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Effects of a short length alcohol on dimyristoylphosphatidylcholine system

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ABSTRACT

We have investigated dimyristoylphosphatidylcholine system with a high concentration of butanol (two molecules per phospholipid) by performing X-Ray diffraction, differential scanning calorimetry and density measurements. A lowering of the main transition temperature for the membrane/alcohol system has been observed. The phospholipid bilayer/alcohol system is characterized by a higher density value than the pure membrane as determined by volumetric measurements. The analysis of diffraction patterns has put into evidence the existence of a more packed structure due to the presence of alcohol. The area of polar head has also been estimated.

1. INTRODUCTION

Even though the effects of anaesthetics on the human body have been known for more than 100 years, the mechanism by which short-chain alcohols affect the lipid portions of biomembranes remains still unsolved. Vesicles or supported bilayers, of known lipid composition, have been studied as model systems with the aim to elucidate the details of the influence of short chain alcohols on the physical and thermodynamic properties of lipid membranes. In particular, phosphatidylcholine bilayers have been the most largely investigated membranes because they represent the major constituent of mammalian cells. These saturated phospholipid bilayers can undergo a series of thermotropic phase transitions which are considered to be relevant for biological membranes [1]. The most extensively studied transition, commonly named main transition, involves the chain melting in the bilayers passing from a low temperature gel-phase, characterized by ordered chains in the zigzag configuration (all-trans), to a high-temperature fluid-phase, characterized by quite disordered chains. Moreover, these lipids undergo another phase transition

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below the main one, the so-called pre-transition, in which the flat membrane in the tilted gel phase transforms into a periodically undulated bilayer and the corresponding phase is named ripple phase. It has been shown that the pre-transition temperature depends on acyl chain length of the lipids constituting the bilayer. [2].

Nowadays, it is well established that the presence of alcohol can significantly modify the phase behaviour of phospholipids. More precisely, experiments on phospholipid membranes have shown that alcohol can induce the interdigitated phase [3,4,5] that, at high alcohol concentrations, replaces the ripple gel phase [5,6]. The interdigitated phase is characterized by a greater order compared to the ripple phase [7]. Furthermore, in an ordinary membrane the hydrophobic thickness is approximately twice the length of the hydrophobic tails of a phospholipid while in the interdigitated phase the hydrophobic thickness is reduced to the sum of the length of the hydrophobic tails of a single phospholipid and the alcohol.

The main consequence of these structural modifications is a lowering of the main transition temperature of phospholipid bilayers [8].

In this study the effects of the butanol molecules on the gel-liquid crystalline transition and on the structure of a multilamellar phosphatidylcholine membrane have been investigated by performing differential scanning calorimetric (DSC), X-Ray diffraction and volumetric measurements.

2. EXPERIMENTAL DETAILS

The phospholipid 1,2-dimyristoyl-sn-glycero-phosphatidylcholine was purchased from Avanti Polar Lipids in powder form and butanol from Sigma Chem. Co. All chemicals have been used without further purification.

The pure lipid samples have been prepared by dissolving DMPC in a 2:1 $\text{CHCl}_3/\text{CH}_3\text{OH}$ solution. All procedures for sample preparation have been executed in a glove box under nitrogen gas to prevent lipid oxidation. Multilamellar vesicles and highly aligned multi-bilayers have been prepared as explained in the following.

2.1 Differential Scanning calorimetry measurements

After the evaporation of $\text{CHCl}_3/\text{CH}_3\text{OH}$ solution in N_2 atmosphere, the appropriated amount of butanol and water has been added to the dry lipid in order to establish a molar ratio of lipid/butanol/water of 1:2:10. The samples have been incubated at 5°C for at least one week before to be studied. Multilamellar vesicles have been prepared by subjecting the samples to ultrasonic

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3 irradiation in an water bath for about 30 minutes at a temperature of $\sim 10^{\circ}\text{C}$ above the melting
4 temperature of the lipid. Afterwards, the lipid and lipid/butanol hydrated samples have been
5 vortexed at room temperature four times.
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8 DMPC and DMPC/butanol have been weighed directly into the aluminium pans of the calorimeter
9 in a quantity of 10.6 mg and 21.4 mg respectively. The samples have been sealed and then
10 equilibrated at the lowest temperature for a period of time ranging from 0.5-2h prior to start the
11 measure. DSC scans have been carried out from 5°C to 25°C by using a Perkin-Elmer Calorimeter
12 Pyris 1 at a scan rate of $1^{\circ}\text{C}/\text{min}$ and repeated three times to check for reproducibility.
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18 19 *2.2 X-Ray diffraction (XRD) measurements*

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22 The DMPC with butanol sample has been prepared by adding the correct aliquot of butanol to lipid/
23 $\text{CHCl}_3/\text{CH}_3\text{OH}$ solution reaching a molar concentration of 1:2 of lipid/alcohol. Afterwards, highly
24 aligned multi-bilayers have been obtained by spreading both pure lipid and lipid/alcohol solutions
25 on silicon plates. The solvent has been removed by evaporation under nitrogen and then, the dry
26 samples have been stored for at least two weeks at 35°C in 100% humidity atmosphere to be
27 hydrated.
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30 X-Ray diffraction measurements have been performed at about 27°C with a D8-ADVANCE Bruker
31 diffractometer in the Bragg-Brentano geometry and by using the $\text{Cu K}_{\alpha 1}$ line ($\lambda=1.542 \text{ \AA}$).
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34 Each scan has covered 2θ from 0.9° to 15° with step size 0.02° , one second per step.
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37 During the measurements the samples have been hosted in a small chamber with 180° cylindrical
38 Kapton windows. Temperature stability has been controlled by a PT100 and humidity has been
39 adjusted by adding water solution into an open reservoir.
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45 46 *2.3 Volumetric measurements*

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49 We have calculated the specific volume of the pure lipid and of the lipid/alcohol system using the
50 Neutral Flotation Method [9,10]. Within the range from ~ 0.9 to $\sim 1.0 \text{ cm}^3/\text{g}$, this technique allows
51 to determine specific volumes of lipids with very high accuracy ($\pm 0.002 \text{ cm}^3/\text{g}$) [9].
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54 The desired amount of lipid has been dispersed in a $\text{D}_2\text{O}-\text{H}_2\text{O}$ mixture and centrifuged on a
55 laboratory centrifuge at 25°C (corresponding to the liquid-crystalline lamellar phase temperature)
56 and at 3500 rpm for a period of 1 up to 12 hours. Vigorous vortexing of the sample is resulted in
57 visually homogeneous sample with a milky appearance.
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5 It's worth observing that all the data acquired are consistent and independent from the specific
6 shape of the sample (vesicle or multi-bilayer) according to the experimental results of Katsaras [11]
7 who has shown that highly aligned DMPC multibilayers and liposomal samples dispersed in an
8 excess of liquid water exhibit the same physical characteristic (e.g. d-spacing and transition
9 temperatures) in all phases.
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14 15 16 3. RESULTS AND DISCUSSION 17

18
19 In Fig. 1 DSC-heating curves of DMPC and DMPC/butanol, in the temperature range between 5°C
20 and 25°C, are shown. The pure lipid membrane undergoes the typical pre-transition and main
21 transition at 11.3°C and 23.4°C respectively as indicated by the presence of two peaks observed in
22 the thermogram at these temperature values. The presence of butanol lowers the melting point of the
23 phospholipid bilayers and, as a consequence, the peak referring to liquid-crystalline transition is set
24 at 17.3°C. As suggested by previous results on model membranes with alcohols [12], the observed
25 decrease of chain-melting phase transition temperature can be considered as indicative of a disorder
26 of lipid chains induced by hydrocarbon chains of alcohol molecules. Furthermore, differently from
27 pure DMPC, the lipid/alcohol system doesn't show any pre-transition in the thermogram. This is in
28 agreement with previous studies on DPPC membrane with short-length chain alcohols which have
29 shown a decreasing of the pre-transition temperature and its disappearance at high alcohol
30 concentrations [12]. It has been argued that alcohol molecules, incorporated in the lipid bilayers,
31 perturb the alkyl-chain confinement of lipids and decrease the cooperativity of the phase transitions.
32 By increasing alcohol concentration, the pre-transition temperature decreases and, finally, the
33 interdigitation of lipid chains is induced. In this regard, the disappearance of the pre-transition has
34 been considered as an unambiguous indication for the complete hydrocarbon interdigitation. [12].
35 It's worth emphasizing that the occurrence of the interdigitated phase is expected at alcohol
36 concentrations above a threshold value equal to 2:1 alcohol/lipid ratio, as it has been observed for
37 DPPC/n-butanol system [6]. This is just the concentration of the lipid/alcohol samples which have
38 been investigated in this paper.
39
40 Owing to these findings, a double interpretation is feasible to explain the absence of the pre-
41 transition into the thermogram of DMPC/butanol system: the addition of alcohol to pure DMPC
42 could have shifted the pre-transition to temperature lower than the investigated experimental range
43 or it can be supposed that the alcohol perturbs the chain packing reducing the structural differences
44 between the gel and the ripple phase.
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3 In Figure 2, the representative diffraction patterns of hydrated DMPC and DMPC/butanol are
4 shown as a function of 2θ . Both diffractograms are characterized by the presence of five peaks
5 indicating that lamellar reflections up to the fifth order have been detectable. Our interest has been
6 focused on the position of these peaks thus, at this step, no treatment of data, with regard to the
7 intensity, has been performed and it has been postponed to further future analysis.

8
9 It is worth emphasizing that the experiments have been carried out at temperature above the main
10 transition temperature thus, both the samples have been investigated in their fluid phase.

11
12 The main feature of the diffraction patterns for both samples is plotted in the inset of figure 2 as a
13 function of the lamellar repeat distance D . The lamellar spacing for pure DMPC is 63 Å in good
14 agreement with literature data [13] while the presence of butanol reduces this value to 51.6 Å. **The**
15 **observed decreased thickness of DMPC with butanol could be a consequence of the disorder**
16 **introduced in the membrane by the short-length alcohol.**

17
18 **As a matter of fact, above the main transition temperature, the membrane is characterized by a high**
19 **fluidity which increases with temperature. In the fluid state, a lipid molecule may expand laterally**
20 **and shrink vertically and the membrane adopts a state in which the attractive interactions between**
21 **the hydrocarbon chains and the repulsive interactions between the headgroups balance each other.**
22 **This occurs when the free energy is minimal with respect to the area of the membrane and the**
23 **bilayer membrane is characterized by the optimal packing of the lipid molecules. As a consequence,**
24 **a reduction of the membrane thickness in the fluid phase respect to the gel phase is expected [14].**

25
26 In previous X-ray studies on membranes with alcohols, in the gel phase, it has been suggested that
27 alcohol molecules penetrate the acyl chain region to some extent, separating the headgroups and
28 creating voids inside the phospholipid bilayer [7,15]. Furthermore, the increase of the polar
29 headgroup area [16], due to the addition of alcohol molecules, gives rise to a reduction of the Van
30 der Waals attraction between lipid acyl chains that, as a consequence, appear to be more disordered.
31 By increasing the temperature, when the membrane/alcohol is in the fluid phase, an increased
32 fluidity of the membrane is revealed [17,18]. The higher mobility of chain tails would lead to an
33 additional chain coiling and to a filling of the energetically unfavorable voids and, as a
34 consequence, to a decrease of the membrane thickness [18].

35
36 Another feasible explanation is that the shortening of the **membrane/alcohol thickness** could arise
37 from a reminiscence of the interdigitated gel phase characterized by a shorter D respect to the pure
38 membrane. In this case the reduction of the bilayer thickness, corresponding to about 11.4 Å, could
39 arise from the replacement of an hydrophobic tail of a single phospholipid of the bilayer with the
40 chain tail of an alcohol molecule, assuming a length of ~ 1 Å for a CH_2 unit, according to a
41 molecular model of alkane [19,20].

The values of the thickness of both bilayers have been used to determine the average polar head area, A . Of course, volume is the connection between area and thickness so densitometric measurements provide a key datum to obtain details on the structure of membranes. In table I, the A values of pure DMPC and DMPC/butanol are reported. The polar head area has been calculated by using the following expression:

$$A = \frac{(V_l + n_w V_w)}{D/2} \quad (1)$$

where V_l is the phospholipid or phospholipid/alcohol volume, n_w is the number of hydration water molecules and V_w is the volume of a single water molecule (conventionally assumed to be 30 \AA^3).

Both molecular volume and the polar head area values for DMPC are in good agreement with data obtained previously by densitometric [9] and X-ray diffraction measurements [21]. It can be observed that the presence of butanol increases the A value according to similar behaviours revealed in other model membranes with alcohols [22,23]. This result suggests that alcohol molecules, replacing the interfacial water molecules, are located next to membrane polar head. More precisely, it is expected that the OH group of alcohol molecules binds to the phosphate group of the lipid [24] increasing the lateral space between the head-groups and leading to an enhanced effective head polar area.

These findings support the idea that also the presence of butanol, as for other short length alcohols, changes bilayer structure and significantly modifies the phase behaviour of phospholipids as it has been observed by the decrease of the melting point temperature in DSC thermogram.

4. CONCLUSION

The combined use of Calorimetric, X-ray diffraction and volumetric measurements performed on pure DMPC and DMPC/butanol systems, has allowed to study the influence of a short length alcohol on a phospholipid membrane in the fluid phase. A thinning of the bilayer and an increasing polar head-group area, following the addition of butanol, have been observed. These structural modifications of the membrane/alcohol system appear to favour a disorder of hydrophobic chain tails facilitating the gel-fluid transition and lowering the melting point temperature respect to the pure membrane.

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TABLE:

TABLE1: Data of Molecular volume, % hydration, number of water molecules per lipid, semi-lamellar repeat distance and polar head area for DMPC and DMPC/butanol.

Sample	Molecular Volume (\AA^3)	% hydration by weight	Number of water molecules per lipid	D/2 (\AA)	Polar head area A (\AA^2)
DMPC	1099 \pm 1	24 \pm 1	9 \pm 1	31.5	43.46
DMPC/butanol	1305 \pm 1	15 \pm 1	7 \pm 1	25.8	61.05

FIGURE CAPTIONS:

FIGURE 1: DSC heating scans of pure DMPC (dashed line) and DMPC/butanol (solid line).

FIGURE 2: 2θ dependence of the X-ray diffraction patterns of DMPC (dashed line) and DMPC/butanol (solid line) at 27°C. In the inset the main peak of the diffraction pattern for both samples is plotted as a function of lamellar repeat distance D.

FIGURES:

FIGURE 1

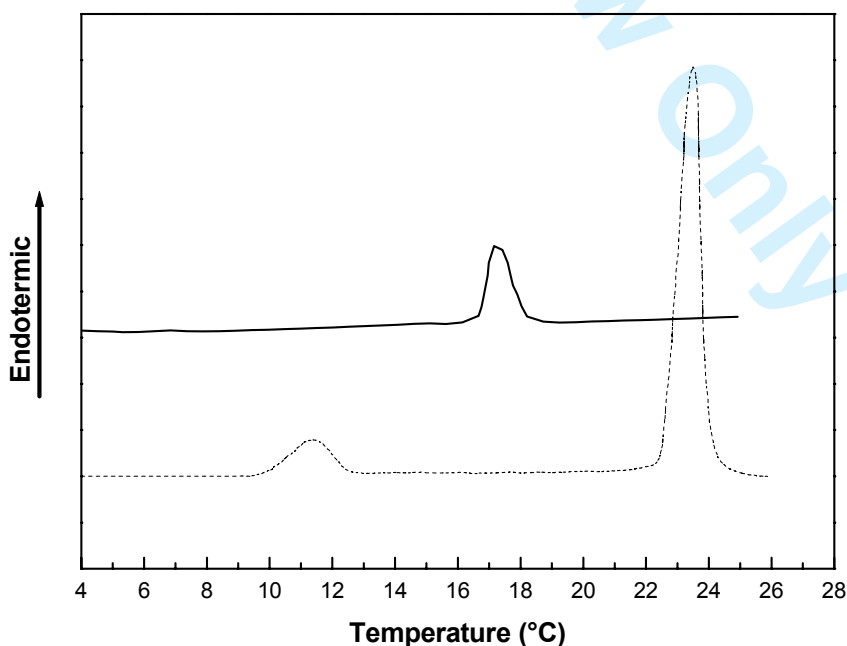
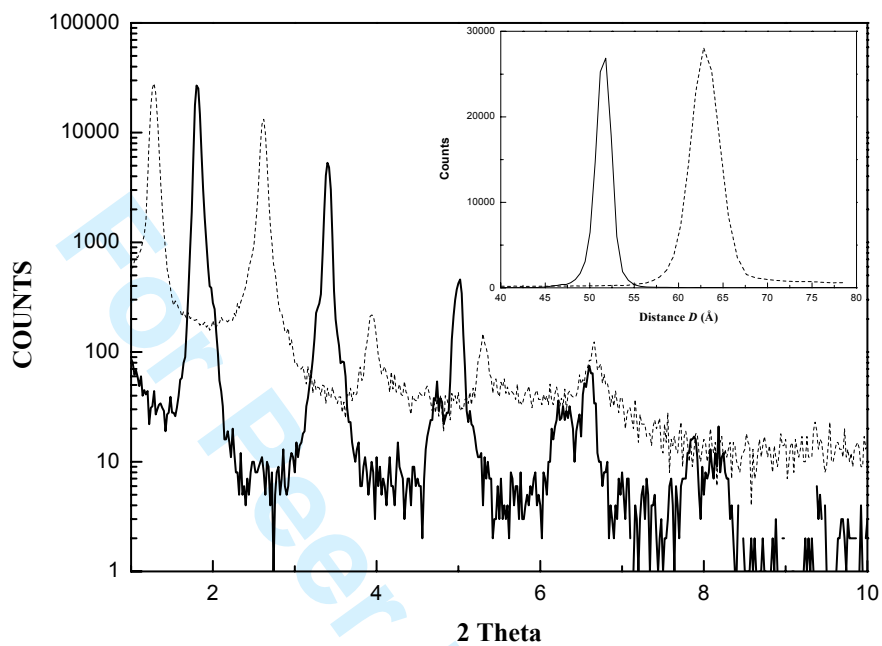


FIGURE 2

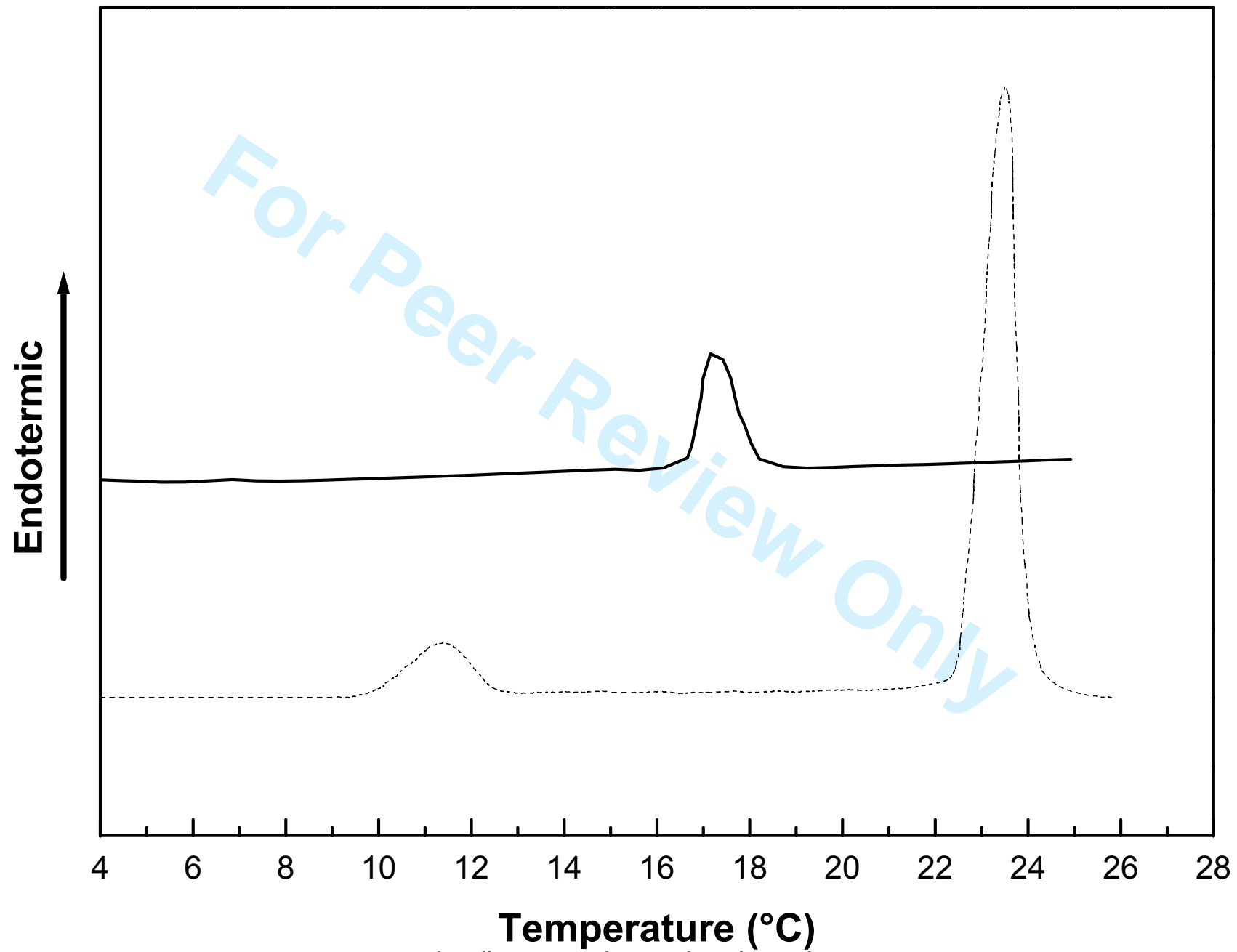


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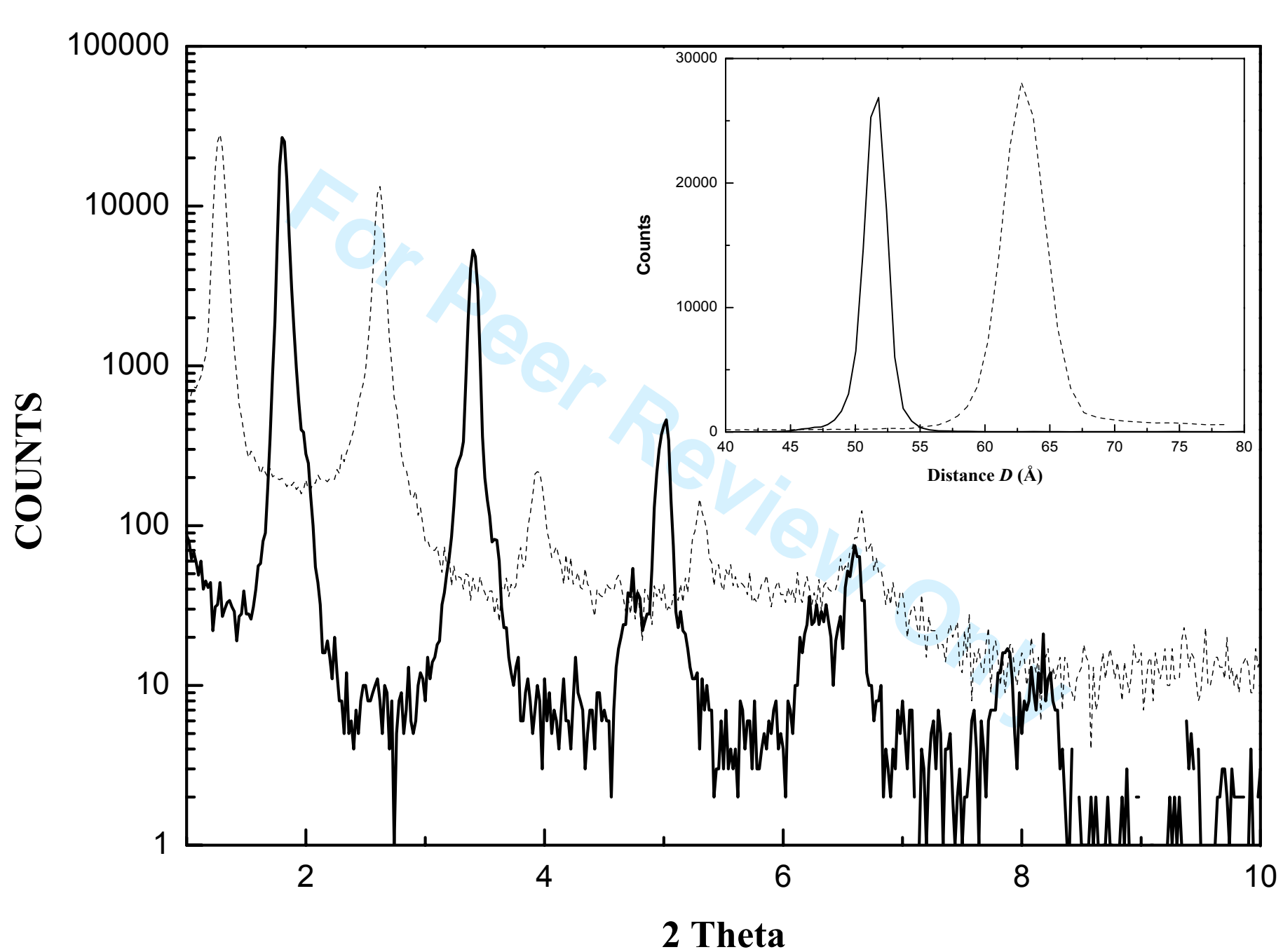
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DMPC/butanol	1305 \pm 1	15 \pm 1	7 \pm 1	25.8	61.05



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