Experimental report

11/07/2022

Proposal: 9-10-1722 **Council:** 4/2021

Title: Defining Short-chain Alcohol Distribution in Phospholipid Multilayers

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Fernanda ALVARADO GALINDO

Experimental team: Sylvain PREVOST

Local contacts: Sylvain PREVOST

Samples: D2O
DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine)

Instrument	Requested days	Allocated days	From	To
D11	1	1	05/09/2021	06/09/2021

Abstract:

Phospholipid (PL) liposomes are of central importance for industrial formulations in pharmacology and cosmetics. Typically, they are obtained by injecting a concentrated ethanolic PL solution (or other alcohol) into water with subsequent application of shear to reduce the liposome size and make them colloidally stable. However, it is not clear to which extent do short-chain alcohols behave as cosurfactants nor their definite location in the phospholipid bilayer (despite their miscibility with water). For example, a fast ultrafiltration on a lipid dispersion did not remove any alcohol, whereas doing it over several hours resulted in complete removal of the ethanol. Solvent contrast provided by SANS would be the ideal tool to further study the sturctural changes of fluid bilayers in the presence of short-chain alcohols.

Defining Short-chain Alcohol Distribution in Phospholipid Multilayers

Experimental report for experiment 09-10-1722 (05/09/2021)

Phospholipid liposomes are particles used in pharmacology and cosmetics as carriers of active ingredients. Typically, they are obtained by injecting a concentrated ethanolic PL solution (or other alcohol) into water with subsequent application of shear to reduce the liposome size and make them colloidally stable. However, it is not clear to which extent do short-chain alcohols behave as cosurfactants nor their definite location in the phospholipid bilayer (despite their miscibility with water). The aim of the experiment was to elucidate the effect of short-chain alcohols on fluid bilayers by profiting of contrast variation between hydrogenated lipids and (partly) deuterated solvents with Small-Angle Neutron Scattering (SANS).

Experimental setup and samples

For this experiment, we prepared DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) liposomes with a constant lipid concentration of 2mg/mL for all systems. The liposomes were extruded with an Avanti mini extruder for 21 times using a 200nm pore size membrane in the alcoholic solutions shown in Table 1. In addition, 4 samples were prepared in D_2O or D_2O/H_2O.

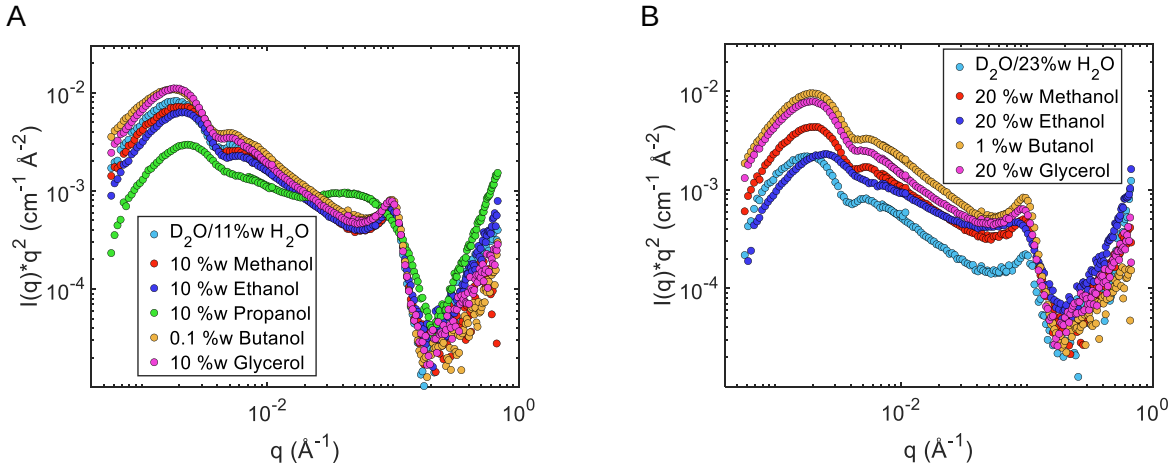
Table 1. Solvent compositions used for liposome preparation. Mixtures were composed of heavy water (D_2O) + (H- or D-) alcohol.

Solvent	Alcohol content (%w)	Form of alcohol
D_2O + Methanol	10%, 20%, 40%	Hydrogenated, deuterated
D_2O + Ethanol	10%, 20%, 40%	Hydrogenated, deuterated
D_2O + 1-Propanol	10%, 20%	Hydrogenated
D_2O + 1-Butanol	0.01%, 0.1%, 0.5%	Hydrogenated, deuterated
D_2O + Glycerol	10%, 20%, 40%	Hydrogenated

A total of 30 samples were measured with their respective background at D11 at 3 detector distances to access the desired Q-range of 0.001 - 0.05 1/Å. The samples at 40%w ethanol and 20%w propanol were unstable, thus they are omitted from the data analysis.

Results and discussion

The Kratky-Porod plots of liposomes in D_2O/H–alcohol are shown in Figure *1*, where the samples at the lowest alcohol concentrations are shown in Figure 1.A, followed by increasing concentrations in Figures 1.B and again in 1.C.



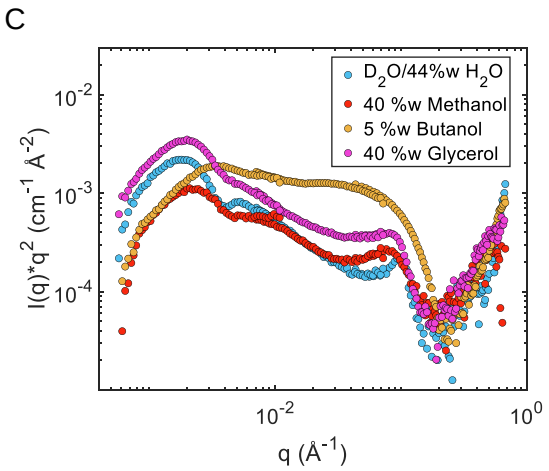


Figure 1. Kratky-Porod plot from SANS scattering curves of DOPC liposomes in different D_2O/H Alcohol solutions.

The scattering curves for most of the systems show a q^{-2} behavior at the mid-q region, characteristic of vesicles. However, the system is not composed exclusively of unilamellar vesicles as the peak q = 0.1 $Å^{-1}$ indicates multilamellarity in the dispersion. Particle morphology is highly influenced by the presence of propanol and at the highest concentration of butanol, as can be seen in Figures 1.A and 1.B, respectively, where the decrease of I(q) deviates from q^{-2}. Therefore, the presence of different morphologies other than core-shell structures is not ruled out, but details on the supramolecular architectures present in the system will be elucidated after the on-going data modelling is finished.

From model-free analysis of the scattering curves, we obtain experimental values for Guinier radius, forward scattering and scattering invariant, amongst others. We note an overall reduction in particle size at increasing alcohol concentration and at increasing alcohol chain length in the case of those with linear aliphatic chains. Furthermore, particles in the D_2O/glycerol solvent have a higher radius of gyration compared to those in D_2O/ethanol. Ethanol and glycerol have the same chain length and differ only in the number of –OH groups.

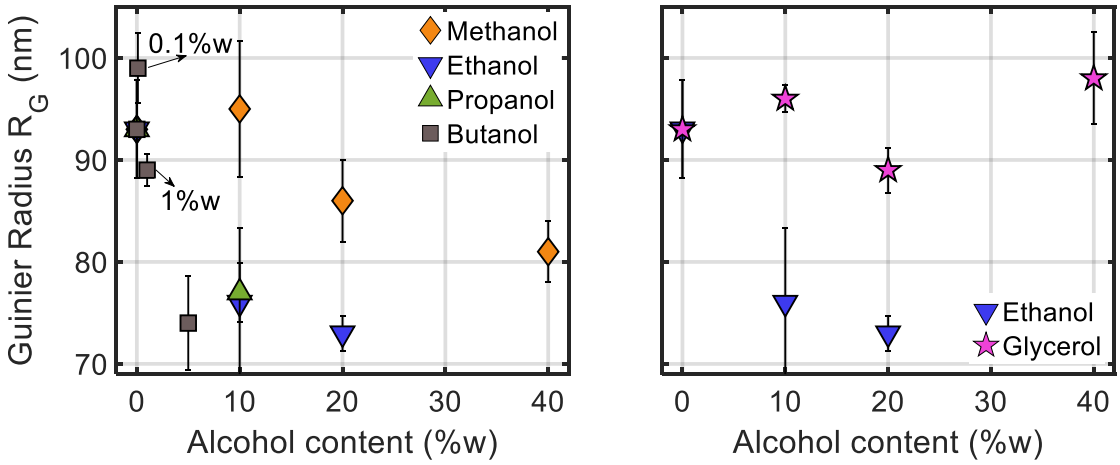


Figure 2. Guinier radius of DOPC liposomes at different D2O/H-alcohol compositions

The scattering invariant (Q*) was also obtained from the SANS scattering curves and the values for the different D_2O/alcohol compositions are shown in Figure 3. In this case, there is a trend of reduction in Q* at increasing alcohol concentrations.

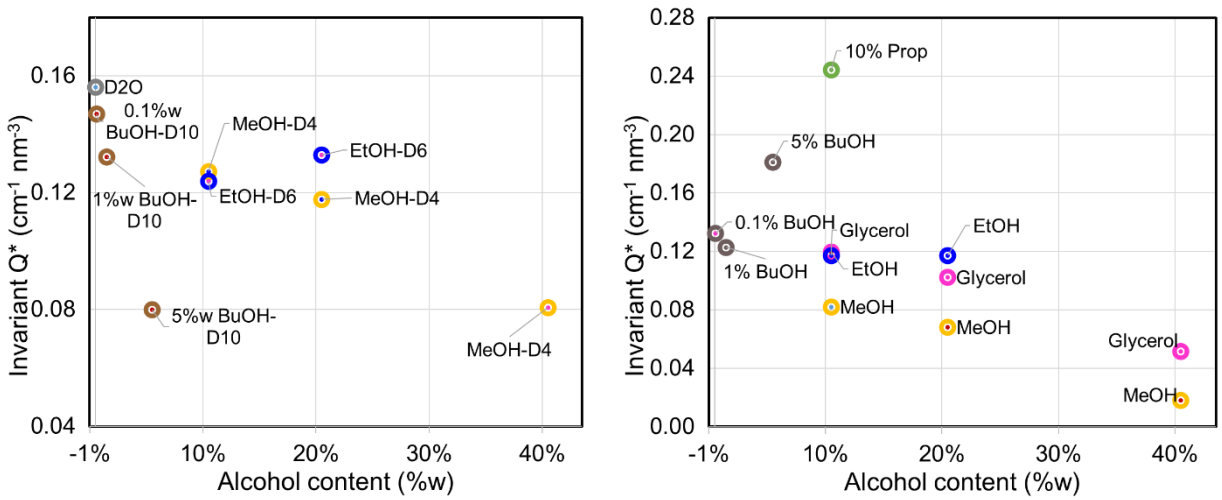


Figure 3. Scattering invariants of DOPC dispersions in different D2O/alcohol solvents. *Left:* D2O + deuterated alcohols. *Right:* D2O + hydrogenated alcohols.

The scattering invariant, directly related to the average quadratic deviation of the scattering length density (and therefore independent of the shape of the scatterers), is defined by the following expression: $Q^* = \int_0^\infty q^2 I(q) dq = 2\pi^2 \phi_1 \phi_2 \Delta SLD^2$, where $\Delta SLD = SLD_{vesicle} - SLD_{solvent}$. Assuming a 2 phase system consisting of lipids and solvent, a decrease in Q* would be related to a reduction in $SLD_{vesicle}$ as a consequence of alcohol incorporation in the membrane. By solving a system of equations, we expect to calculate the volume fraction of the lipid system that consists of alcohol. The analysis is on-going but thus far, we see a trend of increasing alcohol fraction in the bilayer as a function of concentration.

Conclusion

DOPC dispersions in different D_2O/alcohol solvents were studied via SANS. Reduction of R_G and Q* as a function of increasing alcohol content and alcohol chain-length was observed. An exception to this is found in the D_2O/glycerol dispersions, where particle size vs. an alcohol with same chain-length but different number of –OH groups (ethanol), is higher. The analysis is on-going as to find quantitative fractions for all the alcohols in the PL bilayer as well as their effect in particle morphology.