Quantification of PEG40St squeeze out from DSPC/PEG40St monolayers at higher molar ratios

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ABSTRACT

Mixtures of 1,2 Distearoyl-sn-glycero-3-phosphocholine (DSPC) and polyoxyethylene-40-stearate (PEG40St) were prepared at different molar ratios and their miscibility were investigated using Langmuir isotherms. Pure DSPC monolayer exhibited a liquid-condensed (LC) phase whereas PEG40St monolayer exhibited liquid-expanded (LE) phase at the air-water interface at 22 ± 2 °C. At the collapse pressure of 33 mN/m, the PEG40St mean molecular area was calculated to be 28 Å²/molecule for 9:1 composition and 50 Å²/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 PEG40St from Langmuir isotherms of the DSPC/PEG40St mixtures at different molar ratios. Almost 93%, 82%, and 53% of PEG40St 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 PEG40St content. Remaining PEG40St squeezed out at the end of the second collapse plateau, where 20% of PEG40St still contained within the 5:5 composition. It was concluded that increasing PEG40St content would be advantageous to design more stable lipid based microbubbles.
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 phospholcholine, DSPC, and the emulsifier could be Tween-40, DSPE-PEG5000, DSPE-PEG2000, or PEG40St, among which PEG40St 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:PEG40St 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:PEG40St at molar ratio higher than 15 mol% of PEG40St has not been studied in the literature [6,26,27].

The miscibility characteristics of DSPC/PEG40St 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, A12, 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, Σ xi < A > , in the monolayer, A12 = x1 < A1 > + x2 < A2 > . 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 complexities [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, A12 ideal, can be related to the area per molecule, A1, for each component obtained from their pure component isotherms at the specified surface pressure and mole fraction of each species, A1 ideal = x1A1 + x2A2. [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 subphase 

2. Materials and methods

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC, 99%) and Polyoxyethylene-40-stearate (PEG40St) were purchased from Sigma Aldrich (St. Louis, MO). Chloroform (CHCl3, 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 MΩcm. Predetermined amount of components were weighted into a clean vial and dissolved in chloroform resulting a concentration less than 1.0 mg/ml. DSPC/PEG40St 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 cm² with dimensions of 364 × 75 × 7 mm³ 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 MΩcm 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 PEG40St 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 PEG40St, and their binary mixtures at DSPC/PEG40St 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
unchanged as the area of the trough decreased significantly indicating that the DSPC molecules departed from the two dimensional monolayer forming a double layer on the existing 2D monolayer called a collapse. Therefore, the collapse pressure for DSPC was measured to be about 59 mN/m which is in good agreement with the literature [30,39,40]. At the collapse pressure, the mean molecular area for DSPC was found to be about 40 Å²/molecule, which is also in good agreement with the literature [30,39,40]. It was understood that the DSPC monolayer in the liquid-condensed phase showed strong cohesive interactions between C₁₈ aliphatic chains on aqueous subphase and the surface pressures increased significantly with little increase in the mean molecular area, making the DSPC molecules rigid in nature [41-43].

Unlike DSPC, PEG₄₀St monolayer exhibited a non-zero surface pressure even at very low molecular densities. The higher surface pressure suggests the presence of intermolecular interactions in PEG₄₀St chains forming globular aggregates on the surface of the aqueous subphase. The surface pressure for the pure PEG₄₀St increased only little as large changes occurred in its mean molecular area. The negligible surface pressure change indicated that the emulsifier molecules did not form a continuous ordered monolayer at the air-water interface with a rigid condensed phase, but spread as floating clusters due to intermingling of polymer chains on aqueous subphase at the interface in a pancake conformation [44]. As evidenced also from the compressibility (C₋¹) curve for the PEG₄₀St (data not shown), the Langmuir isotherm of PEG₄₀St exhibited a collapse pressure at about 35 mN/m, consistent with the literature [39,45]. There are inconsistencies in the literature about the mean molecular area of PEG₄₀St at its collapse pressure such that the mean molecular areas of 40 Å²/molecule [46], 30 Å²/molecule [27], and 20 Å²/molecule [11] were reported. Here, our measurements showed that the mean molecular area for PEG₄₀St is about 30 Å²/molecule at the collapse pressure of 35 mN/m.

Langmuir isotherms of the DSPC/PEG₄₀St mixtures were located in between the isotherms for pure DSPC and pure PEG₄₀St. There are four discontinuities as the turning points and four plateau regions in the mixture isotherms as illustrated in b on the isotherm for the 8:2 mixture as an example. The values for the surface pressures and the mean molecular areas for the isotherms at different PEG₄₀St content are also shown in Fig. 1c and d respectively. The first plateau region, region-1, is below the first collapse pressure of about 33 mN/m for the mixtures studied and it was lower than 35 mN/m for the collapse pressure of the pure PEG₄₀St. The mean molecular areas at the start of the first collapse, 33 mN/m, were obtained to be about 45 Å²/molecule for the mixtures and it showed a slightly increasing trend with the PEG₄₀St content. The second plateau region, region-2, is the region started at 33 mN/m and ended at 41 mN/m. The second collapse initiated at the surface pressures of about 41 mN/m for the mixtures. Both molecular configurations/reconformations and consequently PEG₄₀St squeeze out took place from the monolayer at the air-water interface through this plateau region. Therefore, the mean molecular area decreased with increasing PEG₄₀St content of the mixtures at 41 mN/m as shown in Fig. 1d. The third plateau region, region-3, is the region starts at 41 mN/m and ends at 42 mN/m and showed slightly increasing trend. A second collapse was seen in this region therefore a significant decrease in the mean molecular area were obtained for the mixtures with increasing PEG₄₀St content. The forth plateau region, region-4, is the region where the shape of the isotherm show somewhat similar trend with the isotherm for the pure DSPC. The surface pressures were obtained to be about 59 mN/m for the studied DSPC/PEG₄₀St mixtures as it was the same with the pure DSPC isotherm. However, the mean molecular area decreased almost linearly with increasing PEG₄₀St content indicating that a significant amount of PEG₄₀St, if not all, squeezed out from the monolayer at the air-water interface.

In the high compressibility region, molecules spread in a more relaxed form and therefore the mixture isotherms exhibited more expanded behavior in the pancake conformation with increasing PEG₄₀St content [32,47]. The DSPC/PEG₄₀St mixture isotherms exhibited a plateau around the collapse pressure of PEG₄₀St and shifted to the left of the DSPC isotherm with increasing emulsifier content. It was reported that the location of the DSPC/PEG₄₀St mixture isotherms up to 15% of PEG₄₀St content was independent of the emulsifier content after the plateau region, exhibiting an isotherm identical to that of pure DSPC [6]. Similar behavior was observed with DSPC/DSP/PPEG₄₀₀₀, DPPC/PEG₄₀St, and Span60/PEG₄₀St mixtures [27,43,48,49]. Our mixture isotherms studied up to 50% PEG₄₀St contents exhibited plateaus broadening with increasing PEG₄₀St content in the monolayer. Enlargement of the plateau was also observed for DSPC/PEG₄₀St mixture elsewhere up to 15% of PEG₄₀St content [6], but isotherms of the Span60/PEG₄₀St mixed monolayers were seen to be intersecting each other at one point [27]. With further compression of the monolayers, additional smaller plateaus were also observed around 42 mN/m. Similar to the first plateau, mixtures exhibited more prominent plateaus with increasing PEG₄₀St content. The increase in the extent of second plateau was also seen in Span60/PEG₄₀St mixtures [27]. The second plateaus were attributed to partial removal of the emulsifier from the mixed monolayers [27]. Scaling of the plateaus with the emulsifier

Fig. 1. (a) The surface pressure–mean molecular area (π–A) isotherms of pure DSPC, pure PEG₄₀St, and isotherms for DSPC/PEG₄₀St mixtures with different compressions at the air/water interface; (b) main turning points marked on the isotherm for the 8:2 mixture, the collapse pressures for each mixture and the plateau regions on the isotherms, and (d) the mean molecular areas at the collapse pressures for the mixtures.
content clearly indicated the presence of more emulsifier in the mixed monolayer and thus their progressive elimination from the monolayers.

The surface pressure-area (π-A) isotherms provide information on the molecular organization and molecular interaction of the components in mixed monolayers. The excess area (A_{ex}) can be calculated from the measured mean molecular area (\langle A_{12}\rangle) of the real mixed monolayer at the given surface pressure minus the mean molecular area of the ideal mixture (\langle A_{12}^{\text{ideal}}\rangle) \cite{30,50}, where x_1 and x_2 are mole fractions of the components, A_1 and A_2 are molecular areas of the pure components at that surface pressure. The zero value for A_{ex} indicates that the components form an ideal mixed monolayer or they are immiscible \cite{30,43}. On the other hand, deviation from ideality signify that the components are miscible and form non-ideal mixed monolayers \cite{30,43}. To gain more insight on the interaction type of the components and their miscibility behavior, excess areas, A_{ex}, of the mixed monolayers were calculated as shown in Fig. 2. As shown in the figure, all mixtures exhibited negative deviations from ideality, highest for the 7:3 composition. These negative deviations were explained by extension of PEG chains into the subphase by conformational changes with compression such that mean molecular area approaches up to the mean molecular area for the stearate chains \cite{43}. Considering the electrostatic repulsions between the polar head groups of the zwitter-ionic phosphatidylcholines \cite{51,52}, shielding effect of neutral PEG40St on these electrostatic repulsions may also play an additional role for creation of a more condensed phase. The negative A_{ex} values also show that the tilted and more relaxed pure DSPC molecules may be transformed into a more ordered configuration in the mixed monolayer as well as when the pure PEG40St molecules in pancake configuration compressed to a more aligned mushroom or brush configuration in the mixed monolayer \cite{35}. Therefore, the measured mean molecular area is smaller than the mean molecular areas measured in their pure ideal states. At higher compressions when the surface pressure increased up to the first collapse pressure of 33 mN/m, the negative excess area calculated for the mixtures decreased significantly and approached to “zero”. The zero excess area indicated that the DSPC/PEG40St monolayer formed phase separated domains and the measured area could be treated as the ideal area at the first collapse pressure, A_{12} = A_{12}^{\text{ideal}} = x_1 A_1 + x_2 A_2. When the surface pressure exceeds the first collapse pressure of 33 mN/m, the calculated excess area became more negative again at the surface pressure of 35 mN/m which is above the collapse pressure of 33 mN/m for the mixture. We believe that there were not only the conformational changes but also a squeeze out of PEG40St molecules in the monolayers above the collapse pressure of the mixture, which would mislead any calculation on neither excess area nor other thermodynamic parameters due to mass loss from the monolayer at the air-water interface.

The mean molecular areas were compared for the DSPC/PEG40St mixtures for different plateau regions as shown in Fig. 3. Mean molecular areas are in expanded conformation and occupies larger area at the air-water interface at lower pressures. Therefore, as shown in the figure, the mean molecular areas were measured relatively larger for the DSPC/PEG40St mixtures at pressures lower than the collapse pressure of the mixture. At the first collapse pressure of 33 mN/m, both DSPC and PEG40St molecules were significantly compressed to their minimal mean molecular areas on the monolayer at the air-water interface. We believe that the compositions of the mixtures were conserved up to the first collapse pressure so that no squeeze out was seen from the monolayer at the air-water interface. There is a significant decrease in the mean molecular area for the mixtures at the second collapse pressure of 41 mN/m. The decrease in the mean molecular area would not only be due to the conformational changes of the molecules, because they are already compressed to their minimal area at the end of the first collapse plateau, but also to the squeeze out of the PEG40St molecules from the monolayer to the subphase at the air-water interface causing the mean molecular area to decrease.

The DSPC and PEG40St molecules were preserved in the monolayer at pressures less than the first collapse pressure. At the surface pressure of 33 mN/m, the mean molecular areas for the mixtures were shown to increase slightly with the emulsifier content as shown in Fig. 1d. When the amounts of the two components were conserved, the measured surface area must be the area occupied by both components (A_{12} = x_1 < A_1 > + x_2 < A_2 >). Because the change in the mean molecular area for DSPC, < A_1 >, is relatively too small at around the collapse pressure, the DSPC molecules can be considered as rigid so that they would be a “reporting molecule” for the PEG40St molecules in the condensed monolayers. Therefore, the mean molecular area for DSPC, < A_1 >, would be approximately equal to its ideal mean molecular area, A_1, obtained from its pure isotherm at the specified surface pressure. At the first collapse pressure where the DSPC and PEG40St molecules were relatively compressed, the average molecular area for the PEG40St molecules can be calculated before they undergo a collapse or squeeze out. As shown in Fig. 4, the mean molecular area for DSPC and PEG40St molecules were obtained from their pure isotherms at the first collapse pressure of 33 mN/m. As shown in the figure, the mean molecular areas for DSPC were about 45 Å²/molecule whereas the mean molecular areas for PEG40St were about 38 Å²/molecule and these mean molecular areas did not significantly changed for different emulsifier contents of the mixtures. The figure shows an increasing trend for the calculated mean molecular area for PEG40St in the mixture at different emulsifier contents. The mean molecular area was calculated to be about 28 Å²/molecule for the 9:1 composition and 50 Å²/molecule for the 5:5 composition. The mean molecular area at the 9:1 composition is somewhat lower than the mean molecular area obtained...
for the pure PEG₄₀St molecule indicating that the PEG₄₀St molecules were well dispersed within the DSPC molecules so that the DSPC molecules compressed the PEG₄₀St molecules at molecular level and associated mostly with the stearate group of the PEG₄₀St rather than PEG chains at the air-water interface. Therefore, the measured mean molecular area is close to the mean molecular area for the stearate group of the PEG₄₀St molecules and a large portion of the PEG chains submerged within the subphase. The mean molecular area was measured to be about 50 Å²/molecule for the 5:5 composition indicating that the PEG₄₀St molecules would be mostly phase separated and compressed over the PEG chains by the DSPC and therefore, higher average molecular areas were obtained for the PEG₄₀St molecules at higher emulsifier contents.

At surface pressures higher than the collapse pressure of any component, the mean molecular area for that component can be taken as the mean molecular area of its pure form at its collapse pressure. The mean molecular areas were measured to be about 40 Å²/molecule for DSPC at its collapse surface pressure of 59 mN/m and 30 Å²/molecule for PEG₄₀St at its collapse surface pressure of 35 mN/m.

The collapse pressures for the DSPC/PEG₄₀St mixtures at the last collapse point were obtained to be about 59 mN/m although the mean molecular area decreased almost linearly with the emulsifier content as shown in Fig. 5a. At the indicated collapse pressures, which are much higher than the collapse pressure of 35 mN/m for the PEG₄₀St, it was reported that all the emulsifier was squeeze-out from the 2D monolayer [6]. In this case, the mean molecular area of the DSPC in the monolayer can be estimated from the measured average molecular area for the mixtures omitting the mean molecular area for PEG₄₀St (Aₓ₂ = x₁A₁ + x₂A₂). Therefore, the measured mean molecular area for the mixture would be equal to the area occupied by the DSPC molecules with a mean molecular area of 40 Å²/molecule, which is reasonable because the variation in the mean molecular area is very small. Fig. 5b shows the measured mean molecular area for the mixtures with respect to the calculated mean molecular area when the sole area occupied by DSPC and the PEG₄₀St component was omitted. As shown in the figure, the diagonal line represents the measured area would be equal to the calculated mean molecular area for the DSPC. However, the measured area for the mixtures is lower than the diagonal indicating that a small amount of DSPC would also be lost from the 2D monolayer at this late stage of the compression.

The squeeze out amount of PEG₄₀St can be estimated from the measured surface area for the mixtures. The measured mean molecular area is the summation of the areas imposed by both DSPC and PEG₄₀St molecules and it was shown in Fig. 1d that the measured surface areas decreased with increasing the emulsifier content at each collapse point. The decrease in the measured surface area indicated that PEG₄₀St molecules were lost at each collapse point and can be calculated using the equation

\[ A_{\text{mix}} = x_1A_1 + x_2A_2 - y \]

where \( A_{\text{mix}} \) is the measured mean molecular area for the mixtures, \( A_1 \) and \( A_2 \) are the mean molecular areas for pure DSPC and pure PEG₄₀St, and \( y \) is the percentage of DSPC contributed to the measured mean molecular area. The remaining of \( y \) indicated the loss percentage of PEG₄₀St from the monolayer. Here, DSPC is a rigid molecule and can be taken as the reporting molecule for the PEG₄₀St because the change in the mean molecular area for DSPC was considered to be minimum or negligible, if any. Fig. 6a shows the estimated percentage of the PEG₄₀St loss from the monolayer into the subphase at different collapse pressures. As shown in the figure, almost 93% of PEG₄₀St was lost at the end of the first collapse plateau for the 9:1 mixture and showed a decreasing trend for the higher PEG₄₀St contents for the mixtures. For instance, almost 82% and 53% of PEG₄₀St were lost for the 7:3 and 5:5 compositions at the end of the first collapse plateau, respectively. Remaining of the PEG₄₀St molecules were lost at the end of the second collapse plateau, where 20% of PEG₄₀St were still present for the 5:5 mixture on the air-water interface. The PEG₄₀St was entirely lost at the last collapse pressure for each mixtures studied.

The collapsed amounts of DSPC were estimated from the measured mean molecular area at the last collapse pressures. The fact that the measured surface area is lower than the estimated mean molecular area for the DSPC indicating that all the PEG₄₀St and some of DSPC were lost from the 2D monolayer [6]. Then, the lost DSPC amount can be estimated from the measured mean molecular area at the last collapse pressure when the PEG₄₀St molecules were omitted (Aₓ₂ = x₁A₁ + z x₂A₂), where \( z \) is the percentage of DSPC contributed to the measured mean molecular area. The remaining of \( z \) indicated the percentage of DSPC loss from the monolayer. Fig. 6b shows the estimated percentage of the collapsed DSPC from the monolayer with different emulsifier contents. The DSPC molecules would also be lost from the monolayer into the subphase by associating with the PEG₄₀St molecules.
molecules. As shown in the figure, about 5% of DSPC molecules were lost for the mixtures, which can be considered relatively negligible.

4. Conclusions

A quantification method was developed to estimate the squeeze out amount of PEG40St from Langmuir isotherms of the DSPC/PEG40St monolayers at different molar ratios. The discontinuities on the Langmuir isotherm were always more extended for the first collapse plateau and relatively shorter for the second collapse plateau. The extent of the plateaus increased in proportional to the PEG40St content of the mixtures for the two collapse regions. The isotherms were analyzed considering that the DSPC molecules are relatively rigid at room temperature and their mean molecular area did not vary with relatively small deviations in the surface pressure, therefore, DSPC molecules were considered “reporter molecules” for the PEG40St. It was also considered that the PEG40St molecules already compressed to their collapse area above its collapse pressures and its area can be taken as its mean molecular area at its collapse pressure at the end of the first and second collapse plateaus. Reproducible results were obtained for the mean molecular area of 40 Å²/molecule and 30 Å²/molecule for DSPC and PEG40St at their collapse pressures of 59 mN/m and 35 mN/m, respectively. It was shown that PEG40St molecules compressed to different degree of molecular orientations depending on its content in the DSPC/PEG40St mixtures at pressures below the first collapse pressure. For instance, the PEG40St mean molecular areas were calculated to be 28 Å²/molecule for 9:1 composition and 50 Å²/molecule for 5:5 composition with an increasing trend with the PEG40St content at the collapse pressure of 33 mN/m. As a result, at the end of the first collapse region, 93%, 82%, and 53% of PEG40St were lost for the 9:1, 7:3, and 5:5 mixtures, respectively, and showed a decreasing non-linear trend. Remaining PEG40St molecules were lost at the end of the second collapse plateau except for 5:5 mixture. At the end of the second collapse plateau, a 20% of PEG40St were found to be still retained with the 5:5 mixture at the air-water interface and it squeezed out from the monolayer at the last collapse pressure for the DSPC. Retaining the PEG40St within the monolayer upon squeeze out is advantageous for the design of stable microbubble shell. Because the DSPC costs about $169/g-DSPC [53] and PEG40St costs about $0.22/g-PEG40St [54], the cost for the 5:5 shell composition of DSPC/PEG40St mixture would be much cheaper compared to the customized composition of 9:1 molar ratio. It was concluded that increasing PEG40St content could not only increase the microbubble stability but also it decreases the product cost for the ultrasound contrast agents.

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References

[22] C.C. Chen, M.A. Borden, The role of poly(ethylene glycol) brush architecture in complement activation on targeted microbubble surfaces, Biomaterials 32 (2011)
S. Kilic

Colloids and Surfaces A 551 (2018) 58-64

6579–6587.


