Density of Lecithin and Its Effective Molecular Volume in Lecithin-Water Dispersions

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Abstract

Liposomes are of major interest as model membrane systems and their potential applications. As the packing structure of lipid molecules in the liposomes will vary with different liposome sizes, it is expected that the density of the lipid in the dispersions will also vary, albeit minutely. The main objective of this study is to obtain accurate density measurements of lipid-water dispersions, and to relate the results to the molecular packing structure of the liposomes. Lipid-water dispersions were prepared by means of sonication. The average sizes of liposomes were estimated via turbidity measurements, while the densities of the lipid dispersions were determined using a digital density meter. The density of the lecithin in the dispersions was calculated and the value found was comparable to the value in the dry state. A thermotropic phase transition at about 40 °C - 45 °C was observed. The results for the density of the lecithin in the dispersions are 1.0579 g cm⁻³ at 25 °C and 0.9961 g cm⁻³ at 50 °C. However, the average values of the effective molecular volume of lecithin in the dispersions are 1.152E-21 cm³ at 25 °C and 1.224E-21 cm³ at 50 °C.

Keywords: Lecithin; Density; Effective Molecular Volume; Dispersion

1. INTRODUCTION

Liposomes are quasi-spherical structures composed of lipid bilayers that encapsulate an aqueous space [1]. Liposomes form spontaneously when lipids are dispersed in an aqueous media, giving rise to a population of liposomes with various sizes. They can be prepared in the laboratory by various methods, such as organic solvent injection [2, 3], sonication [4 - 7], reverse phase evaporation [8] or extrusion [9 - 12].

The study of liposomes is very essential because of their similarity to biological membranes and their medical value as delivery agents for enzymes, drugs, and in genetic manipulation and diagnostic imaging [13 - 16]. Hence, liposomes are of particular interest.

Various investigations have addressed the biological aspects of liposomes, both in vivo and in vitro. The relationship between the structure of the simple lipid membrane and that of the lipid phase of biological membranes has been a matter of some concern. As is well known, the polymorphism of the bilayer arises from alterations in the packing arrangements of lipid hydrocarbon chains, order or disorder isomerizations in intramolecules, and hydrophobic or hydrophilic interactions between water and lipid. Consequently, considerable interest underlying the subject of the molecular packing structure of liposomes in lipid-water systems has been generated.
The occurrence of a thermotropic phase transition is characteristic of lipid-water systems. The study of thermotropic lipid phase transitions in both natural and model membranes has proven to be a productive approach towards the understanding of the structure, organization and interactions present in lipid bilayer assemblies. Generally, the lipid bilayer composed of pure one-component phospholipids, in excess water, can undergo multiple thermotropic phase transitions upon heating [17]. Of these several transitions, the chain-melting transition or the gel to liquid-crystalline phase transition is the main phase transition, which is accompanied by the largest entropy change. A variety of physical techniques have been applied in the study of phase transitions, including differential thermal analysis [18, 19], Raman spectroscopy [20, 21] as well as infrared spectroscopy [22, 23].

The main objective of this study is to obtain accurate density measurements of lipid-water dispersions, and to relate the results to the molecular packing structure of the liposomes. The lipid-water dispersions were prepared by means of sonication to produce liposomes of minute sizes. Measurements of the turbidity as a function of wavelength, range from 400 nm to 800 nm, using a spectrophotometer, provided a means of evaluating the average size of the liposomes in the dispersions. The densities of the lipid-water dispersions were measured using a digital density meter, in the temperature range from 25 °C to 55 °C. A theoretical approach, based on the precise density measurements, was formulated to estimate the effective volume of lipid molecules packed in the liposomes in the dispersions.

2. MATERIALS AND METHODS

2.1 Materials

Synthetic DL-α Phosphatidylcholine, dipalmitoyl (which hereafter abbreviated to DPPC or simply called lecithin) of 99 % purity was purchased from Sigma Chemical Company and was used without further purification. Ultra pure water was used throughout the experiments.

2.2 Preparation of samples

Dispersions of lecithin were prepared by sonicating the lecithin in 25 ml of water using an Artek Sonic Dismembrator 150 with a titanium probe operated at a fixed power setting of 70 % of maximum intensity for 35 min. During the sonication, the probe was maintained at a constant depth of about 1 mm beneath the surface of the sample held in the beaker, which was immersed in a water bath at room temperature (~25 °C). The concentrations of lecithin in the dispersions were 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml and 3 mg/ml (i.e. mass of lecithin in 25 ml of water).

2.3 Turbidity measurements

Measurements of turbidity of the lecithin-water dispersions were carried out using a UV-Visible Spectrophotometer (Shimadzu UV-1601 PC), at the wavelength scan that extended from 400 nm to 800 nm. From the turbidity measurements of the dispersions, the average diameters of the liposomes were estimated according to the approach of Pozharski et al. [24].

2.4 Density measurements
The densities of the lecithin-water dispersions were measured using a vibrating-tube Anton Paar Density Meter DMA 58, in the temperature range from 25 °C to 55 °C. The density meter is utilized to determine the oscillating periods that are automatically converted to liquid densities after calibrations. The proper calibrations of the density meter were achieved at each working temperature, with air and water as references. The accuracy of the density meter has been reported to be 1 part per 100,000 by Salleh et al. [25] and Maham et al. [26].

3. THEORETICAL CONSIDERATIONS

The density of the dispersion, \( \rho \) is expressed as

\[
\rho = \frac{w}{v} = \frac{w_l + w_w}{v_l + v_w}
\]  

(1)

where \( w \) is the value of total mass and \( v \) is the value of total volume; subscript ‘\( l \)’ represents the lecithin and subscript ‘\( w \)’ represents the water. To express the density of the dispersion in terms of more directly measurable parameters, Equation (1) can be written as

\[
\rho = \frac{\rho_w (1 + w_r)}{1 + v_r}
\]  

(2)

where \( \rho_w = \frac{w_w}{v_w} \) is the density of the water, \( w_r = \frac{w_l}{w_w} \) and \( v_r = \frac{v_l}{v_w} \) are the mass ratio and volume ratio of the lecithin to the water respectively. \( w_r \) is constant for a fixed mass of water and lecithin used. The density of the dispersion therefore, is dependent on the value of \( v_r \). The larger is \( v_r \), the smaller the density of the dispersion.

From Equation (2), the volume ratio can be written as

\[
v_r = \frac{\rho_w (1 + w_r) - \rho}{\rho}
\]  

(3)

Thus, the value of \( v_r \) can be calculated from the experimental value of density, \( \rho \), and known values of \( \rho_w \) and \( w_r \). The volume of lecithin in the dispersion is given by

\[
v_l = v_r \times v_w
\]  

(4)

This value can hence be calculated using the value of \( v_r \) from Equation (3) and the volume of the water, \( v_w \).

The total number of lecithin molecules, \( N_l \) present in the dispersion is given by

\[
N_l = \frac{w_l}{M_l} \times N_A
\]  

(5)

where \( M_l \) is the molecular weight of the lecithin and \( N_A \) is the Avogadro’s number. Knowing the value of \( N_l \), the expression for the effective volume of a lecithin molecule packed in the liposomes in the dispersion can finally be expressed as

\[
v_{\text{eff}} = \frac{v_l}{N_l}
\]  

(6)

which is dependent on the volume of lecithin in the dispersion. When the volume of lecithin changes, the effective volume occupied by a lecithin molecule in the dispersion would therefore change, while \( w_l \) is held constant.

Assuming that the above considerations are applicable, the density of the lecithin in the dispersion may also be derived from the relationship
\[ \rho_l = \frac{w_l}{v_l} \]  \hspace{1cm} (7)

where \( \rho_l \) is the density of the lecithin and is dependent on the experimental parameters of \( w_l \) and \( v_l \).

4. RESULTS AND DISCUSSION

4.1 Estimation of liposome sizes

Table 1 summarizes the average sizes of the liposomes generated in dispersions with different concentrations of lecithin. The estimate of 90 nm was found for the average diameters of the liposomes generated in dispersions with concentration of 1 mg/ml and 1.5 mg/ml. The average liposome diameter, however, increased to 120 nm as the concentration of lecithin in the dispersion was increased to 2 mg/ml. Further increases of concentration of lecithin in the dispersion to 2.5 mg/ml and 3 mg/ml caused a more pronounced increase of the average liposome diameter to 170 nm. This variation may be attributed to insufficient sonication process for dispersions with higher concentrations of lecithin.

4.2 Temperature dependence of density

The densities of the dispersions as a function of concentration of lecithin in the dispersion at different temperatures are shown in Table 2 and Figure 1. The densities of the dispersions show only a slight variation with concentration of lecithin. However, the densities of the dispersions show a more significant decrease with increase in temperature. It can be seen that the densities of the dispersions are only a little higher than that of the water. Since there is only a small change in density, the experimental density data for different lecithin dispersions will thus be presented in terms of a more appropriate parameter, which is the fractional change of density, given as

\[ \text{Fractional change of density} = \frac{\text{Density of lecithin dispersion} - \text{Density of water}}{\text{Density of water}} \]  \hspace{1cm} (8)

Temperature dependence of the fractional change of density for dispersions with different concentrations of lecithin is illustrated in Figure 2. It can be seen that a rather abrupt decrease occurred in the temperature range of 40 °C – 45 °C, which was observed as the gel to liquid-crystalline phase transition for the lecithin [19]. This reduction in density at the phase transition region may be attributed to the increase in the effective volume of the lecithin molecules in the liposomes in the dispersions. Note that the higher the concentration of lecithin in the dispersion, the greater the degree of the fractional change of density. This relationship is rather linear particularly for a temperature that is well below the transition temperature (see Figure 3). However, the results for a temperature beyond the transition temperature, the variation with different concentrations of lecithin in the dispersions is not so significant. This may imply that at higher temperatures, the liposomes are larger in size as a result of a more rigorous vibrational motion of the lecithin molecules in the dispersions. The effective volume of the lecithin molecules has increased to the extent such that the density of the lecithin itself is now only about the same as water.

4.3 Theoretical calculation of effective molecular volume
Figure 4 illustrates the volume of lecithin in the dispersions with different concentrations of lecithin over a temperature range of 25 °C to 55 °C. It can be seen that the volume of lecithin in the dispersion increases when the temperature is increased gradually through the phase transition. An obvious feature is a slight increase of the volume of lecithin in the dispersion near the transition temperature of 42 °C. These results are in agreement with the observation reported by Trauble and Haynes [27] that a volume change does accompany the phase transition. Further, as the concentration of lecithin in the dispersion is increased, the volume of lecithin in the dispersion significantly increases. This relationship is linear for temperatures below and beyond the transition temperature (see Figure 5).

The effective molecular volume of lecithin generated in the dispersions with different concentrations of lecithin is summarized in Table 3, which indicates that the effective molecular volume generally increases with increasing temperature. These results also show that the liposomes have undergone an expansion at the phase transition. This expansion can be attributed to a conformational change of the lecithin molecules packed in the liposomes in the dispersion [17]. As the temperature increases, the packing arrangement of the lecithin molecules in the liposomes becomes loosened gradually. Because of the trans to gauche conformational change of the lecithin molecules packed in the liposomes occurring at the phase transition, the effective molecular volume would be larger at temperatures beyond the phase transition than those below the phase transition. Another observation is that the values of the effective molecular volume are generally smaller for dispersions of higher concentrations of lecithin, particularly at a given temperature below the transition temperature. This implies the molecular packing of lecithin is closer for these dispersions with larger liposomes.

From the results of the above calculations, the density of the lecithin in the dispersion, $\rho_l$, can be determined from Equation (7), as the values of the mass and the volume of the lecithin in the dispersions are known. Table 4 summarizes the densities of the sonicated lecithin at temperatures below and beyond the transition temperature. The calculated density data at a room temperature of 25 °C are comparable to that obtained by Sheetz and Chan [6] as well as Chong and Colbow [7]. Nevertheless, slightly higher values are obtained for dispersions of higher concentrations of lecithin. This may imply that liposomes generated in these cases are larger and more closely packed. As expected, the density at the post-transition is only slightly higher than that of water.

From the results tabulated in Table 3 and Table 4, the average values of the density of the lecithin and the effective molecular volume of lecithin in the dispersions at two temperatures are found to be as follows:

\[
\begin{align*}
\rho_l \text{ (at 25 °C)} &= (1.0579 \pm 0.0014) \text{ g cm}^{-3} \\
\rho_l \text{ (at 50 °C)} &= (0.9961 \pm 0.0009) \text{ g cm}^{-3} \\
v_{\text{eff}} \text{ (at 25 °C)} &= (1.152 \pm 0.002) \text{ E-21 cm}^{3} \\
v_{\text{eff}} \text{ (at 50 °C)} &= (1.224 \pm 0.001) \text{ E-21 cm}^{3}
\end{align*}
\]

5. ACKNOWLEDGMENTS

The authors would like to acknowledge the short term research grant provided by Universiti Sains Malaysia.
6. REFERENCES


Figure 1: Density of lecithin dispersion as a function of concentration of lecithin in dispersion at different temperatures.
Figure 2: Temperature dependence of the fractional change of density for dispersions with different concentrations of lecithin.
Figure 3: Fractional change of density as a function of concentration of lecithin in dispersion at 25 °C and 50 °C.
Figure 4: Volume of lecithin in dispersion as a function of temperature for dispersions with different concentrations of lecithin.
Figure 5: Volume of lecithin in dispersion as a function of concentration of lecithin in dispersion at 25 °C and 50 °C.
Table 1: Size estimation of the liposomes generated in dispersions with different concentrations of lecithin

<table>
<thead>
<tr>
<th>Concentration of Lecithin in Dispersion (mg/ml)</th>
<th>Average Diameter (nm)</th>
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<tr>
<td>1.0</td>
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Table 2: Densities of lecithin dispersions at different temperatures

<table>
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<tr>
<th>Concentration of Lecithin in Dispersion (mg/ml)</th>
<th>Density of Lecithin Dispersion (g cm(^{-3})) at 25 °C</th>
<th>30 °C</th>
<th>35 °C</th>
<th>40 °C</th>
<th>45 °C</th>
<th>50 °C</th>
</tr>
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</table>

* Density of water as a comparison.

Table 3: Effective molecular volume of lecithin generated in dispersions with different concentrations of lecithin

<table>
<thead>
<tr>
<th>Concentration of Lecithin in Dispersion (mg/ml)</th>
<th>Effective Molecular Volume of Lecithin (cm(^3)) at 25 °C</th>
<th>30 °C</th>
<th>35 °C</th>
<th>40 °C</th>
<th>42 °C</th>
<th>45 °C</th>
<th>50 °C</th>
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Table 4: Densities of sonicated lecithin at 25 °C and 50 °C

<table>
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<th>Concentration of Lecithin in Dispersion (mg/ml)</th>
<th>(v_i) (cm(^3)) at 25 °C</th>
<th>(\rho_i) (g cm(^{-3})) at 25 °C</th>
<th>(v_i) (cm(^3)) at 50 °C</th>
<th>(\rho_i) (g cm(^{-3})) at 50 °C</th>
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