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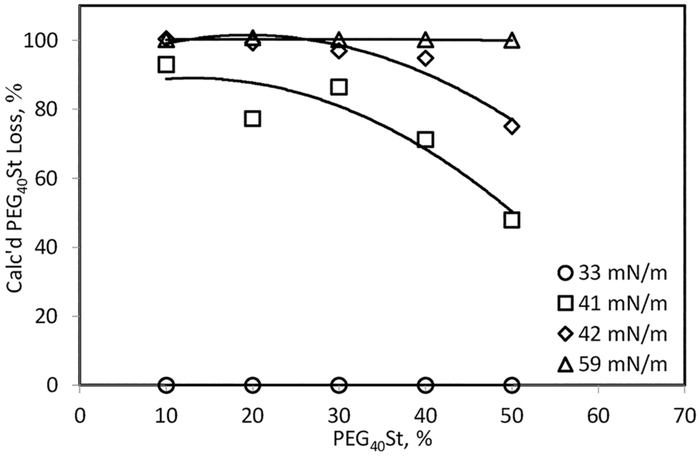
Quantification of $PEG_{40}St$ squeeze out from DSPC/$PEG_{40}St$ monolayers at higher molar ratios

Sevgi Kilic

Department of Chemical Engineering, Izmir Institute of Technology, Urla, Izmir, 35430, Turkey

GRAPHICAL ABSTRACT



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ABSTRACT

Mixtures of 1,2 Distearoyl-sn-glycero-3-phosphocholine (DSPC) and polyoxyethylene-40-stearate ($PEG_{40}St$) were prepared at different molar ratios and their miscibility were investigated using Langmuir isotherms. Pure DSPC monolayer exhibited a liquid-condensed (LC) phase whereas $PEG_{40}St$ monolayer exhibited liquid-expanded (LE) phase at the air-water interface at 22 ± 2 °C. At the collapse pressure of 33 mN/m, the $PEG_{40}St$ mean molecular area was calculated to be 28 Å^2/molecule for 9:1 composition and 50 Å^2/molecule for 5:5 composition, showing an increasing trend with the emulsifier content. A quantification method was developed to estimate the squeeze out amount of $PEG_{40}St$ from Langmuir isotherms of the DSPC/$PEG_{40}St$ mixtures at different molar ratios. Almost 93%, 82%, and 53% of $PEG_{40}St$ displaced for the 9:1, 7:3, and 5:5 mixtures, respectively, at the end of the first collapse plateau and showed a decreasing trend with the $PEG_{40}St$ content. Remaining $PEG_{40}St$ squeezed out at the end of the second collapse plateau, where 20% of $PEG_{40}St$ still contained within the 5:5 composition. It was concluded that increasing $PEG_{40}St$ content would be advantageous to design more stable lipid based microbubbles.

E-mail address: sevgikilic@iyte.edu.tr.

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1. Introduction

Lipid-based microbubbles are micron size gas bubbles used in medicine as ultrasound contrast agent to improve the quality of ultrasound image [1] as well as efficient drug and gene delivery. [1–4] However, phospholipids alone are not capable of forming microbubbles [5,6], and an additional component, emulsifier, is needed [7–10]. The lipid used in a microbubble shell is usually a phosphocholine, DSPC, and the emulsifier could be Tween-40, DSPE-PEG_{5000}, DSPE-PEG_{2000}, or PEG_{40}St, among which PEG_{40}St is particularly attracted in the lipid microbubbles [11–17]. The PEG chains also protect the gas bubble from aggregation in the bloodstream [18,19]. In a microbubble formulation, DSPC:PEG_{40}St mixture with a molar ratio of 9:1 has usually been used [13,15,20–25]. To the best of our knowledge, the miscibility behavior of DSPC:PEG_{40}St at molar ratio higher than 15 mol% of PEG_{40}St has not been studied in the literature [6,26,27].

The miscibility characteristics of DSPC/PEG_{40}St mixtures can be estimated by a relatively simple Langmuir monolayer technique where surface pressure of a monolayer is measured with a Wilhelmy plate while compressing a flat 2D-monolayer at the air-water interface [28,29]. The measured average area per molecule, A_{12}, can be obtained experimentally by dividing the trough area to the number of molecules in the mixture [6]. The measured mean molecular area is the cumulative area occupied by each component, $\Sigma x_i < A_i >$, in the monolayer, $A_{12} = x_1. < A_1 > + x_2. < A_2 >$. However, the true mean molecular area could not be accurately estimated for each component during the measurements due to attractive-repulsive forces between the component molecules and their complexations [30–33]. The mixture could be considered “ideal” if the component molecules have no irregular packing attachments, no complex formations, and form a phase separation in the monolayer [6,30]. In such cases, the ideal average mean molecular area, A_{12}^{ideal}, can be related to the area per molecule, A_i, for each component obtained from their pure component isotherms at the specified surface pressure and mole fraction of each species, $A_{12}^{ideal} = x_1.A_1 + x_2.A_2$ [6,30]. Although the surface pressure-area isotherms enable to investigate the physical interactions of components on the monolayer, deviations from the ideality is possible such that the surface area of the trough is large and bounded by the hydrophobic barriers, the compression is anisotropic and unidirectional, and the surface pressure may not be measured accurately during the transition from a fluid to solid phase [29]. However, these artifacts are small and reliable data can be extracted from the Langmuir mean molecular area-surface pressure isotherms for the monolayers [28].

Different regions of the Langmuir isotherm can give information on the phase behavior of the molecules for each component. It was shown that more ordered and rigid monolayers collapse at relatively higher surface pressures, usually by fracturing, followed by loss of materials to the air side with the formation of multilayered aggregates, or to the sub phase with budding or vesicle formation [25,29,31,34]. When a binary mixture of components having two different collapse pressures were compressed, at least two plateaus will exhibit, one is at the lower collapse pressure for the expanded phase and the other is at the higher collapse pressure for the more rigid component [6,35–38]. The component in the expanded phase usually squeeze out from the monolayer over the plateau region [6,34].

PEG_{40}St was shown to squeeze out from the monolayer at higher compression pressures and, to our knowledge, no studies were found to quantify the amount of PEG_{40}St departed from the monolayer during the collapse plateaus. In this study, we aimed to investigate the DSPC/PEG_{40}St mixtures at higher molar ratios from 9:1 to 5:5 compositions. To the best of our knowledge, the miscibility of DSPC:PEG_{40}St at molar ratios higher than 15 mol% of PEG_{40}St has not been studied in the literature [6,26,27]. The surface pressure versus area isotherms were measured on a Langmuir trough for the mixtures and analyzed for their compression, collapse, molecular area, and squeeze-out properties in a two dimensional (2D) monolayer technique. It was found that PEG_{40}St in 10% composition is easily squeeze-out from the 2D film up to about 93% at the end of the first collapse plateau, at 41 mN/m, whereas almost 60% PEG_{40}St squeezed out from the monolayer for the 5:5 composition, retaining almost 40% of PEG_{40}St molecules in the 2D film. Using PEG_{40}St at higher concentrations was found advantageous not only to increase the stability of the microbubbles but also reduce the product cost for the ultrasound contrast agent.

2. Materials and methods

2.1. Materials

1,2 Distearoyl-sn-glycero-3-phosphocholine (DSPC, 99%) and Polyoxyethylene-40- stearate (PEG_{40}St) were purchased from Sigma Aldrich (St. Louis, MO). Chloroform ($CHCl_3$, 99–99.4%) was purchased from Merck and used as a solvent to prepare spreading solutions. Ultrapure water used as subphase was produced by Millipore purification system with specific resistivity of 18 MΩ.cm. Predetermined amount of components were weighted into a clean vial and dissolved in chloroform resulting a concentration less than 1.0 mg/ml. DSPC/PEG_{40}St mixtures were prepared at molar ratios of 9:1; 8:2; 7:3; 6:4 and 5:5. After chloroform addition, the vials were immediately sealed with screw cap to avoid chloroform evaporation and, if needed, stored in the freezer at -22 °C. Before spreading the solution at the gas-liquid interface, the sealed vial was homogenized using a bath sonicator and continuously stirred by hand-shaking with the cap closed.

2.2. Langmuir isotherm measurements

Langmuir-Blodgett system (KSV minitrough, Finland) with two movable PTFE barriers was used to study the phase behavior of the binary systems and pure components. The surface area of the trough is 273 cm^2 with dimensions of 364 × 75 × 7 mm^3 and a subphase volume of 140 ml. The system was enclosed in a box to minimize possible contamination of air-monolayer-water interface and the disturbance of the monolayer by air currents. Trough was filled with ultrapure water with specific resistivity of 18 MΩ cm produced by a Millipore purification system. Cleanness of the air-water interface was confirmed by closing and opening the barriers and ensuring that surface pressure readings do not differ by more than ± 0.1 mN/m. The spreading solutions were spread on the water subphase via Hamilton micro syringe. The monolayer was allowed to evaporate the chloroform for 20 min. The surface pressure-area (π-A) isotherms were obtained via symmetric compression of monolayers by the two barriers. A compression speed of 5 mm/min was used in all experiments. Each isotherm was performed 4–5 times to ensure reproducibility of the isotherms.

3. Results and discussion

DSPC and PEG_{40}St mixtures at different molar ratios were prepared and their miscibility behaviors were investigated using Langmuir isotherms. The Langmuir isotherms are shown in Fig. 1a where the surface pressure (π) versus mean area per molecule isotherms were measured on a Langmuir trough for pure DSPC, pure PEG_{40}St, and their binary mixtures at DSPC/PEG_{40}St molar ratios of 9:1, 8:2, 7:3, 6,:4, and 5:5. As shown in the figure, the isotherms show similar shape and behavior for all the mixtures, therefore, for simplicity, main discontinuities as the turning points were marked in Fig. 1b by the arrows on the isotherms for the pure components and on the isotherm for their 8:2 mixture. As shown in the figure, pure DSPC monolayer exhibited a liquid-condensed (LC) phase at the air-water interface upon spreading at room temperature. A steep increase in the surface pressure was evident for the DSPC with little compression occurred with its mean molecular area. The steep surface pressure change may indicate that DSPC form a continuous ordered monolayer at the interface with a rigid condensed phase. The surface pressure of DSPC at about 59 mN/m almost