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Published in:
Colloids and Surfaces B: Biointerfaces

DOI:
10.1016/j.colsurfb.2011.02.015

Publication date:
2011

Document version
Early version, also known as pre-print

Citation for published version (APA):

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Compressibility study of quaternary phospholipid blend monolayers

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A R T I C L E I N F O

Article history:
Received 29 March 2010
Received in revised form 1 February 2011
Accepted 7 February 2011
Available online 15 February 2011

Keywords:
Langmuir monolayer
Compressibility
Drug delivery
Ultrasound
Sonosensitivitity
Brewster’s angle microscopy

A B S T R A C T

The mechanical properties of liposome membranes are strongly dependent on type and ratio of lipid compounds, which can have important role in drug targeting and release processes when liposome is used as drug carrier. In this work we have used Brewster’s angle microscopy to monitor the lateral compression process of lipid monolayers containing as helper lipids either distearoyl phosphatidylethanolamine (DSPE) or dioleoyl phosphatidylethanolamine (DOPE) molecules on the Langmuir trough. The compressibility coefficient was determined for lipid blend monolayers containing the helper lipids above, cholesterol, distearoyl phosphatidylcholine (DSPC) and pegylated-DSPC at room temperature. Two variables, the cholesterol fraction and the ratio p between the helper lipid (either DSPE or DOPE) and the reference lipid DSPC, were studied by multivariate analysis to evaluate their impact on the compressibility coefficient of the monolayers. The cholesterol level was found to be the most significant variable for DSPE blends while the ratio p was the most significant one for DOPE blend monolayers. It was also found that these two variables can exhibit positive interaction and the same compressibility value can be obtained with different blend compositions.

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1. Introduction

Liposomes consisting of long-chain, saturated and unsaturated phosphatidylcholine and cholesterol in close to equimolar amounts as well as minor percentages of pegylated phosphatidylethanolamine for a number of years have been recognized as efficient drug carriers for intravenous administration in terms of modulating biodistribution and pharmacokinetics [1] therefore potentially improving activity and reducing toxicity [2] of a given drug. In chemotherapy such sterically stabilized pegylated liposomes, containing anticancer drugs are in routine clinical use due to their long circulation time and ability to specifically accumulate within most solid tumor tissue [3]. In principle, liposomes can accommodate all types of drugs, and markedly reduce leakage rates granting excellent shelf life and in vivo-stability have especially been reported for liposomal formulations, where the drug is loaded via a pH- or ion-gradient [4,5]. Once the liposomes have arrived at the target site, however, their intrinsic tightness may represent a serious barrier against efficient drug release. More recently, various attempts have thus been undertaken to design liposomes, which release their drug payload upon a change in the surrounding biological milieu (pH-sensitive liposomes [6,7]) or upon an external stimulus such as heat (thermosensitive liposomes [8]) or ultrasound (sonosensitive liposomes [9]). For the aforementioned goals, the incorporation of non-bilayer-forming lipids like phosphatidylethanolamines (PE), lysophosphatidylcholine and/or pegylated PE to the formulation has proven successful. Although lipid polymorphism, i.e. which morphology, like lamellar, cubic or inverse hexagonal, lipids adopt, is rather well understood for most (single) phospholipids as well as a range of binary lipid mixtures, little is known on lipid polymorphism of more complex lipid blends. Furthermore the implications of lipid polymorphism for drug carrier design after many years of research are still under intense discussion. Clearly, the liposome membrane can present different mechanical properties depending on type and ratio of lipid compounds, which can have important role in drug targeting and release processes. The aim of the current study thus was to investigate quaternary lipid blends of long-chain, saturated distearoyl phosphatidylcholine (DSPC) with cholesterol, pegylated phosphatidylethanolamine (DSPE-PEG 2000), which are standard for established liposomal drug carriers in combination with selected non-bilayer-forming lipids like distearoyl phosphatidylethanolamine (DSPE) or dioleoyl phosphatidylethanolamine (DOPE) the saturated and unsaturated version of a C18 PE respectively. The latter were chosen because...
recent reports indicate that phosphatidylethanolamines are able to tune sonosensitivities, i.e. ultrasound-triggered release of drugs from pegylated PC/cholesterol-liposomes without impairing their stability in vivo [10].

In a first approximation, we considered two-dimensional lipid monolayers confined at the air–water interface as a model for studying physical properties of liposome membranes. Two-dimensional lipid monolayers have effectively been used as a model membrane mainly for studying miscibility, interaction and other physicochemical properties of materials in biological membranes or artificial vesicles [11,12]. Moreover, the thickness of the membranes can be several orders of magnitude smaller than their extension so that they can be considered like two-dimensional systems for many respects [13].

The Langmuir trough is the apparatus used for studying an artificial lipid monolayer confined to a two-dimensional surface [13], such as the air–water interface, for membrane simulation and studying of two-dimensional packing phases, membrane mechanical properties, structural distortions, molecular interaction and aggregation, among others. In this work, we present a quantitative study on Langmuir monolayer compressibility as a function of lipid composition.

A Brewster angle microscope (BAM) mounted on top of the Langmuir trough can be used for the study of the membrane topography on a length scale of the order of tens of micrometers [14]. In this work, the BAM technique turned out to be a helpful tool for qualitative control of the different lipid monolayer compositions.

2. Materials and methods

Cholesterol from lanolin 99%, C_{27}H_{46}O, MW = 386.67, purchased from Fluka; DSPE, 99%, C_{44}H_{88}NO_{8}P, MW = 790.09, purchased from Genzyme Pharmaceuticals; PEGylated lipid, MPEG-2000-DSPE (DSPE-PEG), 99%, C_{43}H_{82}NO_{10}P(C_{2}H_{4}O)_{45}, MW = 2800, purchased from Genzyme Pharmaceuticals; DOPE, 97%, C_{41}H_{78}NO_{8}P, MW = 744, purchased from Genzyme Pharmaceuticals.

Sample preparation: Each lipid is independently dissolved in chloroform at different concentrations until the solutions appear clear at room temperature: DSPC at 12 mg/ml, DSPE and DOPE at 6 mg/ml, cholesterol at 4 mg/ml and DSPE-PEG at 1 mg/ml. These solutions are then mixed in appropriate ratios for preparation of different series: series A has fixed ratio \( \rho = 2 \) (\( \rho = PE/DSPC \), where PE is either DSPE or DOPE) and different amounts of cholesterol as 0, 10, 20 and 30 mol%; the series B contains fixed amount of cholesterol of 30 mol% and variable molar ratio \( \rho = 0.5, 1, 2, 4 \) and 10. Series A and B have fixed amount of 3 mol% of DSPE-PEG. Samples of pure helper lipids DSPE and DOPE taken directly from the stock solutions are used as references. The amount of 30% of cholesterol is known to have better stability in vivo, reason for it to be chosen as fixed amount in series B of our model membrane system. However, in series A, the intermediate rate \( \rho = 2 \) is studied with lower amounts of cholesterol, 20%, 10% and 0%, in order to test its impact on the compressibility coefficient.

Langmuir film preparation: A Langmuir trough, Nima model 712 BAM, was used at the Soft Condensed Matter Lab, Troika II, European Synchrotron Radiation Facility (ESRF), France. The mixture of lipid-in-chloroform is diluted and the final concentration of each one is around 0.2 mg/ml. Small amount of the diluted solution, from 100 to 300 \( \mu \)l, depending on the sample, was taken by means of a Hamilton glass microsyringe and spread on the surface of the Langmuir trough containing pure water (Elga purificator, resistivity = 18.2 M\( \Omega \)cm). A minimum of 10 min was given for the solvent to evaporate. The monolayer was then compressed to a rate of 10 cm\(^2\)/min (or 1 cm/min in linear movement of the barrier) and the surface pressure was measured by a filter paper Wilhelmy plate coupled to a microbalance. A Brewster angle microscope (BAM), 1 \( \mu \)m resolution, mounted on top of the Langmuir trough was used for monitoring of the microscopic structure of the monolayers, fluidity and membrane folding. All the experiments were performed at room temperature, 22 \( ^\circ \)C. Before initiating the experiment, the trough was exhaustively cleaned using isopropanol and pure water. The Langmuir trough and BAM were kept in a Plexiglas cabinet to avoid dust particle contamination and to prevent the air stream from vibrating the water surface during compression.

3. Results and discussion

The essential part of the present work is the use of the compressibility factor in the comparison of different lipid compositions of the membranes. The BAM images, also the ones not shown here, helped to monitor qualitatively the compression process. We found interesting to show the images for pure DSPE and pure DOPE monolayers because these two lipids have very distinct behavior (powder-like and fluid-like at room temperature, respectively) which is also the main behavior of the blends made with them even when they are not the major component in the mixture. The graph in Fig. 1 shows the pressure–area \((\Pi-A)\) isotherms for pure DOPE and pure DSPE monolayers at room temperature, which are in reasonable agreement when compared with equivalent ones found in the literature [15–18], some differences being due to variations in temperature, compression speed, impurities or other instabilities of polar lipid systems. The area per molecule is more difficult to reproduce while the shape of the isotherm should be reproducible and is characteristic of each material or mixture. However, the higher the speed of the barrier of the Langmuir trough, the lower is the resolution to capture details of the shape of the isotherm curve. When the applied pressure is very low, the lipid molecules behave as a two-dimensional gas and can be described by \( \Pi A = kT \), where \( k \) is the Boltzman constant and \( T \) is the thermodynamic temperature. The corresponding two-dimensional “liquid” and “solid” phases are formed when the external pressure increases, by means of the movement of the lateral barrier of the Langmuir trough, packing the monolayer. Both molecules DOPE and DSPE have negative mean curvature because the cross section of their common head group, is smaller than the cross section of their acyl chains. In the case of DOPE molecule, the kinks of the unsaturated chains increase even
more the cross section of the acylic chain and keep the molecules well separated from each other producing a more expanded Langmuir monolayer than the DSPE one as we observe in Fig. 1 where at the same pressure, DOPE isotherm curve presents a higher area per molecule than DSPE. The first phase transition point is also called “lift-off area” of the isotherm curve. The DSPE monolayer has a lift-off area at 74 Å², while the DOPE monolayer has a larger lift-off area at 199 Å². There is a large dispersion in the range of values for lift-off areas for pure DOPE monolayers found in the literature [15–19], going from 100 to 300 Å². Also for DSPE there is a dispersion of liftoff values reported in the literature [15,20], however not as large as DOPE. BAM pictures taken during different stages of a compression process of pure DSPE and DOPE monolayers are shown in Figs. 2 and 3. Pure DOPE monolayers present a clear fluid aspect observed under the microscope, which will remain as a strong characteristic for all blend monolayers containing DOPE molecules presented in this work. Pure DSPE monolayers present a more abrupt phase transition such as a solid-like behavior and so are all blend monolayers containing DSPE molecules studied here.

The pictures of Fig. 2, taken by BAM, show the two-dimensional organization of pure DSPE monolayer at micrometer resolution. They are labeled from “a” to “f” according to the pressure–area isotherm of Fig. 1. The pictures with more contrast are in the region of low surface pressure of the monolayer evidencing the gas phase, liquid-condensed (LC) phase or the coexisting of both. Fig. 2a–c, shows initially, at pressure near to zero, LC phase domains shaped as small dots, and filaments turning into a net and then merging to bigger plaques. After the phase transition, the monolayer is homogeneously packed and “flat” to the resolution of the microscope; the pictures appear dark with poor contrast of too fine topography of the packed membrane. The last picture in Fig. 2, show bright dots that might be isolated folded domains, or squeeze-outs, just before total collapse.

In comparison with pure DSPE monolayers shown in Fig. 2, the main characteristic of pure DOPE monolayer at room temperature, shown in Fig. 3, is the fluidity and the collective evolution of the membrane in the gas/liquid-expanded phase. Starting from Fig. 3a, the LE domains, initially long-filament shaped, start to arrange into a stretched net, which becomes “rounded” until the inner gas phase change to LE when the monolayer is highly packed. Similar picture was reported in the literature after using AFM to study supported monolayers transferred at low pressure [15]. After the phase transition, the surface becomes homogeneously flat and dark to the Brewster angle microscope. The last picture shows bright dots that might be isolated folded domains at very high packing. N.B.: Fig. 1 shows a kink point for DOPE monolayer at ~26 mN/m but no significant change is observed by BAM pictures in this region.

3.1. Comparing the pressure–area isotherms of mixed lipid monolayers

Fig. 4 shows the pressure–area isotherms for series A containing a fixed ratio ρ = 2 and different amounts of cholesterol, while Fig. 5 shows the pressure–area isotherms for series B containing a fixed amount of cholesterol of 30 mol%, and variable ratio ρ = 0.5, 1, 2, 4 and 10. The lift-off area is always higher for DOPE blend monolayers than DSPE blend monolayers in any studied case. DOPE blend monolayers are more expanded than DSPE blend monolayers as shown in Figs. 4 and 5, i.e., at the same pressure, the area per molecule is higher for DOPE monolayers. The quantity of DSPE–PEG is fixed and equal to 3 mol% for all blends studied here. The lipid DSPE–PEG also gives an expansion effect to the monolayers and it is miscible with DSPC as we can see in the work of Chou et al. [21]. Analyzing the systems without cholesterol, we can observe from Fig. 4a, that the isotherms for DOPE and DSPE are very much distinct. A pure cholesterol isotherm and the mixture with DOPE isotherm is found in the literature, for example in the work of Savva [22] where it is reported a similar behavior shown here. The addition of cholesterol make DOPE and DSPE behavior more alike, in respect of phase transition area and slope of the curve (which will be related to their compressibility, discussed further), as we see in Fig. 4b–d. In Fig. 5, when analyzing the increase in ρ, we have a slight shift towards higher area (better observed in summary discussed further) and the appearing of intermediate phase transitions (kinks on the slope of the curve). For ρ = 10, we can observe at least two intermediate phase transition points, which could be the miscibility transitions from two coexisting phases [23] which is happening at too high amount of DOPE or DSPE lipid inserted on the monolayer. A summary of results seen in Figs. 4 and 5 is shown in Fig. 6, where the lift-off area, or phase transition area, is plotted versus chole-
sterol amount and ratio \(\rho\). In the first graph of Fig. 6 we observe that the addition of cholesterol increases the lift-off area of DSPE monolayers while for DOPE it seems to have a competitive role: a DOPE monolayer without cholesterol presents more expanded behavior or higher phase transition area, than all DOPE monolayers containing cholesterol. The second graph of Fig. 6 we observe that the increase in \(\rho\) increases the phase transition area of DOPE and DSPE monolayers as shown in Fig. 6b, except for \(\rho = 10\), the last point in each curve, which are not following the trend maybe because of the coexistence of phases, already discussed above.

### 3.2. Compressibility coefficient

The compressibility isotherms presented previously in Figs. 4 and 5 show very distinct behavior for DSPE and DOPE monolayers. The fact that the phase transition is always more abrupt for DSPE monolayers can be quantitatively described by the compressibility coefficient \(k\) defined by the following expression [21]:

\[
k = \frac{1}{A} \frac{dA}{d\Pi}
\]

where \(A\) and \(\Pi\) represent the mean area per molecule and the monolayer pressure respectively. The compressibility of the monolayer is said to be an indication of the equilibrium elasticity. The more abrupt is the phase transition, the higher is the differential \(d\Pi/dA\) and stiffer is the monolayer. The compressibility coefficient \(k\), being proportional to the inverse of the differential \(d\Pi/dA\), will be higher for soft monolayers (like the DOPE blends in our study) than for stiff ones (like the DSPE blends).

The graphs in Figs. 7 and 8 show the compressibility coefficient values for the interval in surface pressure corresponding to the region just after the main phase transition of the studied systems. For this interval in surface pressure, the compressibility coefficient can be precisely fitted to a first order exponential decay. The compressibility coefficient \(k\) increases for DSPE monolayers when the cholesterol amount increases (see hollow symbols in Fig. 7). The coefficient \(k\) for DOPE monolayers have a different behavior, related to (or consequence of) the behavior discussed in Fig. 6a: \(k\) follows the trend \(0\% > 30\% > 20\% > 10\%\) of cholesterol (see solid symbols in Fig. 7, and summary in Table 1), which could be interpreted again as a competitive role between DOPE and cholesterol for compressibility.

Fig. 8 shows that the compressibility coefficient increases with an increasing \(\rho\) value for both DSPE and DOPE monolayers except for the highest ratio \(\rho = 10\). We can also observe that a minor quantity of DOPE lipid in the composition of the monolayer, gives the same value of compressibility for much more concentrated DSPE monolayers, for instance, solid squares in Fig. 8, 22 mol% fraction of DOPE (\(\rho = 0.5\)) show even higher value of compressibility than hollow triangles, which contains twice as much DSPE (45 mol%, \(\rho = 2\)).

Table 1 presents fitted values for compressibility coefficient \(k\) at given pressures \(\Pi = 8, 10, 12\) and 14 mN/m, which covers the average pressure range after the main phase transition in both DOPE and DSPE systems. The compressibility coefficient \(k\) is higher for DOPE systems than DSPE systems. Fig. 9 shows the ratio \((k_{dspe} - k_{dope})/k_{dspe}\) for all studied cases. Except for the first and the last points of the graph, we observe that by using DOPE instead of DSPE we have an increase of approximately 50% in compressibility of the monolayer structures, at this pressure region, at room temperature. The first point of the graph (\(\rho = 2\); cholesterol fraction = 0) is the case where no cholesterol was added to the blend and so the fluidity of DOPE monolayer is much higher compared to DSPE blends just by the fact that DOPE is more fluid-like at room temperature. The last point in the graph (\(\rho = 10\); chol = 30) is the case of highest amount of DOPE or DSPE compared to DSPC, which can generate separation of phase and a more complex way to calculate the compressibility.
Fig. 5. Pressure–area isotherms for DOPE and DSPE monolayers with varying molar ratio $\rho$. The graph (a) shows again compressibility isotherm of pure DOPE and DSPE systems already shown in Fig. 1, by the sake of comparison.

Fig. 6. First phase transition evolution for DOPE and DSPE monolayers related to cholesterol content (series A) and ratio $\rho$ (series B).
Table 1

Compressibility coefficient \( k \) (m/mN) for mixed lipid monolayers at four different pressures \( \Pi \) in mN/m. The values of \( k \) were taken from the fitting curve with error of 0.01 m/mN. All monolayers contain fixed amount of DSPE-PEG (3 mol%).

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>( \rho )</th>
<th>chol (mol%)</th>
<th>( \Pi = 8 )</th>
<th>( \Pi = 10 )</th>
<th>( \Pi = 12 )</th>
<th>( \Pi = 14 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PE( ^a )</td>
<td>0</td>
<td>0</td>
<td>0.594</td>
<td>0.11</td>
<td>0.51</td>
<td>0.07</td>
</tr>
<tr>
<td>Series A</td>
<td>2</td>
<td>0</td>
<td>0.52</td>
<td>0.18</td>
<td>0.37</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>0.38</td>
<td>0.22</td>
<td>0.26</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>0.40</td>
<td>0.32</td>
<td>0.27</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>0.46</td>
<td>0.33</td>
<td>0.33</td>
<td>0.22</td>
</tr>
<tr>
<td>Series B</td>
<td>0.5</td>
<td>30</td>
<td>0.36</td>
<td>0.24</td>
<td>0.25</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30</td>
<td>0.40</td>
<td>0.29</td>
<td>0.29</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>0.46</td>
<td>0.34</td>
<td>0.33</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30</td>
<td>0.52</td>
<td>0.42</td>
<td>0.37</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0.45</td>
<td>0.19</td>
<td>0.29</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\( ^a \) "PE" stands for either DOPE or DSPE.
\( ^b \) Same system belonging to the two series A and B at the same time.

3.3. Quantification of influence of tested factors on monolayer compressibility

Until this point the effect of the variables \( \rho \) and cholesterol fraction on the monolayer compressibility have been interpreted as separate factors. There are, however, indications that there might be interaction effects between these two variables. By employing multivariate analysis techniques the impact as well as the significance of each factor can be quantified. Also possible interactions as well as non-linear behavior can be identified and quantified. Since the distribution of experiments within the design space of the current study is not fully balanced, partial least square regression (PLS) was chosen over multiple linear regressions (MLR). PLS analyses were performed separately for both DSPE and DOPE blends using The Unscrambler 9.8 (Camo ASA, Norway). We have used 4 representative values of surface pressure \( \Pi = 8, 10, 12 \) and 14 mN/m shown in Table 1 to perform the multivariate analysis. Prior to calculations the variation of each variable was scaled to unit variance \((1/\sigma)/\sigma\) (standard deviation). Cross validation and jack-knifing \[24\] was used to validate and assess the stability of the models. The significant regression coefficients are presented in Table 2 together with merits of the calculated models.

Blend monolayers of the two main lipids, DSPE and DOPE, behave differently with respect to compressibility as described above. For DSPE blends, cholesterol was identified to have a high impact on the compressibility of the monolayer at all pressures. As seen in Table 2, the regression coefficient of cholesterol is high and statistically significant in all cases for DSPE monolayers reflecting the high impact of this factor on the compressibility. The value of
Table 2

Significant regression coefficients from PLS-1 analysis (p < 0.05) of the compressibility coefficient \( k \) for mixed lipid monolayers at four different pressures \( \Pi \) in mN/m. Investigated variables are ratio \( \rho \) and cholesterol fraction. Separate models for DSPE or DOPE are given.

<table>
<thead>
<tr>
<th>Variables (X)</th>
<th>Effect on compressibility (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DSPE</td>
</tr>
<tr>
<td></td>
<td>( \Pi = 8 )</td>
</tr>
<tr>
<td></td>
<td>( \Pi = 10 )</td>
</tr>
<tr>
<td></td>
<td>( \Pi = 12 )</td>
</tr>
<tr>
<td></td>
<td>( \Pi = 14 )</td>
</tr>
<tr>
<td>Ratio (( \rho ))</td>
<td>0.293</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.415</td>
</tr>
<tr>
<td>Ratio ( \rho )/Cholesterol</td>
<td>0.332</td>
</tr>
<tr>
<td>Cholesterol **2</td>
<td>-0.363</td>
</tr>
<tr>
<td>Optimum number of PCs</td>
<td>2</td>
</tr>
<tr>
<td>Explained X-variance (%)</td>
<td>95</td>
</tr>
<tr>
<td>Explained Y-variance (%)</td>
<td>96</td>
</tr>
<tr>
<td>RMSEC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0141</td>
</tr>
<tr>
<td>RMSEP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0218</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not-significant (p < 0.05).
<sup>b</sup> Root mean square error of calibration.
<sup>c</sup> Root mean square error of prediction.

Fig. 10. Surface plots for compressibility predicted from PLS models calculated in Table 2 (\( \Pi = 12 \) mN/m). (a) DSPE and (b) DOPE.

the regression coefficient of cholesterol is high also compared to the ratio \( \rho \), implying that cholesterol fraction has higher influence on the compressibility than the ratio \( \rho \). For all compressibility pressures (except for 14 mN/m) a positive interaction was identified between the lipid ratio \( \rho \) and cholesterol fraction. The interaction effect is visible as curved lines in the surface plot, created from the model, which is presented in Fig. 10a. The highest compressibility for monolayer blends based on DSPE is identified at high level of cholesterol combined with a high ratio \( \rho \). For DOPE monolayers, blends, on the other side, cholesterol is a less important modulator, as evidenced by a statistically non-significant regression coefficient (p < 0.05). For DOPE monolayers the ratio \( \rho \) was identified as more important. There also seems to be an important interaction between \( \rho \) and cholesterol fraction, even though cholesterol fraction alone did not show any significant influence.

Comparing the surface plots from the two lipids in Fig. 10, it can be seen that higher values of compressibility are obtained for DOPE (Fig. 10b) compared to DSPE (Fig. 10a). Formulations displaying similar degree of compressibility can be identified for both DSPE and DOPE, for instance, the range of compressibility coefficient from 0.16 to 0.19 for DSPE can be obtained with high content of cholesterol but only at high ratios \( \rho \) (from 2 to 4) while for DOPE the corresponding conditions can be obtained at the same range of ratio \( \rho \) but without cholesterol or very low concentrations of cholesterol (≈5 mol%). Also the combination of high content of cholesterol (30 mol%) with very low lipid ratio (\( \rho = 0.5 \)) gives corresponding compressibility values for DOPE according to the predicted model. This finding suggests that the same compressibility or hardness of lipid monolayer can be obtained with both DOPE and DSPE blends just by changing the molar ratio of the blend.

4. Conclusions

Quaternary lipid blends confined at the air–water interface of a Langmuir trough were used as a model for liposome membrane studies. The Brewster angle microscope (BAM) technique facilitated to monitor qualitatively the phase transition during compression of the lipid monolayers. The compressibility coefficient was determined for the region just after the main phase transition of the studied systems.

At room temperature, pure DOPE lipid monolayer presents a fluid and collective evolution seen under the microscope meanwhile pure DSPE monolayer presents a solid-like behavior. Examining the BAM pictures for pure DSPE and DOPE monolayers, together with the pressure–area isotherms, we see that the global structure happens to start at the gas phase, at low pressure. Local microscopic order and different shaped domains coexist with expanded phase before the lift-off area.
There is a more pronounced tendency of our DOPE-based lipid blends as compared to DSPE systems to form coherent fluid phases. Furthermore, DOPE blend monolayers are more expanded than DSPE ones and the compressibility coefficient increases with the ratio \( \rho \). All these tendencies, more coherent fluid phase, more expanded monolayers and higher compressibilities appear to fall together with increased seasonality of the corresponding DOPE-bilayer systems as compared to the DSPE-systems reported in the work of Evjen et al. [10] and the patent of Lauten et al. [25]. Whether there is a mechanistic correlation between the tendencies seen here and the susceptibility of liposomes towards external mechanical triggers remains to be studied more thoroughly. Cholesterol included in the blend monolayers also increased the compressibility coefficient for DSPE monolayers, while such relation is not always evident for DOPE monolayers indicating an interaction between DOPE and cholesterol as found by the multivariate analysis results.

This work shows that by preparing blend monolayers using DOPE instead of DSPE we can have an increase of approximately 50% in compressibility at the studied pressure range at room temperature. A minor quantity of DOPE lipid in the composition of the monolayer, for instance, 22 mol% fraction of DOPE (\( \rho = 0.5 \)), yield the same value or even slightly higher value of compressibility of twice as much concentrated DSPE monolayer (45 mol%, \( \rho = 2 \)) for the studied pressures at room temperature. Also, the same compressibility of lipid monolayer can be obtained using either DOPE or DSPE and appropriate molar ratio of the other components of the blend as predicted by the multivariate analysis.

It remains to be investigated if the observed differences in lateral compressibility of monolayers composed of DSPC and DSPE-PEG with varying contents of cholesterol and phosphatidylethanolamine show some correlation with the susceptibility of comparable bilayers towards pH sensitivity or external mechanical stimuli like ultrasound. The present results will complement structural studies, which are foreseen or under going, using different techniques like grazing incidence X-ray diffraction in lipid monolayers and small angle X-ray scattering in lipid dispersions to be compared with release experiments under ultrasound stimuli.

Acknowledgments

This work was financed by the Norwegian Research Council under the Nanomat Programme. The BAM experiments were performed at the Soft Condensed Matter Lab, Troika II, European Synchrotron Radiation Facility (ESRF) in France. The authors thank Tove J. Evjen (Epitarget AS and University of Tromsø), LPC thanks Thais Rigoletto (Faculty of Chemistry Engineering, UNICAMP, Brazil) for useful discussions during the preparation of the manuscript.

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