Methylene volumes in monoglyceride bilayers are larger than in liquid alkanes

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ABSTRACT

The densities as a function of temperature of four fully hydrated saturated monoglycerides with even chain lengths ranging from eight to fourteen were determined by vibrating tube densitometry and their phase transition temperatures were determined by differential scanning calorimetry (DSC). We find the volume of a methylene group in a monoglyceride bilayer is 2% larger than in liquid alkanes at physiological temperatures, similar to the methylene group volumes found in phosphatidylcholine (PC) bilayers. Additionally, we carefully consider the traditional method of calculating component volumes from experimental data and note potential difficulties in this approach. In the literature, the ratio of terminal methyl volume (CH₃) to methylene (CH₂) volumes is typically assumed to be 2. By analysis of literature alkane data, we find this ratio actually ranges from 1.9 to 2.3 for temperatures ranging from 0 °C to 100 °C. For a rough sense of scale, we note that to effect a 2% reduction in volume requires of order 200 atmospheres of pressure, and pressures of this magnitude are biologically relevant. For instance, this amount of pressure is sufficient to reverse the effect of anesthesia. The component volumes obtained are an important parameter used for determining the structure of lipid bilayers and for molecular dynamics simulations.

1. Introduction

Monoglycerides are a molecularly simple group of compounds that form a diverse, complex array of self-assembled structures with an even broader range of applications (Kulkarni et al., 2011). Encapsulation for drug delivery, scaffolds for protein crystallization, and nanoscale templates are some of the many uses for these compounds (Seddon, 2013). Monoglycerides have been used in food preparation for over 80 years (Wang and Marangoni, 2016), including, for instance, bread making (Hattori et al., 2015). Interestingly, they also are useful anti-microbial, anti-viral, anti-fungal and anti-yeast agents (Thormar and Hilmarsson, 2007; Ruzicka et al., 2003) and have been shown to prevent SIV (the simian version of HIV) transmission in monkeys (Li et al., 2009). The relatively simple nature of these compounds makes them quite appealing to molecular simulation efforts, both as a system worthy of investigation in itself and as a resource to determine force-field parameters for use in more complex systems (Laner et al., 2013).

In this paper, we study hydrated monocaprylin, monolaurin and monomyristin, which are medium chain length, saturated monoglycerides (Fig. 1). Despite promising initial structural X-ray and density measurements (Larsson, 1967; Krog and Larsson, 1968; Krog and Borup, 1973; Larsson and Krog, 1973; Lutton, 1971) there is a lack of systematic, fundamental measurements for hydrated, saturated monoglycerides (Wang and Marangoni, 2016). Moreover, there is a lack of density measurements for hydrated monoglycerides of all types (Reese et al., 2015). So, in more recent work, the density of an unsaturated monoglyceride is assumed to match that of a saturated glyceride with a different chain length (Pezron et al., 1990). In another work, the density of an unsaturated glyceride is simply posited (Nyame Mendeny Boussambe et al., 2017). The dictum “form follows function” is universally acknowledged by biophysicists and, indeed, the virucidal properties of monoglycerides are acknowledged to depend on structure (Thormar and Hilmarsson, 2007). Given the widespread use of these compounds, the biological importance of structure and their use as benchmark compounds in molecular modeling, we see a compelling need for systematic density measurements and begin to address that need with this work.
The samples were scanned at 0.5 °C intervals, equilibrating at each cooling scan from 40 or 50 °C to 25 or 10 °C, followed by a heating scan. The rate of changes, using this equation:

\[ \text{changes in the period of oscillation when the density of the solution and below. Between 2 and 8 samples were prepared for each lipid. The temperature is to avoid the clumping that occurs at room temperature.} \]

\[ \text{DMA 5000M density meter which was also held at 50 °C before the} \]

\[ \text{mix the sample. The vial was then placed in a 50 °C oven before being} \]

\[ \text{sealed vial was placed into an 80 °C oven until the lipid dispersed} \]

\[ \text{runs with significant leakage (> 0.1 mg of lost mass) were not used.} \]

\[ \text{The DSC was set to cycle between two temperatures at a set ramp} \]

\[ \text{cycles so that the lipid and water are first allowed to mix. We also} \]

\[ \text{data from long runs (runs consisting of greater than twenty cycles), and also anomalous data, such as transition peaks that appear during only the first cycle, or twin peaks that appear in a cycle or two of one scan but never again over the same region.} \]

\[ \text{2. Materials and methods} \]

\[ \text{2.1. Densitometry} \]

\[ \text{Densitometry was carried out on the four monoglycerides from Nu-} \]

\[ \text{Chek Prep, Inc. (Elysian, MN), each with purity > 99%. Samples of} \]

\[ \text{monolaurin and monomyristin were individually prepared by weighing} \]

\[ \text{2 g milliQ water into a 6 ml Nalgene vial using a} \]

\[ \text{gentle, manual swirling was used to mix the sample. The} \]

\[ \text{blank was placed into an 80 °C oven until the lipid dispersed} \]

\[ \text{runs with significant leakage (> 0.1 mg of lost mass) were not used.} \]

\[ \text{The DSC was set to cycle between two temperatures at a set ramp} \]

\[ \text{cycles so that the lipid and water are first allowed to mix. We also} \]

\[ \text{data from long runs (runs consisting of greater than twenty cycles), and also anomalous data, such as transition peaks that appear during only the first cycle, or twin peaks that appear in a cycle or two of one scan but never again over the same region.} \]

\[ \text{3. Results and discussion} \]

\[ \text{3.1. Phase behavior} \]

\[ \text{For the DSC scan for each phase transition (Fig. 2), we extracted the} \]

\[ \text{enthalpy, peak position (temperature) (Table 1) and full width at half} \]

\[ \text{maximum (FWHM), with the latter two quantities being dependent on} \]

\[ \text{the enthalpy of the transition is large and the hysteresis is small, the} \]

\[ \text{hysteresis might well be dominated by limitations in heat transfer to} \]

\[ \text{the intersection of these two lines (Fig. 3) (Cook et al., 2012; Reese et al., 2015; Toombes et al., 2002).} \]

\[ \text{The difference between the transition temperatures seen on heating} \]

\[ \text{and cooling is the hysteresis and both the hysteresis and the width of} \]

\[ \text{the transition depend on the ramp rate. Potentially, both the sample} \]

\[ \text{and the instrument can contribute to the hysteresis. For instance, if} \]

\[ \text{the enthalpy of the transition is large and the hysteresis is small, the} \]

\[ \text{the temperature is to avoid the clumping that occurs at room temperature.} \]

\[ \text{Between 2 and 8 samples were prepared for each lipid. The} \]

\[ \text{sealed vial was placed into an 80 °C oven until the lipid dispersed} \]

\[ \text{runs with significant leakage (> 0.1 mg of lost mass) were not used.} \]

\[ \text{The DSC was set to cycle between two temperatures at a set ramp} \]

\[ \text{cycles so that the lipid and water are first allowed to mix. We also} \]

\[ \text{data from long runs (runs consisting of greater than twenty cycles), and also anomalous data, such as transition peaks that appear during only the first cycle, or twin peaks that appear in a cycle or two of one scan but never again over the same region.} \]
Table 1
The phase transition temperatures to within 1 °C for medium chain mono-
glycerides. Wide DSC scans were conducted to determine the relative locations of
each phase transition, and then subsequent scans were done to analyze
specific transitions. As discussed in the text, the FWHM (full width half max-
imum) was strongly dependent on sample size and so is not reported. We note
that the $L_c \rightarrow L_a$ transition was seen on heating cycles only and the $L_a \rightarrow L_c$
transition was seen only on cooling cycles. The $L_a \rightarrow L_p$ transition was seen
on cooling, provided that the sample had been heated sufficiently to transition to
the $L_p$ phase, and then the $L_p \rightarrow L_a$ transition was seen on heating, provided
the sample had not been cooled below the $L_p \rightarrow L_c$ transition. Because the $L_p \rightarrow L_c$
transition for monocaprin was below the temperature range of our instrument,
we do not report an enthalpy for the $L_c \rightarrow L_a$ transition, as we are unable to
ensure that the sample was fully in the $L_c$ phase.

<table>
<thead>
<tr>
<th>Glyceride</th>
<th>Phase transition</th>
<th>$T_{p}$ (°C)</th>
<th>$\Delta H$ (kJ/mol)</th>
<th>$T_{c}$ (°C)</th>
<th>$\Delta H$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomyristin</td>
<td>$L_g \rightarrow L_c$</td>
<td>7.5</td>
<td>13</td>
<td>35.8</td>
<td>15</td>
</tr>
<tr>
<td>Monolaurin</td>
<td>$L_g \rightarrow L_a$</td>
<td>-5</td>
<td>12</td>
<td>17.2</td>
<td>15</td>
</tr>
<tr>
<td>Monocaprin</td>
<td>$L_c \rightarrow L_a$</td>
<td>-7.8</td>
<td>9</td>
<td>20.0</td>
<td>-</td>
</tr>
</tbody>
</table>

report the hysteresis and width data, as they are essentially reflections
of instrumental limitations. It is worth noting that on the other extreme,
for samples with large hysteresis and small enthalpies, one is not
straining the heat transfer capabilities of the instrument and these
quantities exhibit no sample size dependence. This is typically the case
for transitions involving non-lamellar phase(s) (Cook et al., 2012; Reese
et al., 2015; Toombs et al., 2002), where the hysteresis is much larger
and the enthalpies are an order of magnitude smaller; in these cases one
does not see sample size dependence. Consequently, in these cases, the
kinetics reflect the intrinsic nature of the phase transition.

3.2. Phase assignments

It is well known that medium and long chain length alkanes are
crystalline at low temperatures and undergo a major transition to ro-
tator phase(s) before making a final major melting transition to a fluid
(Cholakova and Denkov, 2019). The crystalline $\rightarrow$ rotator $\rightarrow$ liquid

phase sequence in alkanes is of necessity deeply connected to the
analogous $L_c \rightarrow L_q \rightarrow L_a$ phase sequence seen in membrane forming
lipids (Cholakova and Denkov, 2019; Lewis et al., 1987; Lewis and
McElhaney, 1993). Our assignment of phases is in agreement with this
framework, with the enthalpies being of appropriate magnitudes and
the increase of the temperature of a given phase transition increasing
with longer chain length as it should. That having been said, direct
evidence of these phase assignments is desirable; as it is for other
structural parameters, the literature is incomplete. However, we do
note that the $L_c$ and $L_a$ phases for monolaurin have been identified via
NMR (Lawrence and McDonald, 1966), with an $L_c \rightarrow L_a$ transition
temperature that matches that of our work to within a few degrees. The
NMR paper additionally mentions a procedure for generating a second
solid phase that transitions to the $L_a$ phase at about 15 °C. We would
identify this second solid phase as the $L_q$ phase; the procedure and the
reported $L_q \rightarrow L_a$ transition temperature are entirely consistent with our
results. It is also worth noting that at water concentrations above ap-
proximately 50%, as are our samples, the fluid phases swell, forming a
milky dispersion. With this swelling, the sharp X-ray diffraction lines go
away; however, there are still multi-lamellar vesicles, as evidenced by
characteristic Maltese crosses seen under polarized microscopy
(Larsson, 1967).

3.3. Volumes of alkanes and monoglycerides

The volumes of each monoglyceride were recorded over a tem-
perature range from 10 °C to 50 °C (Fig. 4). The transition from the $L_q$
to the $L_a$ phase is apparent for monomyristin and monolaurin. Monocaprin
was in the $L_a$ phase throughout the temperature range studied. The
situation for monocaprylin is more complex, as in excess water above
23 °C, it should be in the fluid isotropic phase and in the $L_a$ phase below
23 °C (Larsson, 1967). However, given the highly dilute samples used in
densiometry and the constant agitation, it is not clear which phase (or
phases) monocaprylin is in over this range. The volumes were fit to a
linear function

\[ V = V_0 + \frac{dV}{dT} T, \]

where $V$ is volume and $T$ is temperature, with $V_0$ and $\frac{dV}{dT}$ being fit

3
In a like manner, but to a lesser extent, the values of the linear function were solved to yield a linear dependence on chain length; monomyristin, for which we only obtained the $\Delta V$ for a range exceeding 20 °C, namely monocapryn, monocaprin and monolaurin. We note that the $\Delta V$ for these lipids follow a well-behaved pattern, and so we omit it from our analysis (Fig. 6).

From this point, we restrict ourselves to considering only melted alkanes and the monoglycerides for which we have melted phase data for a range exceeding 20 °C, namely monolaurin, monocaprin and monolaurin. We note that the $\Delta V$ for these lipids follow a well-behaved linear dependence on chain length; monomyristin, for which we only have melted phase data over a narrow temperature range, does not follow this pattern, and so we omit it from our analysis.

### 3.4. Alkane component volume analysis

The standard and often implicit assumption of component volume analysis is that the volumes of like components, say methylenes, are identical in a series of compounds of different chain length at a given temperature. We will call this the component volume assumption; other methods and assumptions have been recently introduced by some of us (Nagle et al., 2019), but the component volume assumption is the one we will consider in this paper. Consequently, in context of the component volume assumption, we model the volume of an alkane as

$$V_{\text{alkane}} = m V_{\text{CH}_2} + 2 V_{\text{CH}_3}$$

where $V_{\text{alkane}}$ is the volume of the alkane, $m$ is the number of methylenes, $V_{\text{CH}_2}$ is the methylene volume and $V_{\text{CH}_3}$ is the volume of a terminal methyl. Using this model to analyze original data from the American Petroleum Institute (API) (Rossini et al., 1953) and more modern results (Banipal et al., 1991), we see generally close agreement with our analysis of the API data. It is also interesting to include an analysis of a longer chain subset of the API data due to Yoshimura et al.

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**Table 2**

Volume data for the studied monoglycerides supplemented by alkane volume data from Banipal et al. (1991) The $V_0$ and $\frac{\Delta V}{\Delta T}$ were results of linear fits done for each lipid over the indicated temperature range, excluding data points where a lipid was transitioning from one phase to another. Plots of these quantities can be seen in Fig. 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chem. form.</th>
<th>Phase</th>
<th>Mol. wt.</th>
<th>Temp. range</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$V_0$</td>
</tr>
<tr>
<td>Octane</td>
<td>C$<em>8$H$</em>{18}$</td>
<td>Liquid</td>
<td>114.229</td>
<td>45–90</td>
<td>260.9</td>
</tr>
<tr>
<td>Nonane</td>
<td>C$<em>9$H$</em>{18}$</td>
<td>Liquid</td>
<td>128.225</td>
<td>45–90</td>
<td>288.0</td>
</tr>
<tr>
<td>Decane</td>
<td>C$<em>{10}$H$</em>{22}$</td>
<td>Liquid</td>
<td>142.282</td>
<td>50–100</td>
<td>314.5</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>C$<em>{12}$H$</em>{24}$</td>
<td>Liquid</td>
<td>226.441</td>
<td>45–100</td>
<td>475.1</td>
</tr>
<tr>
<td>Monocaprylin</td>
<td>C$<em>{13}H$</em>{26}O$_4$</td>
<td>b</td>
<td>218.293</td>
<td>10–40</td>
<td>345.8</td>
</tr>
<tr>
<td>Monolaurin</td>
<td>C$<em>{17}H$</em>{34}O$_4$</td>
<td>Liquid</td>
<td>246.343</td>
<td>25–50</td>
<td>398.9</td>
</tr>
<tr>
<td>Monomyristin</td>
<td>C$<em>{19}H$</em>{34}O$_4$</td>
<td>Liquid</td>
<td>274.401</td>
<td>10–14</td>
<td>426.9</td>
</tr>
<tr>
<td>Nonane</td>
<td>C$<em>{20}H$</em>{42}O$_4$</td>
<td>Liquid</td>
<td>302.455</td>
<td>17–40</td>
<td>452.2</td>
</tr>
<tr>
<td>Monocaprin</td>
<td>C$<em>{21}H$</em>{42}O$_4$</td>
<td>Liquid</td>
<td>36–50</td>
<td>500.6 0.592</td>
<td></td>
</tr>
</tbody>
</table>

* Our fit of the data from Banipal et al. (1991).
* The phase monocaprylin is in is uncertain; see text for details.
(1985). Our own analysis of this subset is in agreement with these results and we note that they demonstrate a deviation from the component volume assumption for alkanes at approximately the 0.5% level.

Pleasingly, the terminal methyl component volumes from Banipal et al. (1991) and Rossini et al. (1953) match up as well (Fig. 8), with the same minor discrepancies as noted before. One interesting result is the determination that the temperature dependence of the terminal methyl volume is much greater than that of the methylenes, with their volume ratio varying from 1.9 to 2.3 over 0 °C to 100 °C. The temperature dependence of this ratio was first noted by Yoshimura et al. (1985).

However, this ratio has been traditionally assumed to be fixed at 2 (see, for example, Uhríková et al., 2007). For lower temperatures, this is a reasonable assumption for the terminal methyl volume; it is less good at higher temperatures. It should be noted that this assumption is far more problematic when attempting to determine the temperature dependence of the terminal methyl volume. This is readily apparent when we consider the coefficient of thermal expansion $\alpha_{vol}$ which is

$$\alpha_{vol} = \frac{dV}{dT} \left( \frac{1}{V} \right).$$ (5)

Table 3 has the $V_0$ and $dV/dT$ values for alkane methylenes and the alkane terminal methyls (the remainder term in alkanes is composed of two terminal methyls). Using these values, for a methylene group, $\alpha_{vol}$ is $7 \times 10^{-4}/°C$; for the terminal methyls, $\alpha_{vol}$ is about three times larger, or $20 \times 10^{-4}/°C$.

### 3.5. Phosphatidylcholine component volumes

The volumes of phosphatidylcholine (PC) lipids are perhaps the best studied of the various lipid families. Uhríková et al. (2007) both made new measurements and incorporated numerous literature values in order to determine the temperature dependent methylene volume. They generally find good agreement between their work and the various literature sources, resulting in a PC methylene volume that is about 2% larger than the alkane methylene (Table 9 and Fig. 9). In order to put...
resulting in an inflated value.

The following is an effort to extract just the methylene volume temperature dependence and decouple it from the terminal methyl. Because the assumption that the terminal methyl volume is twice that of the methylene, the methylene volume, \( V_{CH_2}^U \), reported by Uhríková et al. (2007) is effectively

\[
V_{CH_2}^U = \left( \frac{1}{n_{CH_2} + 2n_{CH_3}} \right) (n_{CH_2} V_{CH_2} + n_{CH_3} V_{CH_3}),
\]

(7)

where \( n_{CH_2} \) and \( n_{CH_3} \) are the number of methylenes and terminal methy-" indulge "s respectively and \( V_{CH_2} \) and \( V_{CH_3} \) are the actual volumes of these groups.

Taking the derivative with respect to temperature and solving for \( \frac{dV_{CH_2}}{dT} \) yields

\[
\frac{dV_{CH_2}}{dT} = \left( 1 + 2 \left( \frac{n_{CH_3}}{n_{CH_2}} \right) \right) \frac{dV_{CH_2}}{dT} - \left( \frac{n_{CH_3}}{n_{CH_2}} \right) \frac{dV_{CH_3}}{dT}.
\]

(8)

Since Uhríková et al. studied PC lipids with varying chain lengths, we opt to take the average ratio of methyl groups to methylene groups across the lipids studied in that work, which is \( \frac{n_{CH_3}}{n_{CH_2}} \approx 13 \). We approximate the value of \( \frac{dV_{CH_3}}{dT} \) by using the result from our analysis of alkane data. The resulting value for \( \frac{dV_{CH_3}}{dT} \) is 0.026 \( \lambda^3/\text{C} \), which is a substantial reduction from \( \frac{dV_{CH_2}}{dT} \), which is 0.032 \( \lambda^3/\text{C} \). Additionally, we note that Uhríková et al. (2007) also conventionally assumed a fixed PC headgroup volume. It turns out the question of what the headgroup does is quite subtle; the interested reader is directed toward Nagle et al. (2019) for an in depth approach to this question.

3.7. Monoglyceride component volume analysis

One can also model monoglycerides utilizing the component volume assumption, with each component assumed to follow a linear dependence on temperature. In the case of saturated monoglycerides there are three components: methylenes, a terminal methyl and a glycerol head group. We group the glycerol head group and the terminal methyl into a remainder volume term, which means that the equation for the volume of a saturated monoglyceride is

\[
V_{MG} = n_{CH_2}V_{CH_2} + V_{rem},
\]

(9)

where \( V_{MG} \) is the total volume of the monoglyceride, \( n_{CH_2} \) is the number of methylene groups, \( V_{CH_2} \) is the volume of one methylene group and \( V_{rem} \) is the remainder volume of the terminal methyl group and the glycerol head group. It should be noted that this equation can also be applied to alkanes; there the \( V_{rem} \) term would correspond to the two terminal methyls.

Fitting our \( L_m \) MG data for monocaprylin, monocaprin and monolaurin to this equation, we find that \( V_0 \) and \( dV/dT \) are respectively 26.72 \( \lambda^3 \) and 0.030 \( \lambda^3/\text{C} \) for the methylene groups and 185.56 \( \lambda^3 \) and 0.096 \( \lambda^3/\text{C} \) for the remainder group (Table 3). The fit is excellent, with the data deviating from the fit by less than 1 \( \lambda^3 \) at all points.

With these results in hand, we can estimate the density of monolein, an 18 carbon tail length MG with one cis double bond. Utilizing component volumes from the fit of our MG data and a constant value of \( V_{C=C} = 45 \lambda^3 \) for the double bond component (Arun et al., 1998), we should have

\[
V_{monolein} = V_{rem} + 14V_{CH_2} + V_{C=C}.
\]

(10)

For monolein, this results in a \( V_0 = 604.6 \lambda^3 \) and a \( dV/dT = 0.516\lambda^3/\text{C} \). Alternatively, one could estimate \( V_{monolein} \) by

\[
V_{monolein} = V_{monopalmitin(extrapolated)} + V_{C=C}.
\]

(11)

where \( V_{monopalmitin(extrapolated)} \) is found by extrapolating from the volumes of monocaprylic, monocaprin and monolaurin. Both methods give effectively the same result, with the volumes matching to well within 0.1

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Fig. 9. The volume per methylene group as a function of temperature. Methylene volumes from different lipid systems are color coded. Alkane, PC (phosphatidylcholine), MG (monoglyceride) and PE (phosphatidylethanolamine) are, respectively, red, black, blue, and green. *Data from Yang et al. (1986), as quoted by Marsh (2010); *Data from Schmidt and Knoll (1985) as quoted by Marsh (2010). *Data from Table 2 in Uhríková et al. (2007).* Adjust-justment to Uhríková’s data without the assumption \( V_{CH_2} = 2V_{CH_3} \) (see Section 3). *Data from Koyanova and Hinz (1990) as quoted by Marsh (2010). An interesting side point is this lone PE data point; it is not immediately clear how to interpret this point, except to note that further investigation into PE systems is certainly merited.

this difference in context, it is useful to consider how much pressure is required to effect a volume change, namely

\[
dP = -k \frac{dV}{V_0},
\]

(6)

where \( dP \) is the pressure change, \( K \) is the bulk modulus and \( dV/V_0 \) is the fractional change in volume. Assuming a bulk modulus of order \( 10^9 \) Pa, typical for organic fluids, we get that a pressure increase of \( 2 \times 10^9 \) Pa or 200 atmospheres is required to accomplish a 2% reduction in volume. Now, in our case, the methylene volume is 2% larger, but this nonetheless gives a sense of scale of the stresses that must be in play. Heimburg and Jackson (2007) and references therein note that pressure changes of this magnitude are comparable to those required to reverse anesthesia, suggesting that this volume difference is potentially biologically significant and may even have relevance to the unsolved question of the mechanism of anesthesia.

It is also worthwhile to consider the rate of the methylene volume change as function of temperature. Uhríková et al. (2007) has a rate of 0.032 \( \lambda^3/\text{C} \), which is a good deal larger than the rate of 0.018 \( \lambda^3/\text{C} \) seen for alkanes (Table 3). It could be that the rates are indeed radically different in the different systems, but we think other considerations would bring them into closer alignment. The literature concerning component volumes of lipids (Marsh, 2010; Koenig and Gavrisch, 2005; Uhríková et al., 2007) assumes the ratio \( V_{CH_2}/V_{CH_3} \) to be a constant 2. As noted earlier, this is a reasonable assumption at lower temperatures that starts to break down at higher temperatures. However, the assumption is much more problematic when attempting to determine the temperature dependence of the methylene group volume since, as we have shown earlier, the coefficient of thermal expansion for a terminal methyl is three times greater than that of a methylene group.

3.6. Phosphatidylcholine component volumes – adjusted

Therefore, it would be good to re-evaluate the Uhríková et al. (2007) results since they, as has been conventional, assumed a fixed methylene/terminal methyl ratio of 2 and also considered a fixed ratio of 1.9. Consequently the large temperature dependence of the terminal methyl group gets folded into their result for the methylene group,
With a little bit of algebraic manipulation, we end up with

\[
\alpha V m \approx - dV m / dT \approx (m + 1) \frac{\alpha V_m}{T} \left( \frac{dV_m}{dT} \right)
\]

where \( m \) is the number of chain lengths. This work has carried out both differential scanning calorimetry and vibrating tube densitometry in an effort to obtain precise thermal and volume information for a series of monoglycerides of different chain lengths between 8 and 14 carbons. By decomposing the lipid component volumes as a function of temperature, we find that the volume of a CH₃ group in a monoglyceride bilayer is 2% larger than in liquid alkanes. We have also shown by means of a simple model that when the average methylene volume is chain length dependent, the traditional group component method can generate artifacts, including a methylene volume that is shifted off the actual volume. In alkanes, this shift is about 0.5% and was first noted by Yoshimura et al. (1985). Note that this shift is relevant in determining the actual volume, but does not directly impact the diﬀerence between the bilayer volume and alkane volume, as both volumes should be shifted by comparable amounts by the group method. In interpreting the temperature dependent slope of the methylene volumes determined by the group method, it is important to realize that it can be oﬀ by as much as 40%. Consequently, it could be the case that the temperature dependent slope of the actual average methylene volumes in bilayers might well be similar to that in

\[
\frac{dV_{CH2\text{ToyActual}}}{dT} \approx \frac{dV_{CH2\text{ToyActual}}}{dT} - (m + 1) \frac{\alpha V_m}{T} \left( \frac{dV_m}{dT} \right)
\]
alkanes, despite the apparent difference yielded by the group method. By analyzing the literature alkane data, we find that the ratio of volumes of CH₂/CH₃ ranges from 1.9 to 2.3 for temperatures ranging from 0 °C to 100 °C. The 2% larger average methylene volumes in monoglyceride and PC bilayers as compared to liquid alkanes is noteworthy, as pressures of hundreds of atmospheres are required to shift volumes by several percent. Future investigative efforts will determine if this is a general feature of lipid bilayers and will examine the effects of this difference on the many biological compounds present in cell membranes.

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