# Experimental report

11/07/2022

**Proposal:** 9-10-1707 **Council:** 4/2021

**Title:** The Influence of short-chain alcohols on the Rigidity of PhospholipidMembranes

**Research area:** Soft condensed matter

**This proposal is a resubmission of 9-13-960**


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

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN15 | 2 | 2 | 10/09/2021 | 12/09/2021 |


## 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. Subsequent application of shear reduces the liposome size and makes them colloidally stable. Despite the widespread use of alcohols for production of liposome formulations, the influence exerted in the fluid bilayer not been studied systematically, yet. Specifically, the membrane rigidity, where one can hypothesize that alcohol molecules either get incorporated in the amphiphilic bilayer at the head groups or modify the hydration of the head group.

# The Influence of short-chain alcohols on the Rigidity of Phospholipid Membranes

Experimental report for experiment 09-10-1707 (10 - 12/09/2021)

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. Despite the widespread use of alcohols for production of liposome formulations, the influence exerted in the fluid bilayer has not been studied systematically, yet. The aim of the experiment was to elucidate the effect of short-chain alcohols on membrane rigidity of fluid bilayers by Neutron Spin-Echo (NSE) Spectroscopy.

## 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. 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. A total of 6 samples were measured with their respective backgrounds. The sample corresponding to 20% propanol did not form core-shell structures and is omitted from the data analysis.

Table 1. Solvent compositions used for DOPC liposomes studied by NSE

| Solvent | Alcohol content (%w) |
|---|---|
| $D_2O$ | 0% |
| $D_2O$ + Methanol D4 | 20% |
| $D_2O$ + Ethanol D6 | 20% |
| $D_2O$ + 1-Propanol D8 | 20% |
| $D_2O$ + 1-Butanol D10 | 5% |
| $D_2O$ + Glycerol D8 | 20% |

The samples were measured at 4 different configurations, namely at wavelengths of 12, 10, 8 and 6 Å at detector angles of 3.5, 5.5, 7, and 7.5 degrees spanning a Q range from 0.023 to 0.16 1/Å. In addition, in-situ DLS was measured to keep track of colloidal stability.

## Results and discussion

The intermediate scattering functions S(q,t) of the 5 samples are depicted in Figure 1 . The decay in the intermediate scattering functions measured at different qs were fitted to the following expression: $(q,t) = exp(-\mathcal{D}q^2t) * exp(-\Gamma_{ZG}q^3t)^{2/3}$. Relaxation rates ($\Gamma_{ZG}$) obtained from the fits are reported in Figure 2.

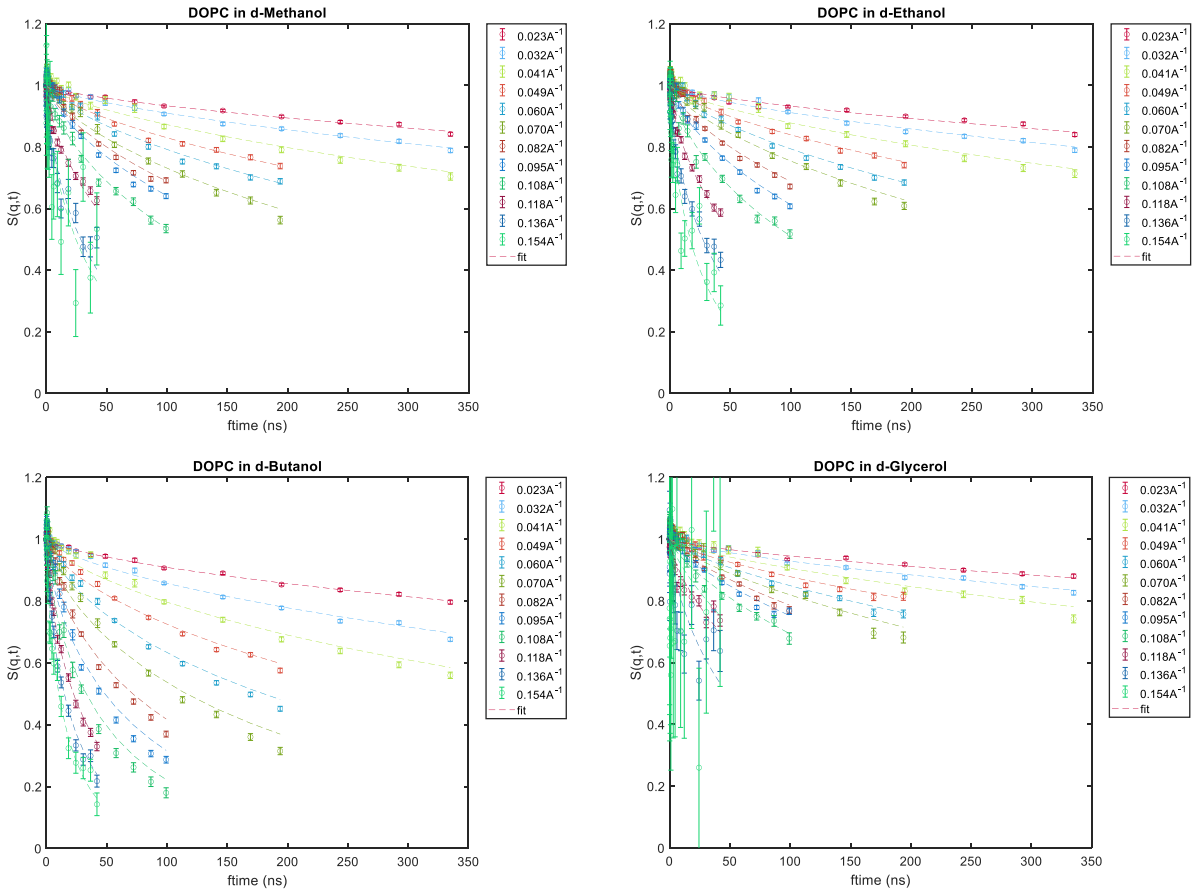


Figure 1. NSE data for DOPC (0.2%w) liposomes in D2O or D2O/D-alcohol solutions

The higher relaxation rates at lower q values correspond mostly to diffusion phenomena rather than membrane undulations. It is also noteworthy the marked difference of $\Gamma_{ZG}$ in liposomes dispersed in $D_2O$/butanol solution, which show a relatively q-independent $\Gamma_{ZG}$ compared to the rest of the samples.

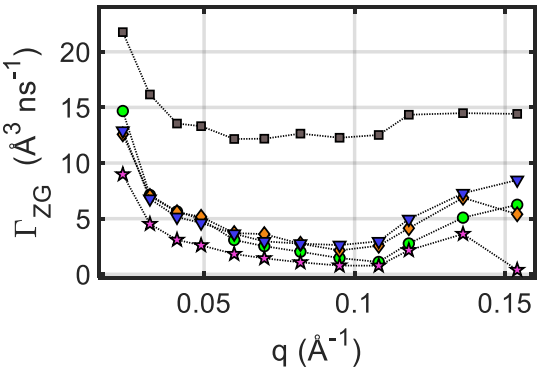


Figure 2. Relaxation rate $\Gamma_{ZG}$ DOPC liposomes at different in D2O + deuterated alcohol mixtures as a function of the scattering vector q

Thereafter, membrane rigidity $\boldsymbol{\kappa}$ was derived from the Zilman-Granek (ZG) expression $\Gamma_{ZG} = 0.0069\sqrt{\frac{k_bT}{\boldsymbol{\kappa}}}\frac{k_bT}{\eta}$. The values of bilayer rigidity decrease with increasing alcohol chain-length,

suggesting softening of the membrane. Already the presence of methanol reduces $\boldsymbol{\kappa}$ by half from 23 $k_bT$ of the sample in pure D2O to 13 $k_bT$ at an MeOH concentration of 20%w. The rigidity is further reduced by half in ethanol down to a value close to 6 $k_bT$. According to our calculations, the softest membrane would correspond to that of liposomes in the glycerol solution.

Table 2. Average relaxation rates and calculated membrane rigidites for DOPC liposomes studied by NSE

| Solvent | $\bar{\Gamma}_{ZQ}$ (Å$^3$ns$^{-1}$) | Membrane rigidity $\boldsymbol{\kappa}$ (J) |
|---|---|---|
| $D_2O$ | 4.72 | ~23$k_bT$ |
| $D_2O$ – 20%w MeOH-D4 | 5.13 | ~13$k_bT$ |
| $D_2O$ – 20%w EtOH-D8 | 5.42 | ~6$k_bT$ |
| $D_2O$ – 20%w Glycerol D8 | 2.60 | ~2$k_bT$ |
| $D_2O$ – 5%w BuOH–D10 | 14.15 | ~4$k_bT$ |

In conclusion, we studied by NSE the bending fluctuations of DOPC fluid bilayers, from which we obtained their bending moduli ($\boldsymbol{\kappa}$) at different solvent compositions. $\boldsymbol{\kappa}$ of PL liposomes decrease with increasing alcohol chain-length. These results seem to be in accordance with the literature, as each additional $CH_2$ seems to have a stronger effect on membrane properties[1]. However, the marked decrease of liposomes in glycerol needs to be verified as normally larger amounts of it do not affect biological membranes to such an extent.

(1) [1] Traube Justus Liebigs Annalen der Chemie 1891, 27–55