another reason could be due to the steric effect attributed from the long polyethoxylated group. Therefore, DPPE-PEG5000 in a monolayer tends to occupy larger area than DPPE-PEG2000. Nevertheless, both of the isotherms displayed the liquid-expanded and liquid-condensed phase transition at surface pressure 5-10 mN m^{-1}. This observation might be caused by extension of the long polyoxyethylene chain from the air-aqueous interface. In this transition, it is suggested that polyoxy-ethylene chain in mushroom conformation thereby modify their structure to rodlike or fibrilar structure for short polyoxyethylene chain and to an elongated coiled conformation for long polyoxyethylene chain[13] as shown in Fig. 3a and 3b. However, both of the isothermal curves in this study do not converge at high surface pressure. At high surface pressure, both of the isothermal curves in this study do not converge. This result is similar to the mixed monolayer composing L-α-distearoylphosphatidylcholine (DSPC) and distearoylphosphatidyl-ethanolamine poly(ethylene glycol)2000 (DSPE-PEG2000)[10]. However, it is contradictory to the study reported by Kuhl *et al*.[9] for mixed monolayer of distearoylphosphatidylethanolamine (DSPE)/DSPE-PEG2000. It is reported that at high surface pressure, the long polyethoxylate chain does not affect the molecular packing in a monolayer and as a consequent of this long polyethoxylate chain extend into the aqueous subphase[14]. The effective hydrocarbon tail end area, a_{eff} and bulkiness of the head group may play a significant role in determining the convergence of the isotherm at high surface pressure. However, this result indicates that the changes of isotherm profile depend mainly on the polymerization degree of ethoxylate group, while the phosphate head group is of secondary importance. Whereupon at higher degree of polymerization for DPPE-PEG5000, inefficiency in molecular close packing in the monolayer resulting a larger area per molecule than DPPE-PEG2000 (Fig. 3b).

The surface pressure-area isotherm of the pure C18 unsaturated fatty acid monolayer and mixed monolayer of C18 unsaturated fatty acid with DPPE-PEG2000 and DPPE-PEG5000 at various concentration are represented in Fig. 4.

The surface pressure-area isotherms of pure C18 unsaturated fatty acid is similar to typical "liquid" surfactant isotherm as indicated by the occurrence of liquid expanded state. The limiting molecular areas for fatty acids are 32 $Å^2$ per molecule and 42 $Å^2$ per molecule for oleic acid and linoleic acid, respectively. The presence of two cis double bond in molecular structure of linoleic acid prevent the molecules from close packing, therefore, limiting area per molecule for linoleic acid is larger than oleic acid with only one double bond.

The effect of DPPE-PEG2000 and DPPE-PEG5000 to C18 unsaturated fatty acid monolayers are shown in Fig. 4 (a1 and b1) and (a2 and b2), respectively. We observed that the incorporation of polyethoxylated phospholipids into the C18 fatty acid monolayers do not cause a remarkable change in the surface pressure-area isotherms curve at low mole fraction of polyethoxylated phospholipids. However, typical plateau transitions of polyethoxylated phospholipid are observed in the surface pressure-area isotherm as increase the mole fraction of polyethoxylated phospholipid. A distinct feature is observed that the isotherms are shifted towards lower molecular area than that pure fatty acid. This suggested the out of plane protrusion of long polyethoxylate groups from the two dimensional monolayer. A plausible reason for the occurrence of protrusion is due to solubility of the polyethoxylate group[14].

The presence of interaction between the components in monolayer film is reflected as dependency of the curve position and shape to the composition of mixture. According to surface phase rule[15], components in a mixed monolayer at condensed and collapse state is considered miscible if the collapse pressure is different from either of the collapse pressures of the pure component monolayer. As shown in Fig. 4, collapse pressures for the mixed monolayer isotherms are dependent on the composition of DPPE-PEG2000 or DPPE-PEG5000. Thus, it can be deduced that the mixed molecules are compatible within the investigated mole fraction range as predicted by the phase rule.

Another indirect way to determine the miscibility and ideality of mixed monolayer at the air-water interface is through evaluation of excess area per molecule, A_{exc} for the mixture at constant surface pressures. Equation 1 was used to calculate the A_{exc} values for binary C18 unsaturated fatty acid/polyethoxylated phospholipid, (eqn. 1).

$$A_{exc} = A_{12} - A_{id} = A_{12} - (A_1X_1 + A_2 X_2) \quad (1)$$

where A_{12} is area per molecule obtained experimentally from surface pressure-area mixed monolayer isotherm and A_{id} is defined as ideal area per molecule that calculated according to additivity rule at specific mole fraction of the pure component. A_1 and A_2 individually are the area per molecular for pure C18 unsaturated fatty acid and pure polyethoxylated phospholipid at the same surface pressure. Mole fraction of the pure C18 unsaturated fatty acid and pure polyethoxylated phospholipid are represented by X_1 and X_2, respectively. If an ideal or immiscible mixed monolayer is formed at a given surface pressure, molecules in system do not interact with each other, hence the value of excess area, A_{exc} will be zero and a linear plot of A_{12} as a function of mole fraction will be obtained. However, there are always deviations from linearity as a result of intermolecular forces either attraction or repulsion among the molecules in the mixed monolayer. Hence, a non-ideal behavior of C18 unsaturated fatty acid/polyethoxylated phospholipid mixed systems is also confirmed by non-linear course of the A_{12} as a function of mole fraction for DPPE-PEG2000 and DPPE-PEG5000 as shown in Fig. 5. The dotted lines correspond to area per molecule that calculated on the basis of additive rule. Deviations from ideality (dotted line) are observed for almost all of the C18 unsaturated fatty acid/polyethoxylated phospholipid mixed monolayers. This has proven

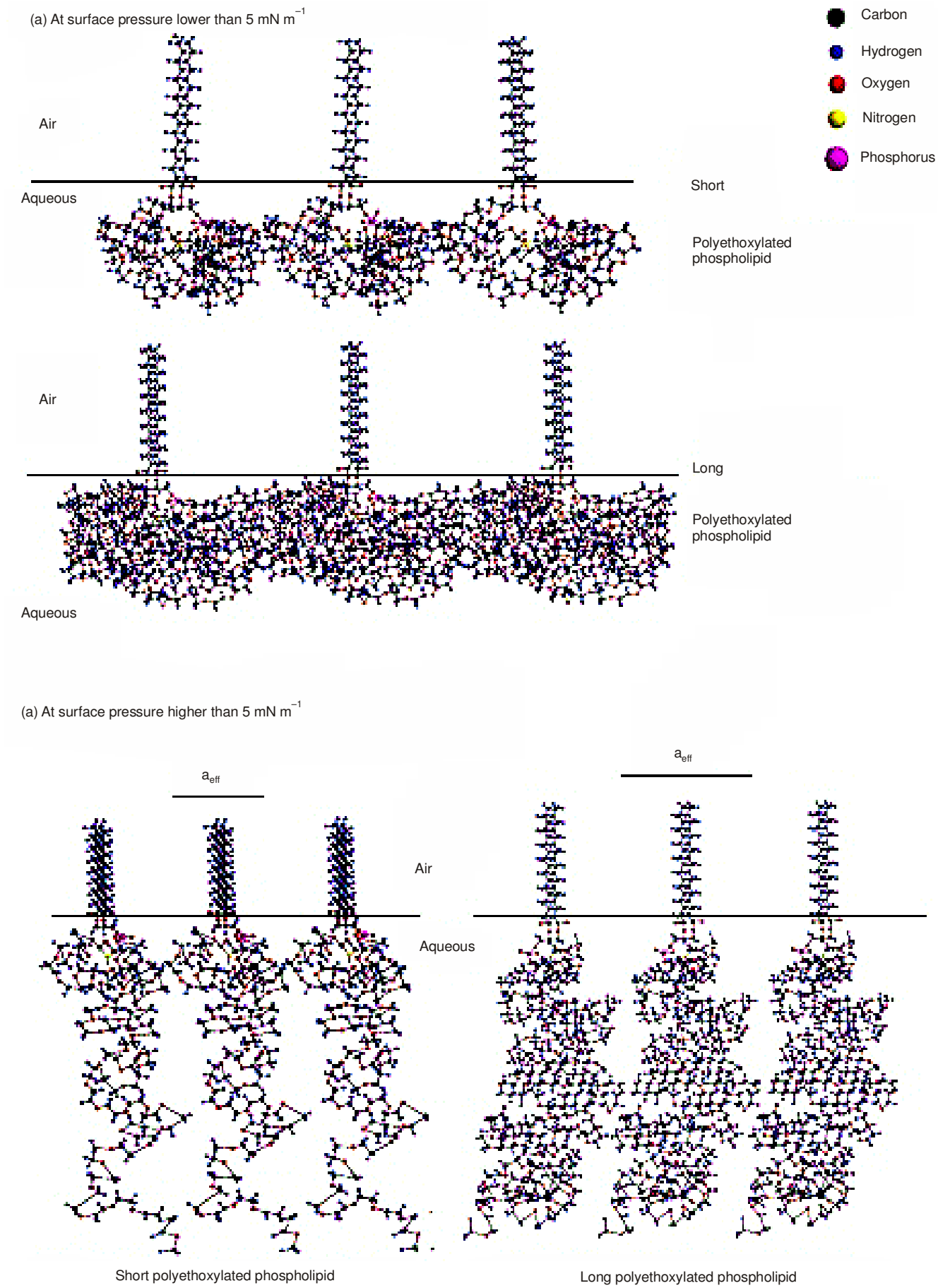


Fig. 3. Conformation of short and long polyethoxylated phospholipid at (a) low surface pressure and (b) high surface pressure

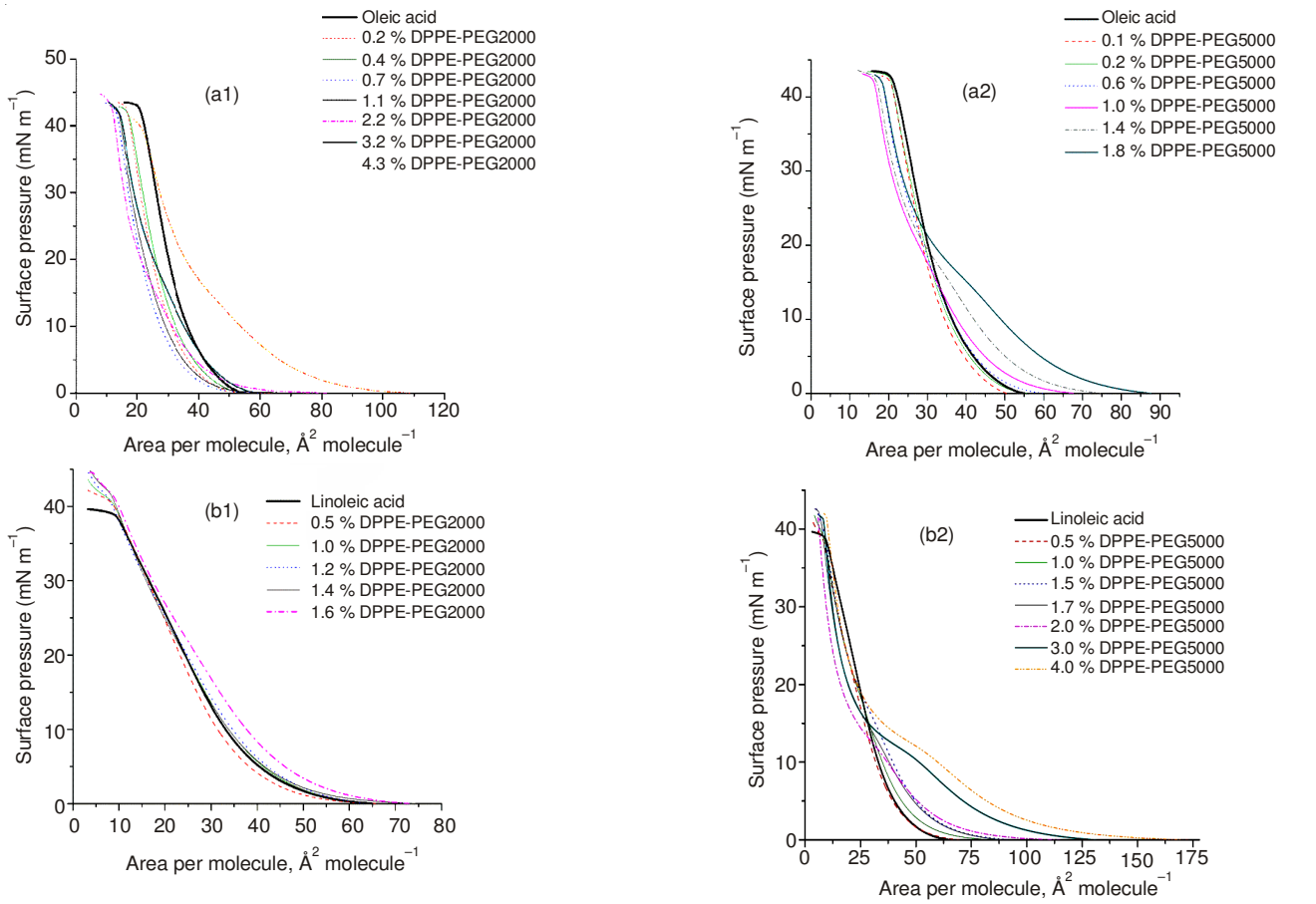


Fig. 4. Surface pressure-area isotherms of (a) oleic acid and (b) linoleic acid mixed with (1) DPPE-PEG2000 and (2) DPPE-PEG5000 at the air-aqueous interface at 25 °C

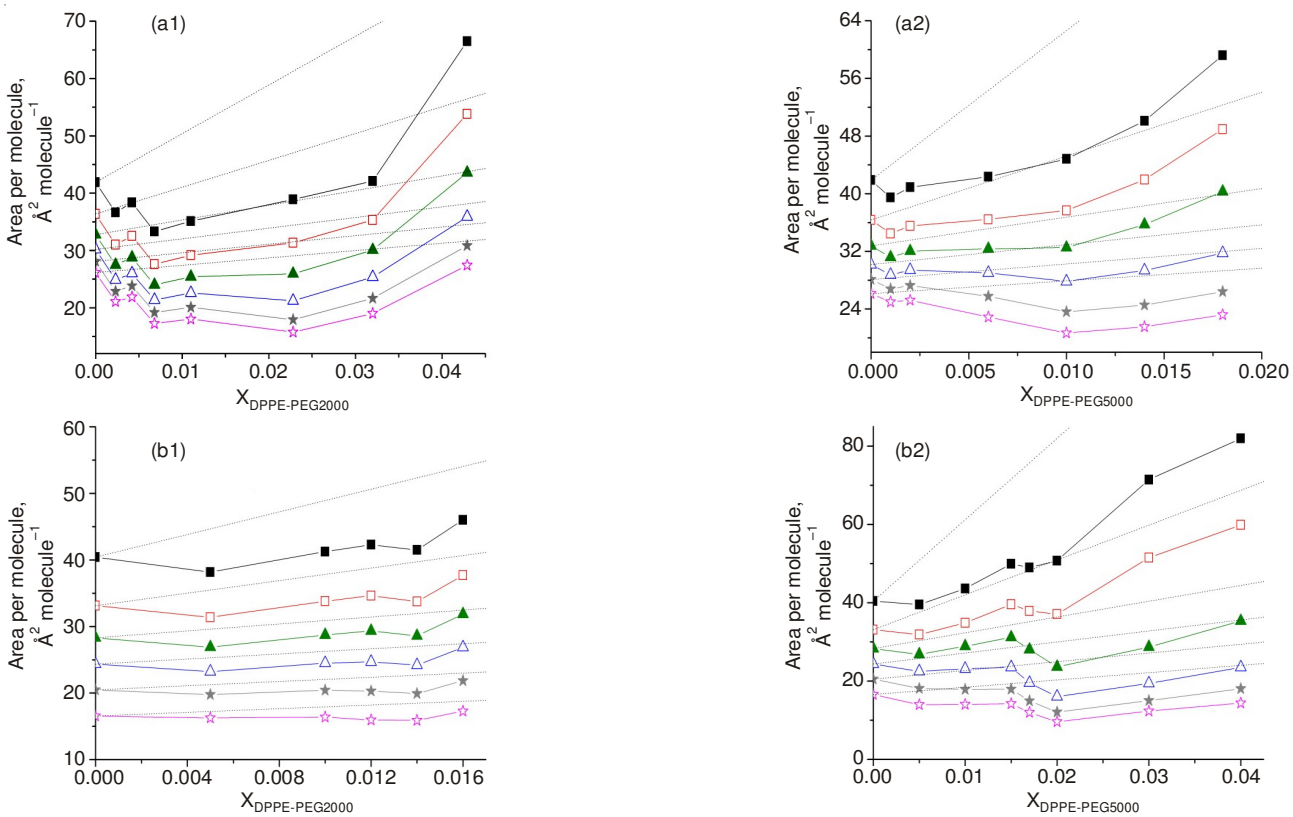


Fig. 5. Area per molecule as a function of composition for mixed monolayers of (a) oleic acid, and (b) linoleic acid with (1) DPPE-PEG2000 and (2) DPPE-PEG5000 at various constant surface pressures. ■ = 5 mN m^{-1}, □ = 10 mN m^{-1}, ▲ = 15 mN m^{-1}, △ = 20 mN m^{-1}, ★ = 25 mN m^{-1}, ☆ = 30 mN m^{-1}

the miscibility of the monolayer components in this composition range and surface pressure region.

The influence of DPPE-PEG2000 and DPPE-PEG5000 on the C18 unsaturated fatty acid monolayer can be further analyzed in a more precise manner on the basis of compression modulus (C_s^{-1}). These values were obtained by numerical calculation of the first derivative from the isotherm data points according to eqn. 2.

$$C_s^{-1} = -A\left(\frac{\partial \pi}{\partial A}\right)^T \quad (2)$$

C_s^{-1} is useful for evaluation of the conformational change in acyl chain upon compression of the mixed monolayer. The larger the C_s^{-1} value, the more compact arrangement of the acyl chain and hence less flexible of the monolayer at the air-aqueous interface. Fig. 6 shows the plots of C_s^{-1} as a function of mole fraction for polyethoxylated phospholipid at various constant surface pressures. C_s^{-1} values for pure fatty acid are indicated at zero polyethoxylated phospholipid content. As the number of double bond in the hydrocarbon chain of fatty acid increases, the value of C_s^{-1} is found to be smaller. The compressibility of the linoleic acid monolayer is greater than oleic acid monolayer. The present of double bond with kinks and bends in the molecules induce π-π repulsion interaction that hinders closed packing of the monolayer at air-aqueous interface could be the reason for the low C_s^{-1} value especially in the case of linoleic acid. This value is also drastically affected by present of impurities in the monolayer.

In this study, the C_s^{-1} values for pure unsaturated fatty acids in Fig. 6 were included for comparison purposes. The result obtained shows that C_s^{-1} for pure oleic acid monolayer is increasing with surface pressure. This implies oleic acid monolayer is more resistance to compression at high surface pressure. However, C_s^{-1} for linoleic acid displays slightly different results, where C_s^{-1} increase from surface pressure 5 mN m^{-1} to 15 mN m^{-1}. This implies the molecules are approaching closer to each other especially the hydrocarbon tail with large surface area, whereby the head group is still far from each other. Further compression of the monolayer at surface pressure higher than 15 mN m^{-1} resulted in a drop of C_s^{-1}. The plausible explanation could be the hydrocarbon tail of linoleic acid with two double bonds are interacting and hence slipped into the gap in between the hydrocarbon region.

The values of C_s^{-1} for the mixed monolayer are dependent on the molecular weight of polyethoxylated phospholipid and degree of unsaturation of the fatty acid molecules. As the degree of polymerization increases, their effect on compressibility is more pronounce. In addition, the higher the degree of unsaturation at the hydrocarbon chain of fatty acid, the less rigid the molecular packing in the monolayer. Hence, it is

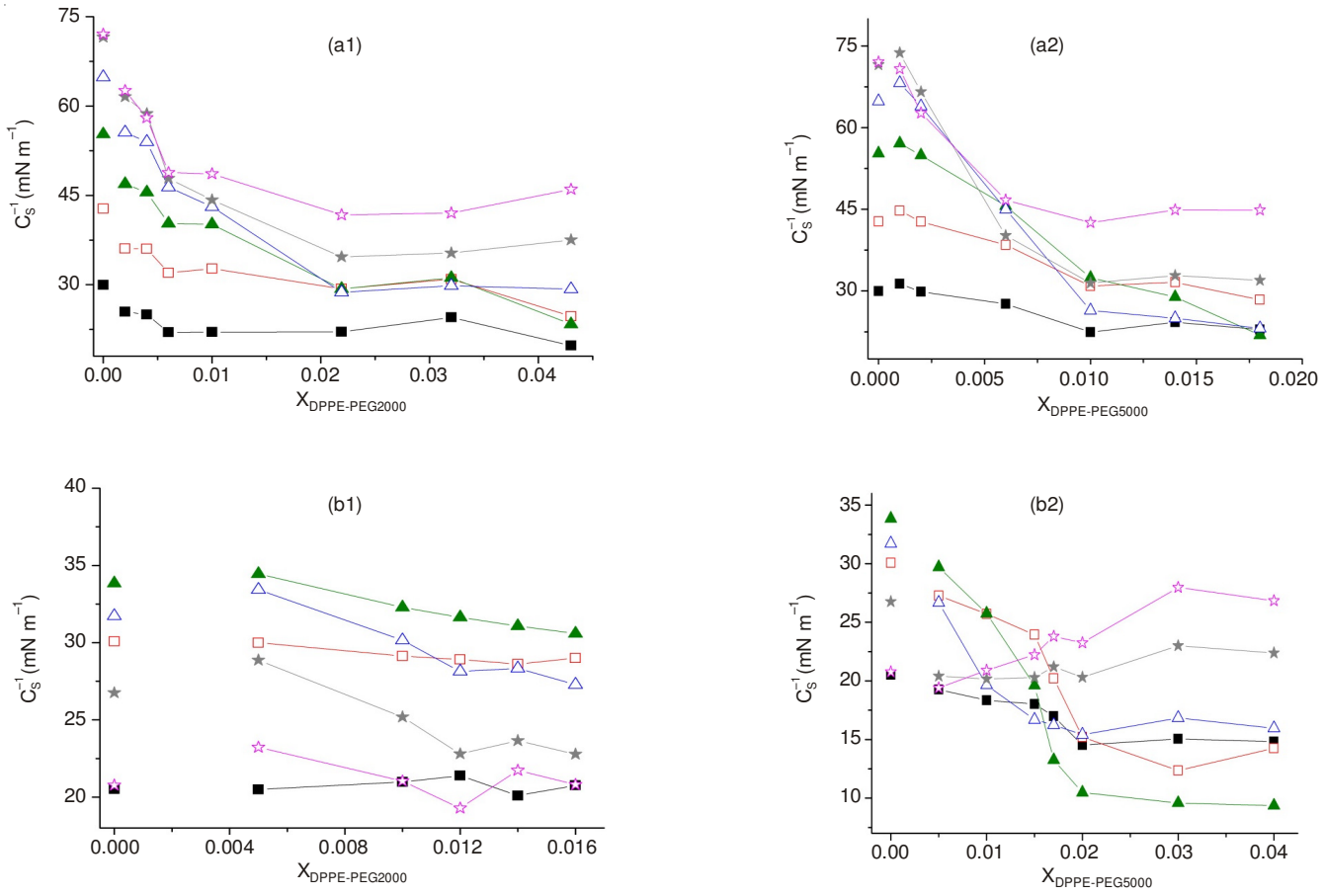


Fig. 6. Compression modulus (C_s^{-1}) for mixed monolayer of (a) oleic acid and (b) linoleic acid as a function of mole fraction (1) DPPE-PEG2000 and (2) DPPE-PEG5000 (d-f) at 25 °C at various constant surface pressures. ■ = 5 mN m^{-1}, □ = 10 mN m^{-1}, ▲ = 15 mN m^{-1}, △ = 20 mN m^{-1}, ★ = 25 mN m^{-1}, ☆ = 30 mN m^{-1}

obvious that the addition of DPPE-PEG2000 and DPPE-PEG5000 into C18 fatty acid monolayer caused variation on C_s^{-1} values with respect to surface pressure (Fig. 6).

Nevertheless, the effect of DPPE-PEG5000 is more pronounced on the monolayer compared to DPPE-PEG2000. This might be due to DPPE-PEG5000 possesses longer polyethoxylate chain, hence larger surface coverage per molecule and less water soluble than DPPE-PEG2000 that cause a significant effect during compression of the mixed monolayers.

The compression modulus of the mixed monolayers is much more dependent on the surface pressure than the composition of polyethoxylated phospholipids as can be realized from Fig. 6. Addition of polyethoxylated phospholipids into the C18 unsaturated fatty acid monolayer do not change the trend of C_s^{-1} with respect to degree unsaturation. In the studied mole fraction range of polyethoxylated phospholipids, oleic acid with polyethoxylated phospholipid still remains its C_s^{-1} value to be highest in comparison to mixture of polyethox-ylated phospholipid with linoleic acid. Nevertheless, polyethoxylated phospholipids have imposed an effect on the conformation of hydrocarbon chain in C18 unsaturated fatty acid mixed monolayers.

Once compatibility of the mixed monolayer has been identified, the strength of interaction between molecules in a mixed monolayer relative to the interaction between molecules in a pure monolayer can be quantitatively evaluated from the excess Gibbs free energy of a mixture at the interface (ΔG_{exc}), as expressed in equation3[16,17].

$$\Delta G_{exc} = N_A \int_0^{\Pi} A_{exc} d\Pi = N_A \int_0^{\Pi} [A_{12} - (X_1 A_1 + X_2 A_2)] d\Pi \quad (3)$$

ΔG_{exc} can be directly evaluated from surface pressure-area mixed monolayer isotherm. Wherein A_{12} is the area occupied per molecule in the mixed monolayer, X_1, X_2 are defined as mole fraction of C18 unsaturated fatty acid and polyethoxylated phospholipid, respectively. A_1 and A_2 are identified as area per molecule in individual pure monolayer and N_A is Avogadro's number. π is surface pressure. By applying equations 3, values for ΔG_{exc} were calculated at various compositions of polyeth-oxylated phospholipid and surface pressure as illustrated in Fig. 7. It can be seen that ΔG_{exc} is dependent on the composition of polyethoxylated phospholipid. Hence, addition of polyethoxylated phospholipids into the C18 unsaturated fatty acid monolayer induce the changes of molecular packing. By analyzing Fig. 7(a1) and (a2), we noticed

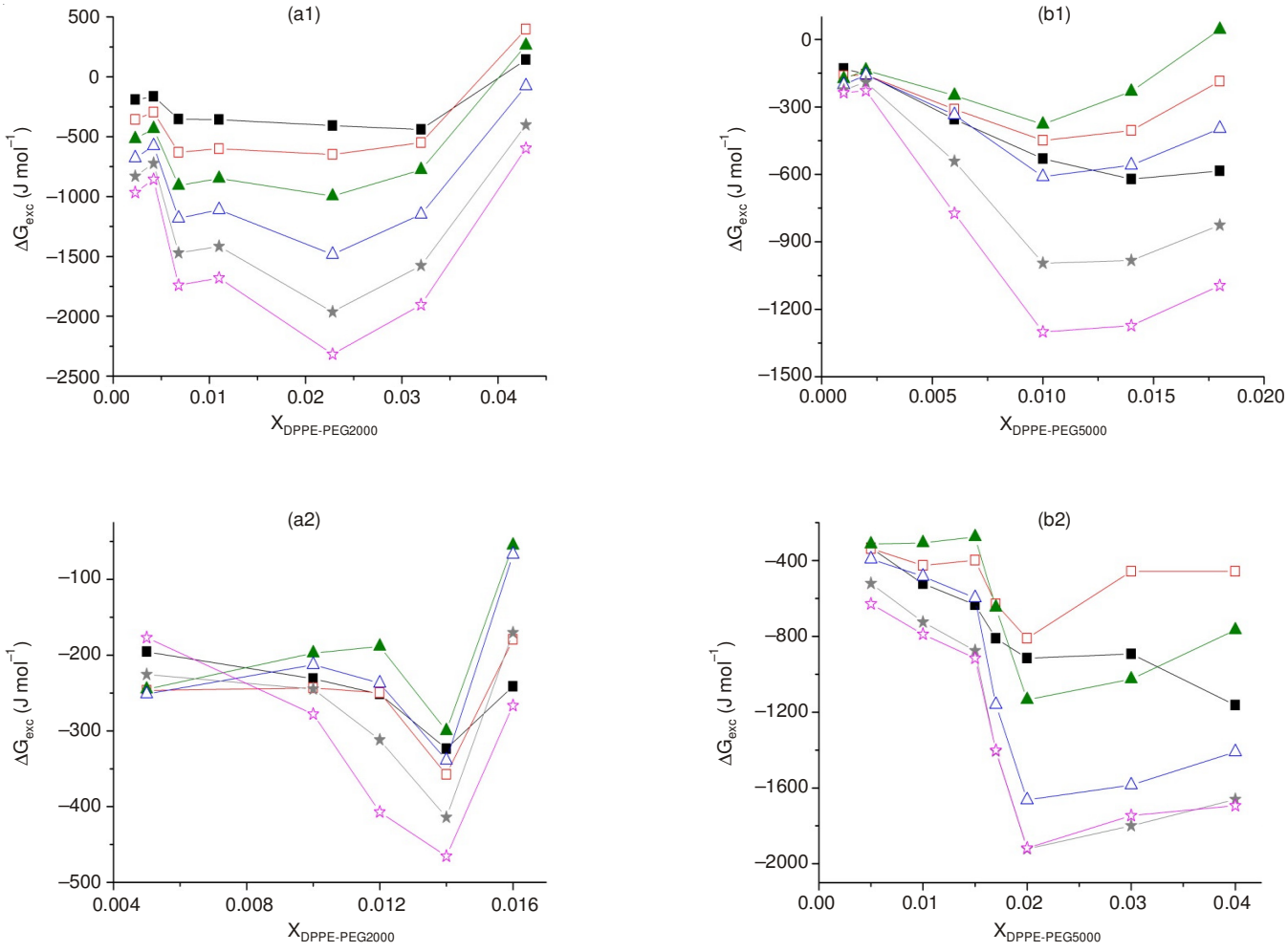


Fig. 7. Excess gibbs free energy (ΔG_{exc}) for mixed monolayer of (a) oleic acid and (b) linoleic acid at a range compositions of (1) DPPE-PEG2000 and (2) DPPE-PEG5000 at various constant surface pressures. ■ = 5 mN m^{-1}, □ = 10 mN m^{-1}, ▲ = 15 mN m^{-1}, △ = 20 mN m^{-1}, ★ = 25 mN m^{-1}, ☆ = 30 mN m^{-1}

that the excess free energy values for monolayer of oleic acid/DPPE-PEG2000 are more negative than oleic acid/DPPE-PEG5000. This indicates that oleic acid interacts stronger with DPPE-PEG2000 than DPPE-PEG5000. A plausible reason for this observation could be due to the smaller hydrophilic head group of PEG in DPPE-PEG2000 compares to DPPE-PEG5000 that fits the gap between the head group of oleic acid. On contrary, the thermodynamic effect accompanying incorporation of DPPE-PEG5000 molecules into linoleic acid monolayer is stronger than in the case of oleic acid. ΔG_{exc} for mixed system of linoleic acid/DPPE-PEG5000 is more negative than mixed linoleic acid/DPPE-PEG2000 illustrated in Fig. 7(b1) and (b2). As mentioned earlier, packing of linoleic acid molecules at air-water interface are less dense compared to oleic acid as a result of more unsaturation are presence in the molecular structure. Therefore, the gap between head group of linoleic acid is higher than oleic acid. In order to reduce the intermolecular distance and form a stronger van der Waals interaction, DPPE-PEG5000 with bulkier head group than DPPE-PEG2000 enables formation of closely packed monolayer. Similar explanation is also applicable to the result of less negative ΔG_{exc} values for DPPE-PEG2000 mixed with linoleic acid compared to oleic acid. This effect is especially pronounced as the monolayers were compressed to higher surface pressure as a consequence of smaller distance between the molecules.

Nevertheless, emergence of a minimum at every surface pressure studied in the plot of ΔG_{exc} as a function of mole fraction implies the most favorable composition with the strongest interaction between the mixed molecules at that particular mixture. The minimum ΔG_{exc} for mixture of oleic acid with DPPE-PEG2000 is observed at $X_{DPPE\text{-}PEG2000} = 0.02$. This mixture is with higher $X_{DPPE\text{-}PEG2000}$ than those for linoleic acid ($X_{DPPE\text{-}PEG2000} = 0.01$) with two *cis* unsaturated double bond. However, the amount of DPPE-PEG5000 required to achieve minimum ΔG_{exc} in the mixture with oleic acid ($X_{DPPE\text{-}PEG5000} = 0.01$) is lower compared to linoleic acid. Linoleic acid still maintains its ability to accommodate DPPE-PEG5000 ($X_{DPPE\text{-}PEG5000} = 0.02$) in order to achieve the minimum ΔG_{exc}. This value provides a guideline on the appropriate amount of PEGylated phospholipid required in the formation of fatty acid liposome. Nevertheless, the results obtained from this studied are relatively lower than phospholipid/polyethoxylated phospholipid monolayer which recorded a miniumum of ΔG_{exc} at 5-7 mol % of PEG-lipid[10,18]. The plausible reason may due to differences of molecular structure in fatty acid and phospholipid.

Conclusion

In this work, the influence of DPPE-PEG2000 and DPPE-PEG5000 on C18 unsaturated fatty acid monolayer have been studied. The interactions within these mixed monolayers were determined by their physicochemical properties. DPPE-PEG2000 is found compatible with all the C18 unsaturated fatty acid as evidence from the excess area analysis. Similar results were also obtained for DPPE-PEG5000 mixed with linoleic acid. However, oleic acid at low mole fraction is not compatible with DPPE-PEG5000. In general, addition of DPPE-PEG2000 or DPPE-PEG5000 into the C18 unsaturated fatty acid monolayer increased the compressibility of the monolayer with respect to surface pressure, which means the presence of polyethoxylated phospholipid enhance the membrane fluidity. It is also revealed that the attractive and repulsive interactions between the mixed molecules were affected by the composition of DPPE-PEG2000 and DPPE-PEG5000. According to the excess Gibbs free energy, the most stable combination of mixing is determined by unsaturation degree and the molecular weight of the polyethoxylated phospholipid. This can be explained by the intermolecular forces or geometric accommodation between the molecules. In conclusion, thermodynamically more stable system can be formed for almost all of the studied mixed monolayer system with an exception of for mixture of oleic acid/DPPE-PEG5000. This finding provides guidance and information on formulation of PEGylated C18 unsaturated fatty acid liposome.

ACKNOWLEDGEMENTS

This research work was supported by the Department of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS) with project number FP001-2013A.

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