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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 (CH_3) to methylene (CH_2) 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 monocaprilyn, monocaprin, 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 Mendendy 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.

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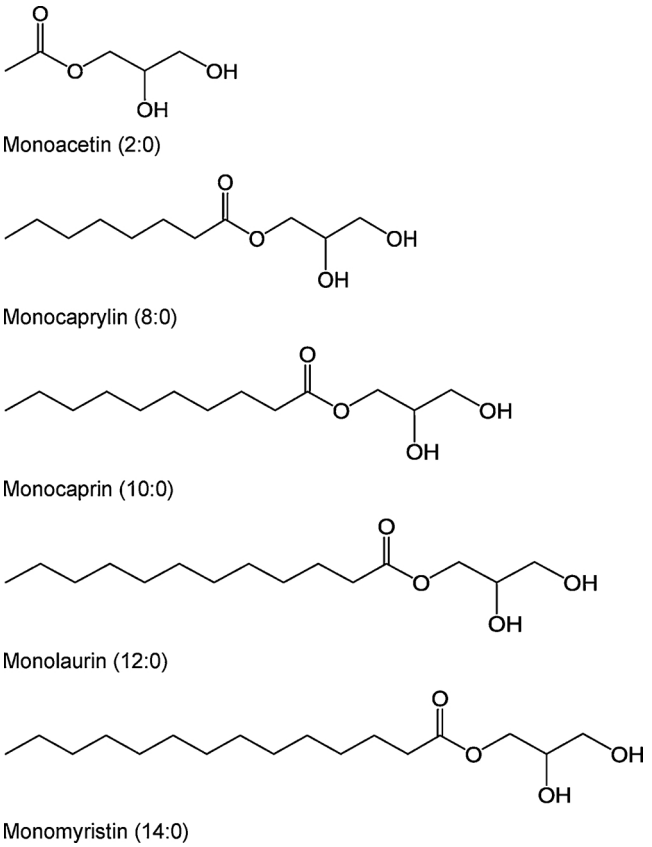

Fig. 1. The structures of the monoglycerides studied in this work as well as monoacetin, which is a monoglyceride with no methylenes in the tail. The names of the compounds along with their hydrocarbon tail length are also given.

2. Materials and methods

2.1. Densitometry

Densitometry was carried out on the four monoglycerides from Nu-Chek Prep, Inc. (Elysian, MN), each with purity > 99%. Samples of monolaurin and monomyristin were individually prepared by weighing ~10 mg lipid with ~2 g milliQ water into a 6 ml Nalgene vial using a Mettler AE 163 analytical balance, with an accuracy of 0.1 mg. Samples of monocaprilyn and monocaprin were prepared in a like manner, but with ~50 mg of lipid being mixed with 1.3 g of milliQ water. Each sealed vial was placed into an 80 °C oven until the lipid dispersed without clumping (10–15 min). Gentle, manual swirling was used to mix the sample. The vial was then placed in a 50 °C oven before being loaded into a 1 ml BD syringe (Becton, Dickinson & Co., Franklin Lakes, NJ) also held at 50 °C. The sample was then loaded into the Anton-Paar DMA 5000M density meter which was also held at 50 °C before the first cooling scan. The reason for maintaining these monoglycerides at high temperature is to avoid the clumping that occurs at room temperature and below. Between 2 and 8 samples were prepared for each lipid. The Anton-Paar DMA 5000M density meter uses a vibrating tube to sense changes in the period of oscillation when the density of the solution changes, using this equation: $\rho_{sample} - \rho_{air} = \kappa(\tau_{sample} - \tau_{air})^2$, where κ is an instrumental constant that depends on atmospheric pressure and room temperature.

The samples were then scanned multiple times each, starting with a cooling scan from 40 or 50 °C to 25 or 10 °C, followed by a heating scan. The samples were scanned at 0.5 °C intervals, equilibrating at each temperature before the density was recorded. Samples were heated at a rate of ~12 °C/h, and cooled at ~4 °C/h. The molecular volume V_L was calculated using the equation (Hallinen et al., 2012)

$$V_L = \frac{\mathrm{MW}_L}{0.6022\rho_s}\left[1 + \frac{m_W}{m_L}\left(1 - \frac{\rho_s}{\rho_W}\right)\right], \quad (1)$$

where MW_L = lipid molecular weight, ρ_s = density of the lipid solution, ρ_W = density of water, m_W = mass of water and m_L = mass of lipid. Using this method, the result from a small, precise weight percent (0.5% for monolaurin and monomyristin, 4.3% for monocaprilyn and monocaprin) is extrapolated to 100% to determine the lipid molecular volume. After each scan, the instrument was cleaned with ethanol followed by copious washing with water. Measurements of the density of pure water served as a check on the cleaning process.

2.2. Differential scanning calorimetry

The monoglycerides monomyristin (14:0), monolaurin (12:0), monocaprin (10:0), and monocaprylin (8:0) were obtained from Nu-Chek Prep, Inc. (Elysian, MN), and used without further purification. Differential scanning calorimetry was used to study the lamellar phase transitions of these lipids. Lipid-water samples with greater than 70% deionized water by weight were made in aluminum Tzero pans from TA instruments (New Castle, DE). Both lipid and deionized water were transferred to pans using a 10 µL Drummond Microdispenser (Broomhall, PA), and samples were subsequently closed by sealing aluminum lids to pans using a Tzero Press. Samples were made using 0.5–2 mg of lipid and 7–8.5 mg of deionized water. Samples were then placed in a model Q20 Differential Scanning Calorimeter (DSC) from TA instruments for temperature scanning. Before and after the scans, samples were weighed, to ensure that there was no leakage. Data from runs with significant leakage (> 0.1 mg of lost mass) were not used.

The DSC was set to cycle between two temperatures at a set ramp rate. Temperature limits were set between −40 and 80 °C depending on which transition was studied. Cycles were repeated up to 4 times at ramp rates of 0.2, 0.1, 0.05, and 0.02 °C/s. Following the completion of a test, the data recorded from the DSC was then analyzed using TA Universal Analysis 2000 software. We exclude data from the initial cycles so that the lipid and water are first allowed to mix. We also exclude 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.

3. Results and discussion

3.1. Phase behavior

For the DSC scan for each phase transition (Fig. 2), we extracted the enthalpy, peak position (temperature) (Table 1) and full width at half maximum (FWHM), with the latter two quantities being dependent on the ramp rate. In order to determine the equilibrium phase transition temperature we take the peak transitions of heating and cooling for all the ramp rates from a given test and then plot $T_{heating}$ vs. $T_{cooling}$ to a linear fit. We then plot the line $T_{heating} = T_{cooling}$ on the same graph, and the phase transition temperature is the intersection of these two lines (Fig. 3) (Cook et al., 2012; Reese et al., 2015; Toombes et al., 2002).

The difference between the transition temperatures seen on heating and cooling is the hysteresis and both the hysteresis and the width of the transition depend on the ramp rate. Potentially, both the sample and the instrument can contribute to the hysteresis. For instance, if the enthalpy of the transition is large and the hysteresis is small, the hysteresis might well be dominated by limitations in heat transfer to and from the sample by the DSC, as opposed to intrinsic properties of the material. In this case, one should observe that hysteresis and transition widths should increase with sample size. This is indeed what we observe for the main transitions for these compounds and so we do not

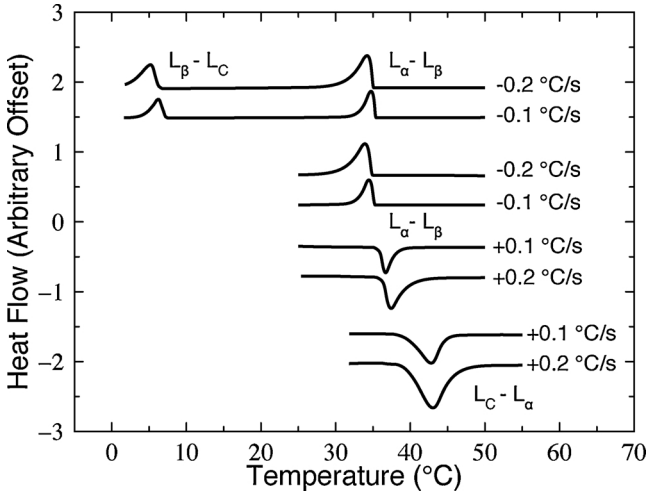

Fig. 2. DSC scans for monomyristin in excess water. An arbitrary vertical offset for each scan has been added for visual clarity. The phase transition is indicated to the right of each peak. Ramp rates for each scan are to the right of each scan. Negative ramp rates denote cooling scans and positive ramp rates denote heating scans.

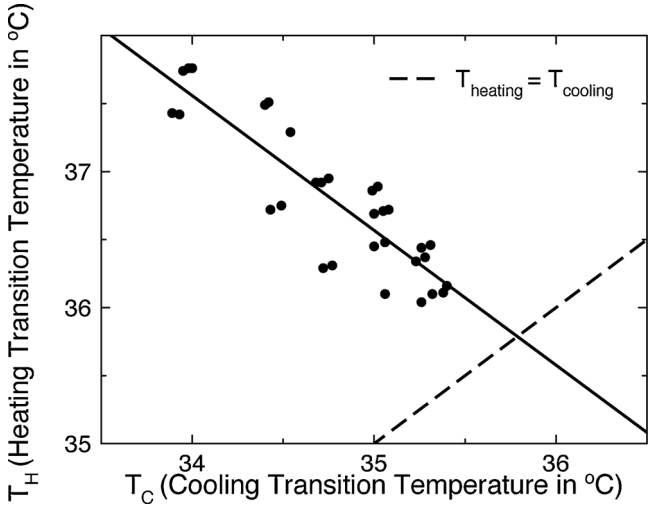

Fig. 3. Transition temperature data for various cycles of a monomyristin sample. The sample was heated and cooled at rates of 0.2, 0.1, 0.05, and 0.02 °C/s. Data are fit with a line for the transition temperature on heating as a function of transition temperature on cooling. As we ramp the sample more slowly, the hysteresis approaches zero, such that we would expect to find the same transition temperature on both heating and cooling, were we to ramp infinitely slowly. This is the equilibrium transition temperature, and it is found at the intersection of the dashed line of best fit with the line $T_{heating} = T_{cooling}$.

Table 1
The phase transition temperatures to within 1 °C for medium chain monoglycerides. 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 maximum) was strongly dependent on sample size and so is not reported. We note that the $L_C \rightarrow L_\alpha$ transition was seen on heating cycles only and the $L_\beta \rightarrow L_C$ transition was seen only on cooling cycles. The $L_\alpha \rightarrow L_\beta$ transition was seen on cooling, provided that the sample had been heated sufficiently to transition to the L_α phase, and then the $L_\beta \rightarrow L_\alpha$ transition was seen on heating, provided the sample had not been cooled below the $L_\beta \rightarrow L_C$ transition. Because the $L_\beta \rightarrow L_C$ transition for monocaprin was below the temperature range of our instrument, we do not report an enthalpy for the $L_C \rightarrow L_\alpha$ transition, as we are unable to ensure that the sample was fully in the L_C phase.

Glyceride	Phase transition					
	$L_\beta \rightarrow L_C$		$L_\beta \leftrightarrow L_\alpha$		$L_C \rightarrow L_\alpha$	
	T_o	ΔH	T_o (°C)	ΔH	T_o (°C)	ΔH
Units	(°C)	(kJ/mol)	(°C)	(kJ/mol)	(°C)	(kJ/mol)
Monomyristin	7.5	13	35.8	15	42.0	48
Monolaurin	−5	12	17.2	15	33.5	33
Monocaprin	–	–	−7.8	9	20.0	–

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; Toombes 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 rotator phase(s) before making a final major melting transition to a fluid (Cholakova and Denkov, 2019). The crystalline → rotator → liquid phase sequence in alkanes is of necessity deeply connected to the analogous $L_C \rightarrow L_\beta \rightarrow L_\alpha$ 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_α phases for monolaurin have been identified via NMR (Lawrence and McDonald, 1966), with an $L_C \rightarrow L_\alpha$ 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_α phase at about 15 °C. We would identify this second solid phase as the L_β phase; the procedure and the reported $L_\beta \rightarrow L_\alpha$ transition temperature are entirely consistent with our results. It is also worth noting that at water concentrations above approximately 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 temperature range from 10 °C to 50 °C (Fig. 4). The transition from the L_β to the L_α phase is apparent for monomyristin and monolaurin. Monocaprin was in the L_α phase throughout the temperature range studied. The situation for monocaprilyn is more complex, as in excess water above 23 °C, it should be in the fluid isotropic phase and in the L_α phase below 23 °C (Larsson, 1967). However, given the highly dilute samples used in densitometry and the constant agitation, it is not clear which phase (or phases) monocaprilyn is in over this range. The volumes were fit to a linear function

$$V = V_0 + \frac{dV}{dT}T, \tag{2}$$

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

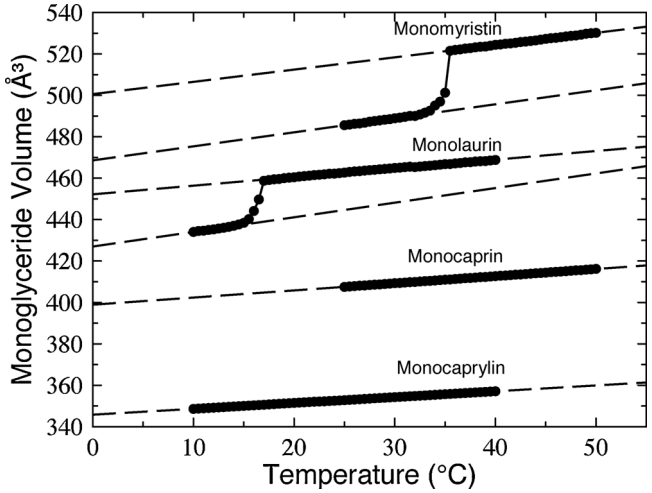

Fig. 4. Volumes of the monoglycerides as a function of temperature. Each curve is labeled with the name of the corresponding lipid. A linear function was fit to each lipid, excluding data points where a phase transition was occurring. The values of the linear fits can be seen in Table 2. For monomyristin and monolaurin two lines were fit with the lower line corresponding to when the lipid is in the L_β phase and the upper line corresponding to when the lipid is in the L_α phase. Monocaprin was in the L_α phase over the temperature range scanned. It is unclear which phase or mixture of phases monocaprylin is in; see text for details.

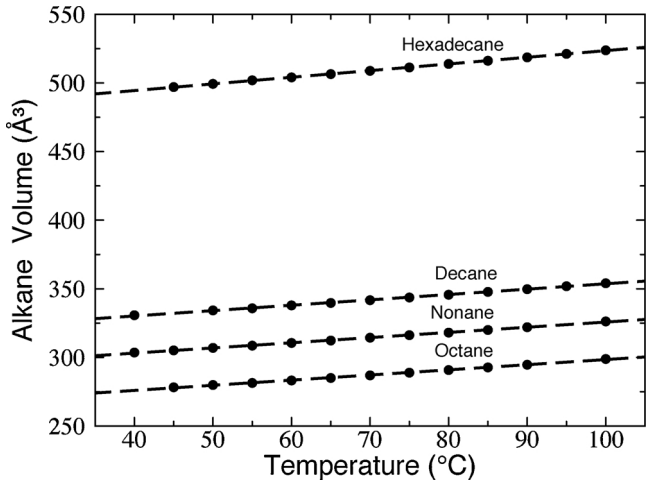

Fig. 5. Volumes of alkanes from Banipal et al. (1991) as a function of temperature. Each curve is labeled with the name of the corresponding lipid. A linear function was fit to each lipid and the values of the linear fits can be seen in Table 2. All of the alkanes were in the liquid phase.

constants. In addition to our data, we also fit alkane data from Banipal et al. (1991) (Figs. 4 and 5 and Table 2 for plots of the data and the values of the fit constants). In general, the data were very well fit by a linear function, with the minor caveat that the narrow temperature range of data for the L_β phase of monolaurin necessarily means that the $\frac{dV}{dT}$ value is less reliable for this phase. In a like manner, but to a lesser degree, the $\frac{dV}{dT}$ value for monomyristin in the L_α phase is also less reliable than those values for the other monoglycerides.

Given that we have both volumetric and enthalpic data, we can utilize the Clausius-Clapeyron equation to find how the melting temperature depends on pressure. The Clausius-Clapeyron equation can be solved to yield

$$dT_m/dP = T_m \Delta V/\Delta H, \quad (3)$$

where dT_m/dP is the rate of change of the melting transition ($L_\beta \rightarrow L_\alpha$)

Table 2
Volume data for the studied monoglycerides supplemented by alkane volume data from Banipal et al. (1991) The V_0 and $\frac{dV}{dT}$ 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.

Compound	Chem. form.	Phase	Mol. wt.	Temp. range	Volume	
					V_0	$\frac{dV}{dT}$
Units			(g/mol)	(°C)	(Å^3)	(Å^3/°C)
Octane[a]	C_8H_{18}	Liquid	114.229	45–90	260.9	0.375
Nonane[a]	C_9H_{20}	Liquid	128.225	40–90	288.0	0.377
Decane[a]	$C_{10}H_{22}$	Liquid	142.282	50–100	314.5	0.391
Hexadecane[a]	$C_{16}H_{34}$	Liquid	226.441	45–100	475.1	0.485
Monocaprylin (8:0)	$C_{11}H_{22}O_4$	[b]	218.293	10–40	345.8	0.282
Monocaprin (10:0)	$C_{13}H_{26}O_4$	L_α	246.343	25–50	398.9	0.345
Monolaurin (12:0)	$C_{15}H_{30}O_4$	L_β	274.401	10–14	426.9	0.706
		L_α		17–40	452.2	0.419
Monomyristin (14:0)	$C_{17}H_{34}O_4$	L_β	302.455	25–32	468.6	0.677
		L_α		36–50	500.6	0.592

[a] Our fit of the data from Banipal et al. (1991).
[b] The phase monocaprylin is in is uncertain; see text for details.

as a function of pressure P, T_m is the melting transition temperature, ΔV is the change in volume and ΔH is the enthalpy. For monomyristin's $L_\beta \rightarrow L_\alpha$ transition, $\Delta V = 30$ Å^3/molecule (Fig. 4), $T_m = 35.8$ °C and $\Delta H = 15$ kJ/mole (Table 1). Incidentally, the ΔV of monomyristin matches the volume of a single water molecule. Using the above values, we find $dT_m/dP = 37$ K/kbar for monomyristin. This is of the same order, but higher, than the values for PC and PE lipids, which range from 21 K/kbar to 28 K/kbar, depending on chain length and head group.

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 monocaprilyn, monocaprin and monolaurin. We note that the $\frac{dV}{dT}$ 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 (Fig. 6).

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

$$V_{\text{alkane}} = mV_{\text{CH2}} + 2V_{\text{CH3}} \quad (4)$$

where V_{alkane} is the volume of the alkane, m is the number of methylenes, V_{CH2} is the methylene volume and V_{CH3} 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 in the methylene volumes obtained (Fig. 7), with only minor discrepancies at 40 °C and 100 °C. For completeness, we also show the methylene volumes also ultimately calculated from API data (see figure caption for details) by Koenig and Gawrisch (2005), which also agree 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.

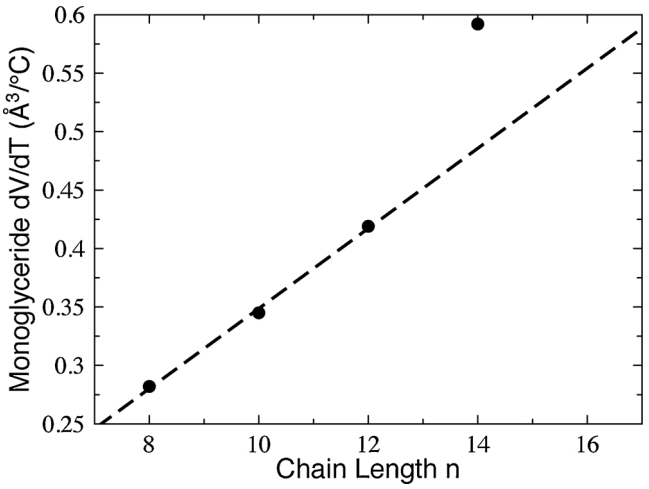

Fig. 6. Rates of volumetric change due to temperature for the monoglycerides in fluid phases. Note the rates for monocaprilyn, monocaprin and monolaurin exhibit a regular, linear dependence on chain length, as shown by the dashed line, which is a fit to these data points. The measured rate for monomyristin falls well outside this pattern; we believe this is in part due to the relatively limited temperature range of data we have for monomyristin, as compared with the other monoglycerides.

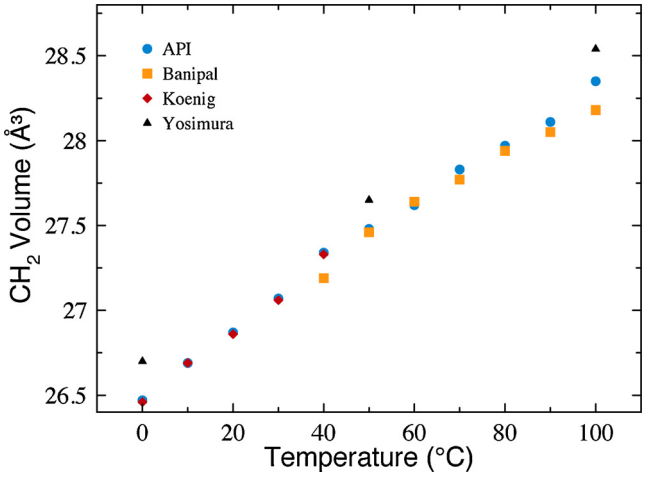

Fig. 7. Methylene volumes for alkanes as determined utilizing the component volume assumption. Note the generally excellent agreement between the venerable American Petroleum Institute (API) Research Project results (Rossini et al., 1953) and more modern results performed with vibrating tube densitometry (Banipal et al., 1991). These are the only two original data sets shown. For reference, we also show Koenig's calculated values (Koenig and Gawrisch, 2005), which use alkane data (Small, 1986) that ultimately originated from the API (Rossini et al., 1953). Apart from minor rounding differences, Koenig and Gawrisch's results line up exactly, as expected, with our analysis of the API data. We also include Yoshimura et al.'s analysis (Yoshimura et al., 1985) of a *longer chain subset* of the API results, showing that there is a slight chain length dependence in the methylene volume.

(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).

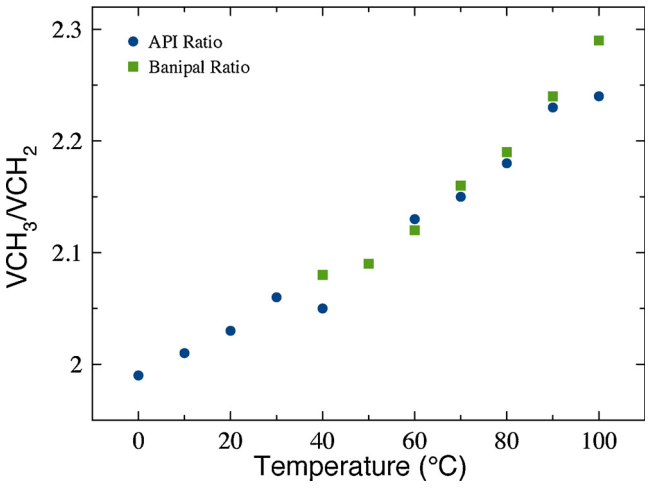

Fig. 8. The ratio of linear fits for the volumes of methyls to methylenes from our fit of the alkanes from Banipal et al. as a function of temperature. A common assumption in the literature is that the volume of a methyl group is twice the volume of a methylene. At lower temperatures this is a reasonable approximation, but breaks down at higher temperatures.

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 α_{vol}, which is

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

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, α_{vol} is 7×10^{-4}/°C; for the terminal methyls, α_{vol} is about three times larger, or 20×10^{-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

Table 3
Methylene and remainder component volumes for alkane, PC and monoglyceride systems. A plot of this data can be seen in Fig. 9.

Compound	Temp. (°C)	CH_2		Remainder	
Units		V_0 (Å^3)	$\frac{dV}{dT}$ (Å^3/°C)	V_0 (Å^3)	$\frac{dV}{dT}$ (Å^3/°C)
Alkanes[a]	0–100	26.52	0.018	104.73	0.218
PC (Uríková)[b]	20–40	26.71	0.032	*	*
PC (Uríková)[c]	20–40	26.90	0.026	*	*
Glycerides (L_α)[d]	10–50	26.72	0.030	185.56	0.096

[a] Our fit of the alkane data from the API (Rossini et al., 1953).
[b] Data from Table 2 in Uhríková et al. (2007).
[c] Adjustment to Uríková's data without the assumption $V_{CH3} = 2V_{CH2}$ (see text).
[d] Fit of our measurements of monocaprilyn, monocaprin and monolaurin.

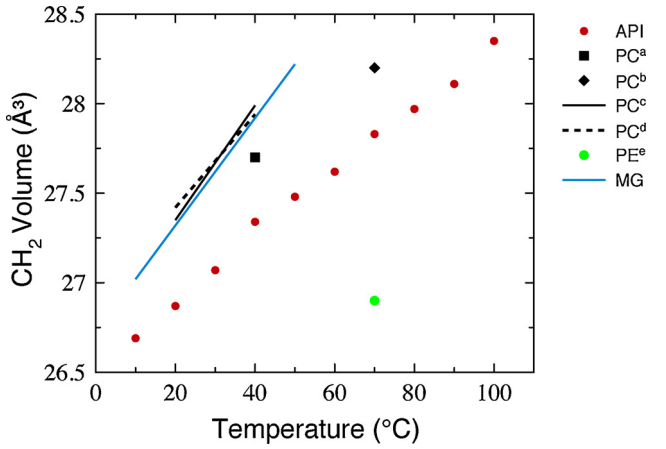

Fig. 9. The volume per methylene group as a function of temperature. Methylene volumes from different lipid systems are color coded. Alkane, PC (phosphatidylcholine), MG (monoglyceride) and PE (phosphatidylethanolamine) are, respectively, red, black, blue, and green. [a]Data from Yang et al. (1986), as quoted by Marsh (2010). [b]Data from Schmidt and Knoll (1985) as quoted by Marsh (2010). [c]Data from Table 2 in Uhríková et al. (2007) [d]Adjustment to Uríková's data without the assumption $V_{CH3} = 2V_{CH2}$ (see Section 3). [e]Data from Koynova 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

$$dP = -K\frac{dV}{V_0}, \tag{6}$$

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 2×10^7 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 Å^3/°C, which is a good deal larger than the rate of 0.018 Å^3/°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 Gawrisch, 2005; Uhríková et al., 2007) assumes the ratio V_{CH3}/V_{CH2} 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, 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^U_{CH2}, reported by Uhríková et al. (2007) is effectively

$$V^U_{CH2} = \left(\frac{1}{n_{CH2} + 2n_{CH3}}\right)(n_{CH2}V_{CH2} + n_{CH3}V_{CH3}), \tag{7}$$

where n_{CH2} and n_{CH3} are the number of methylenes and terminal methyls respectively and V_{CH2} and V_{CH3} are the actual volumes of these groups.

Taking the derivative with respect to temperature and solving for $\frac{dV_{CH2}}{dT}$ yields

$$\frac{dV_{CH2}}{dT} = \left(1 + 2\left(\frac{n_{CH3}}{n_{CH2}}\right)\right)\frac{dV^U_{CH2}}{dT} - \left(\frac{n_{CH3}}{n_{CH2}}\right)\frac{dV_{CH3}}{dT}. \tag{8}$$

Since Uríková 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 $\frac{n_{CH2}}{n_{CH3}} \approx 13$. We approximate the value of $\frac{dV_{CH3}}{dT}$ by using the result from our analysis of alkane data. The resulting value for $\frac{dV_{CH2}}{dT}$ is 0.026 Å^3/°C, which is a substantial reduction from $\frac{dV^U_{CH2}}{dT}$, which is 0.032 Å^3/°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

$$V_{MG} = n_{CH2}V_{CH2} + V_{rem}, \tag{9}$$

where V_{MG} is the total volume of the monoglyceride, n_{CH2} is the number of methylene groups, V_{CH2} 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_α MG data for monocaprilyn, monocaprin and monolaurin to this equation, we find that V_0 and dV/dT are respectively 26.72 Å^3 and 0.030 Å^3/°C for the methylene groups and 185.56 Å^3 and 0.096 Å^3/°C for the remainder group (Table 3). The fit is excellent, with the data deviating from the fit by less than 1 Å^3 at all points.

With these results in hand, we can estimate the density of monoolein, 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 $V_{C=C} = 45$ Å^3 for the double bond component (Armen et al., 1998), we should have

$$V_{\text{monoolein}} = V_{rem} + 14V_{CH2} + V_{C=C}. \tag{10}$$

For monoolein, this results in a $V_0 = 604.6$ Å^3 and a $\frac{dV}{dT} = 0.516$Å^3/°C. Alternatively, one could estimate $V_{monoolein}$ by

$$V_{\text{monoolein}} = V_{\text{monopalmitin(extrapolated)}} + V_{C=C}, \tag{11}$$

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

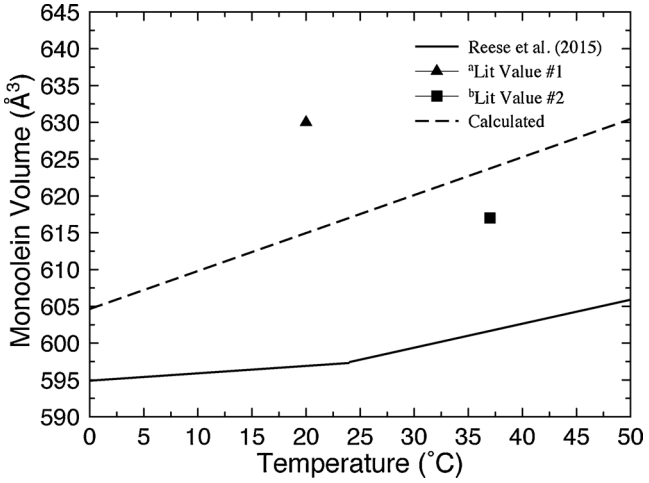

Fig. 10. Experimental and estimated temperature dependent volumes of monoolein. The solid line is from an earlier experimental result of ours (Reese et al., 2015). Our estimated volume (see text) is shown as a dashed line. On average, our earlier experimental result is about 3% lower than our current estimated volume. [a] Lit. value #1 from Kraineva et al. (2005). [b] Lit. value #2 from Vacklin et al. (2000).

%. A plot of the estimated density and the results of experimental measurements is shown in Fig. 10. We note that this calculated result conflicts with an earlier measurement of ours (Reese et al., 2015).

3.8. Toy model of methylene volumes

Physically, the decrease in methylene volume with increasing chain length can be readily understood as a consequence of the more efficient packing of longer chains. However, it is not immediately clear why utilizing the component volume assumption gives results that appear to indicate opposite of physical reality. In order to better understand potential artifacts that can arise if the systems aren't in exact agreement with the component volume assumption (i.e., that the average methylene volume is independent of chain length), let's construct a simple toy model.

Suppose

$$V_{\mathrm{CH2ToyActual}}[m] = (1 - m\alpha)V_{\mathrm{CH2\_0}}, \tag{12}$$

where $V_{\mathrm{CH2ToyActual}}[m]$ is the actual average methylene volume for a lipid containing m tail methylenes at temperature T, with α and $V_{\mathrm{CH2\_0}}$ being temperature dependent constants. Note that in this section α is a dimensionless constant and is *not* the coefficient of thermal expansion. If we assume, as we do, a positive value for α, this means that the average methylene volume is decreasing with increasing chain length, as seen in alkanes (Yoshimura et al., 1985) and phosphatidylcholine bilayer simulations (Nagle et al., 2019). Then, ΔV or the change in methylene volumes between adjacent chain lengths, is given by

$$\begin{aligned}\Delta V &= V_{\mathrm{CH2ToyActual}}[m+1] - V_{\mathrm{CH2ToyActual}}[m] \\ &= \alpha V_{\mathrm{CH2\_0}}.\end{aligned} \tag{13}$$

We can then calculate $V_{\mathrm{CH2ToyGroup}}$ or the methylene volume found by the group method (and assuming that headgroup and terminal methyl volumes cancel out) by

$$\begin{aligned}V_{\mathrm{CH2ToyGroup}} &= (m+1)V_{\mathrm{CH2ToyActual}}[m+1] - \mathrm{mV}_{\mathrm{CH2ToyActual}}[m] \\ &= (1 - ((m+1)^2 - m^2)\alpha)V_{\mathrm{CH2\_0}}.\end{aligned} \tag{14}$$

With a little bit of algebraic manipulation, we end up with

$$V_{\mathrm{CH2ToyGroup}} = V_{\mathrm{CH2ToyActual}} - (m+1)\Delta V. \tag{15}$$

From this, we immediately see how a small volume dependence on chain length is amplified by the group method, in particular by a factor of $m + 1$. So in using the group method, even a tiny 0.1% chain length difference between adjacent chain lengths results in a 1% (or greater) difference between the group method methylene volume and the actual methylene volume. We also note that this shows that the group method will tend to yield an average methylene volume that is *lower* than the actual volume, if the average methylene volume is dropping as a function of chain length. It turns out, this means that the group method can give rise to a highly counter-intuitive artifact, as detailed below. For alkanes, the average methylene volume initially drops as a function of chain length, before stabilizing for the longer chain lengths (Yoshimura et al., 1985). Hence, the shorter chain lengths have a higher average methylene volume than the longer chain lengths. So, for the longer chain lengths, the stability as a function of chain length means that the group method applied to the longer chain lengths yields $\Delta V = 0$ and so $V_{\mathrm{CH2ToyGroup}} = V_{\mathrm{CH2ToyActual}}$. Now consider applying the group method to all the chain lengths; in this system we have an average methylene volume that is decreasing with chain length and so $V_{\mathrm{CH2ToyGroup}} < V_{\mathrm{CH2ToyActual}}$. So, despite the fact we've added in alkanes with *larger* average methylene volumes, the group method yields, artifactually, a *smaller* average methylene volume! This can be seen in Fig. 5 and Yoshimura et al. (1985).

We can also see how the temperature dependence of the chain length dependence introduces an even more dramatic artifact into the slope of the methylene volume. Taking the derivative of the above with respect to temperature and dropping a negligible term results in

$$\frac{\mathrm{dV}_{\mathrm{CH2ToyGroup}}}{\mathrm{dT}} \approx \frac{\mathrm{dV}_{\mathrm{CH2ToyActual}}}{\mathrm{dT}} - (m+1)\frac{d\alpha}{\mathrm{dT}}V_{\mathrm{CH2\_0}} \tag{16}$$

In order to estimate the actual methylene thermal expansion rate, we can use that for the methylenes in alkanes, resulting in $dV_{\mathrm{CH2ToyActual}}/dT \approx 0.015$ Å^3/°C. If we assume an alpha of 0.001, which says there is a 0.1 % difference between the average methylene volumes between adjacent chains, and also assume that alpha changes by about -2% per degree Celsius, then we have $d\alpha/dT \approx -2 \times 10^{-5}$/°C. Using $m = 10$ and $V_{\mathrm{CH2ToyActual}} \approx 30$ Å^3, we find that $dV_{\mathrm{CH2ToyGroup}}/dT$ ends up being over 40% larger than $dV_{\mathrm{CH2ToyActual}}/dT$. This artifact has especially noteworthy consequences for extracting the temperature dependence of lipid headgroup volume. If one is unaware of this artifact, a naive attempt at calculating the headgroup volume results in a headgroup volume that contracts with increasing temperature, which is physically unlikely. In order to correctly extract the headgroup volume, substantial simulation work is required; see Nagle et al. (2019) for how to properly deal with this important issue and for the application of this technique to phosphatidylcholines.

4. Conclusion

This work has carried out both differential scanning calorimetry and vibrating tube densimetry 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 CH_2 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

alkanes, despite the apparent difference yielded by the group method. By analyzing the literature alkane data, we find that the ratio of volumes of CH_3/CH_2 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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