# Location of sugars in multilamellar membranes at low hydration.

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**Abstract**
Severe dehydration is lethal for most biological species. However, there are a number of organisms which have evolved mechanisms to avoid damage during dehydration. One of these mechanisms is the accumulation of small solutes (e.g. sugars), which have been shown to preserve membranes by inhibiting deleterious phase changes at low hydration. Specifically, sugars reduce the gel to fluid phase transition temperatures of model lipid/water mixtures. However, there is debate about the precise mechanism, the resolution of which hinges on the location of the sugars. In excess water, it has been observed using contrast variation SANS that the sugar concentration in the excess phase is higher than in the interlamellar region (Demé and Zemb, J. App. Cryst., 2000, 33:569). This raises two questions regarding the location of the sugars at low hydrations: first, does the system phase separate to give a sugar/water phase in equilibrium with a lipid/water/sugar lamellar region (with different sugar concentrations); and second, is the sugar in the interlamellar region uniformly distributed, or does it concentrate preferentially either in close proximity to the lipids, or towards the centre of the interbilayer region. In this paper we present the preliminary results of measurements using contrast variation SANS to determine the location of sugars in lipid/water mixtures.

*Keywords:* Desiccation; Cryobiology; Dehydration; Membranes; Sugars; SANS; Phase Transitions

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## Introduction

Severe dehydration (or desiccation) is lethal for most biological species, whether the dehydration occurs in dry atmospheres or as the result of freeze-induced dehydration. However, there are a number of organisms which have evolved mechanisms to avoid damage during dehydration. One such mechanism is the accumulation of sugars and other small solutes, which is correlated with desiccation tolerance. The presence of small solutes has been shown to preserve membranes by inhibiting deleterious phase changes at low hydration (eg [1]).

The effects of solutes on lipid phase transitions have been studied extensively using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (FTIR), which are capable of determining transition temperatures. However, there remains disagreement in the literature about the mechanism by which the solutes affect the lipid phase behavior. The debate revolves around whether the effects are caused by direct interactions between the solutes and the lipids (e.g. [2, 3]), or by non-specific effects related to the volumetric, osmotic and solution properties of the solutes (e.g. [4-7]).

There are two important questions to be addressed here: (i) do all solutes remain between bilayers during dehydration, or is there some phase separation leading to a membrane/solute/water phase in equilibrium with a solute/water phase at a different concentration?; and (ii) for solutes that remain between the bilayers, are they homogeneously distributed in the interbilayer space, or are they preferentially concentrated either near the membranes, or in the middle of the intermembrane region?

Despite the interest in the problem, no experiments have been carried out to answer these questions, partly because of the experimental difficulty they pose. The only quantitative study has been carried out at full hydration by Demé and Zemb, who used Small Angle Neutron Scattering (SANS) to measure the concentration of sugars between membranes in the presence of an excess phase [8].

In this paper we report the preliminary results of similar measurements, but at an intermediate hydration. We show that these methods can be applied to these systems, and discuss future research directions.

## Methods

The synthetic phospholipid 1,2-Palmitoyl-*sn*-Glyero-3-Phosphocholine (DPPC) was obtained from Avanti Polar-Lipids and used without further purification. The dry lipid was weighed, then hydrated by the addition of excess water, mixed by vortex mixing until it was homogeneous. Samples were then dehydrated over a 32% saturated salt solution for one week to reduce the water content and ensure the osmotic pressure in the samples was the same. For the contrast matching experiments, samples were hydrated with the appropriate $D_2O/H_2O$ ratio, and equilibration was carried out over salts saturated with the same $D_2O/H_2O$ ratio. Prior to measurement the final mass was determined. Samples with glucose were prepared by hydrating the dry lipid with a specific volume of a glucose/water solution at a known concentration, so the exact number ratio of glucose to lipid molecules was known.

Small angle Neutron Scattering was conducted on the Berlin Neutron Scattering Center's instrument V4 (Berlin, Germany). The SANS data were directly collected onto a two-dimensional detector using a neutron wavelength of 6 Å with spread (fwhm) $\Delta\lambda/\lambda = 10\%$. Data

was collected at sample to detector distances of 4m and 12m, giving a combined $q$ range of $4.76\text{x}10^{-3} < q < 9.45\text{x}10^{-2}$ Å$^{-1}$. The 2-D patterns were corrected for background, empty cell scattering, and individual detector pixel sensitivity and then radially averaged to express the intensity as a function of $\boldsymbol{q}$ ($q=4\pi/\lambda.sin(\theta/2)$ where λ is the wavelength of the neutrons and θ is the scattering angle).

**Results & Discussion**

Figure 1 shows a double logarithmic plot of the SANS data for pure DPPC and DPPC:glucose 2:1. The Bragg peak from the multilayers is just to the right of the highest q measured, at 62.7Å repeat spacing. The broad peak for the 100% D2O sample is due to some contamination, and these data are not used in the following analysis. The low q data shows an approximately $q^{-4}$ Porod-type decay.

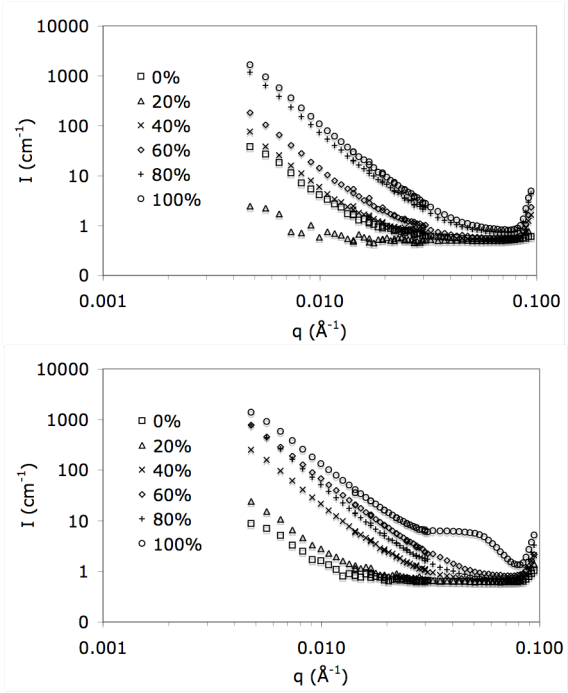


Figure 1: log I vs. log q for pure DPPC (top) and DPPC:glucose 2:1 (bottom) for increasing water contrast.

The purpose of the SANS is to use contrast matching to determine sugar concentrations. Figure 2 shows a plot of the square root of intensity vs. volume fraction of $D_2O$ for several values of q. As can be seen the contrast match points (CMP) for the two samples are significantly different, and within the errors are independent of q. The CMPs are 0.170±0.005 for pure DPPC and 0.086±0.004 for DPPC:glucose, which correspond to scattering length densities of $6.2\text{x}10^{-7}$ Å$^2$ and $3.68\ \text{x}\ 10^{-8}$ Å$^2$ respectively.

In order to determine whether all of the sugar resides between the membranes we apply a similar analysis as that of Demé and Zemb [8]. For the pure lipid sample the overall volume fractions are $\Phi_L$=0.615 and $\Phi_W$ =0.385. For the DPPC/glucose samples the volume fractions are $\Phi_L$=0.431, $\Phi_W$ = 0.516 and $\Phi_S$ =0.053.

At the CMP at low q the scattering from the lamellar region and the excess fluid region be equal:

$$_{L}\#_{L} + \ _{W}\#_{W} + \ _{S}\#_{S} = \ _{W}\#'_{W} + \ _{S}\#'_{S}$$

where: $\rho_L$, $\rho_W$, $\rho_S$ are the scattering length densities of the lipid, water and solute respectively; $\psi_L$, $\psi_W$, $\psi_S$ are the volume fractions of the components in the membrane region; and $\psi'_W$ and $\psi'_S$ are the volume fractions of the water and solute in the excluded phase. The overall volume fraction, and the partial volume fractions in each phase must each add to unity:

$$\Phi_L + \Phi_W + \Phi_S = 1$$
$$\psi_L + \psi_W + \psi_S = 1$$
$$\psi'_W + \psi'_S = 1$$

Finally, the volume fractions of water and solute must satisfy the following conditions:

$$_{W} = \#_{W}v + \#'_{W}(1 \quad v)$$
$$_{S} = \#_{S}v + \#'_{S}(1 \quad v)$$

where v is the volume fraction of the membrane phase relative to the total volume. The scattering length densities of the lipid and water were determined from fig. 2. The scattering length density of the D-glucose is calculated to be $3.99 \times 10^{-6}$ Å$^2$. The overall volume fractions are also known. Solving these equations leads to values of $\psi'_S = 0.117$ and $\psi'_W = 0.883$. Note that these values do not rely on any assumptions about the membrane phase.

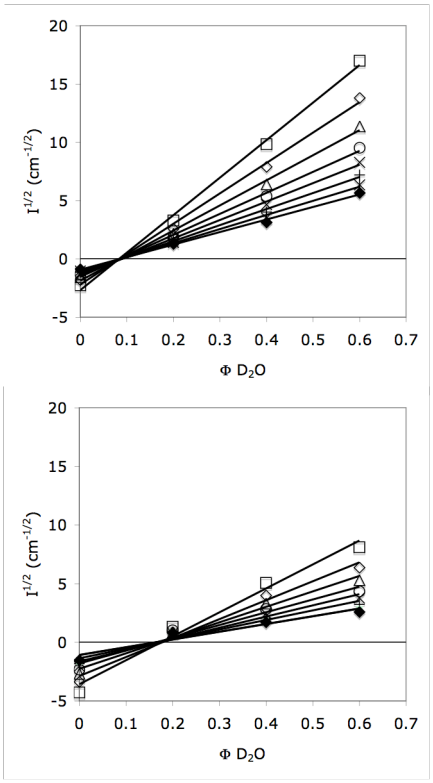


Figure 2: Square root of I vs. volume fraction of $D_2O$. The plots show the contrast match points for several values of q for pure DPPC (bottom) and DPPC:glucose 2:1 (top). As can be seen the contrast match points are q independent.

To determine $\psi_W$ and $\psi_S$ we need to know $\psi_L=d_l/d^*$, where $d_l$ is is bilayer thickness and $d^*=62.7\pm0.2$ Å is the repeat spacing. For DPPC in the gel phase at maximum swelling the lipid thickness is $d_l=44$ Å [9]. This yields a lipid volume fraction in the membrane phase of $\psi_L=0.7$. Using this value yields $\psi_S=0.013$, $\psi_W=0.285$, and $v=0.61$.

**Discussion/Conclusions**

The results confirm the findings of Demé and Zemb [8] that the method can be used to determine solute concentrations in membranes/water/solute mixtures with two phases present. Unlike the samples of Demé and Zemb however, the samples measured here are (just) at full hydration, so it is not clear, *a priori*, whether or not a separate glucose/water phase should exist for the mixed lipid/solute sample. The analysis unequivocally shows that there is a separate phase – if there were not, the solution to the equations would yield a lipid thickness of 27Å, which is unphysical. In addition, the analysis confirms that the concentration of solute is higher in the excluded phase (0.117) than it is in the membrane phase (0.044), compared with the overall solute fraction in water of 0.093. So the concentration in the membrane phase is about half the overall concentration.

The analysis of these preliminary results makes a number of assumptions. The most severe assumption is that the membrane scattering density is the same in the lipid/water mixture as it is in the lipid/water/solute mixture. However, this will only be true in general at maximum swelling. At lower hydrations, this will be an approximation only, and other methods may need to be applied to determine the true bilayer thickness in the lipid/solute samples. However, it is clear that the application of this method to membrane/solute mixtures at low hydrations will yield a great deal of information. More extensive experiments are currently being planned.

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