Experimental report

26/07/2022

Proposal:	**9-13-905**	**Council:**	10/2019
Title:	High temperature phase behaviour of a fatty acid/alcohol membrane		
Research area:	Soft condensed matter		

This proposal is a continuation of 9-13-788

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Samples:	decanol decanoic acid

Instrument	Requested days	Allocated days	From	To
D33	3	2	19/08/2020	21/08/2020

Abstract:

Our project studies a suitable model membrane for the first forms of life.
This system is made of short, single chain fatty acids/alcohols, molecules that were readily available at the onset of life. It has been proven to form vesicles at ambient temperature in a wide range of pH and amphiphile/buffer concentrations.
However, recent solid state NMR experiments show an unexpected high T transition which is only triggered by the fatty alcohol presence.
SANS measurements on D16 were performed on identical samples and conditions to better identify the high T new phase, but only the unbinding transition (leading to unilamellar vesicles) was observed.
We propose to perform SANS experiments on D33 in order to 1) access a lower range of scattering vector to detect changes in the vesicle shape/size/dispersity; 2) explore the effect of the high magnetic field, experienced by the samples during the NMR measurements, that may have a role on the new phase appearance/characteristics.
The results will be of high value as they concern the extreme temperature conditions that constituted the Earth environment when life appeared.

REPORT – EXPERIMENT 9-13-905 on D33 (19-21/08/2020) and INTER-464 on IN15 (28 – 30/07/2019)

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Scientific background: The origin and nature of the first cell membranes represent open questions for science. Because of the supposed lack of molecular complexity on the early planet, the first cell membranes were most likely composed of simple, single chain fatty acids [1] and alcohols, raising questions as how they could withstand the very variable and extreme surrounding environment [2] of the oceanic hydrothermal vents where they probably originated [3].

Our current project considers a mixture of Capric acid with a fatty alcohol of equal chain length, the decanol (named hereafter C10 mix). The system is made of short, single chain amphiphilic molecules that were readily available in the prebiotic environment. To explore the capability of them to form stable vesicles, as well as studying the high temperature/pressure behavior, is important in order to determine whether the system could be a suitable candidate as model protomembrane architecture. To account for the supposed extreme environmental condition, the membrane model can contain an additional hydrophobic molecule such as an alkane chain as eicosane or squalane.

Among the many employed techniques, extruded vesicles of C10 mix have been previously characterized by SANS on D33 (doi:10.5291/ILL-DATA.9-13-788) at ambient temperature. The results allowed to identify the presence of unilamellar vesicles, having a membrane thickness of about 20 Å, as expected.

The same samples, at a higher concentration and in form of MLVs, were studied by means of solid state NMR at the I.E.C.B. in Bordeaux, with the aim of exploring the amphiphile chain dynamics at both ambient and higher temperatures (up to 80 °C). The results revealed an unexpected phase transition seen at 80 °C uniquely for the C10 mix sample. In fact, in the C10 mix at 80 °C the signal measured gives the signature of the fatty acids being aligned with respect to the magnetic field (12 T).

The characteristics of this new high temperature phase, as well as the reason for its presence only when the vesicles contain decanol, are of primary importance for our study, because the high temperature constitutes the most probable environmental condition for the first living forms. For this purpose, a series of test SANS measurements on D16 was made on identical samples. The obtained data showed the unique effect of a progressive MLV disruption, also known as unbinding transition and described in literature for soft lamellar phases of surfactants and lipids [4], leading to unilamellar structures. No exotic phases were found, hence questioning whether the effect was due to the high constant magnetic field. No information on the vesicle size/shape could be obtained with the available D16 Q range.

We proposed now to perform SANS experiments on D33 and NSE experiments on IN15 to identify and corroborate the results obtained with NMR and SANS on D16 and to characterise the new phase.

The measured samples were C10 mix (Capric acid + decanol) in presence or not of eicosane or squalene.

Experiment and results The experiment was performed in August 2020 on the diffractometer D16 and in July 2019 on IN15. In Figure 1, the collected SANS curves are shown together with the best fits obtained. The coexistence of two distinct assemblies (bilayers and droplets, respectively) can be judged qualitatively (by visual inspection of the curves) as well as quantitatively (by fitting the SANS data). In particular, because of the faster decay of the droplet signal (q^{-4}), the lamellar form factor is dominating in the region at $q > 0.04$ Å^{-1}: this allows for a straightforward discrimination of the fraction of sample that self-assembles into bilayer structures, by proper scaling the q^{-2} in the middle-high q region.

The most important difference is observed in the data acquired at T = 60 C. At this temperature, the C10mix sample shows a very small fraction of lamellar assemblies, while most of the sample appears to have formed large dense spheres (droplets) because of the temperature increase. Instead, samples containing intercalated alkanes retain a larger fraction of lamellar structures at this temperature. Note that, at this temperature, the SANS curves of the samples containing eicosane and squalane are found in an intermediate state where it was not possible to fit the fraction of droplets at low q with a single dense sphere form factor. For this reason, the fitting at T = 60 C for these two samples was performed only in the q range where the lamellar form factor predominates ($q > 0.04$ Å^{-1}), to estimate the corresponding fraction. These results demonstrate the membrane stabilizing role of both kind of alkanes, up to at least T = 60 C. Moreover, they show that the result of the high temperature conformational transition on ULV systems is the formation of a lipid droplet phase.

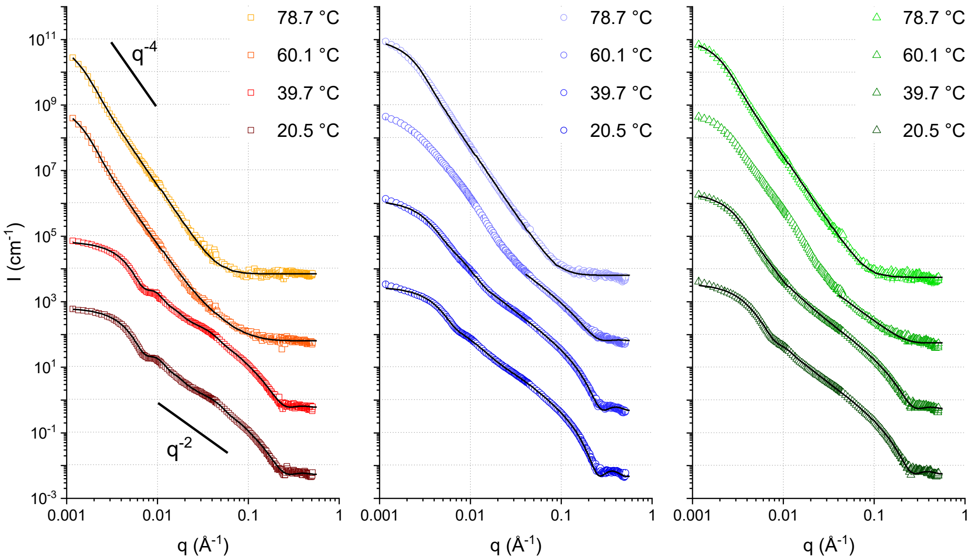

Figure 1. SANS curves of extruded samples at the temperatures investigated. (**a**) C10mix sample. (**b**) C10mix + 2% h-eicosane sample. (**c**) C10mix + 2% h-squalane sample. The black lines are the best fits to the data. The curves are vertically shifted for clarity.

As the above experiments have demonstrated, the presence of alkanes in the membrane has a significant impact in modifying the phase boundaries and structural properties of the protomembranes. To gain further insight into these effects, we looked at the consequences of temperature on membrane dynamics using NSE spectroscopy performed on extruded vesicles at temperatures in the range 20 C < T < 70 C.

Due to the size distribution of the droplets and from the theoretical definition of the intermediate scattering function (shown in Figure 2a), NSE data give us selective information on the membrane phase and are not affected by the presence of the lipid droplets. The results in Figure 2b allow for several interpretations. First, there is a clear temperature effect on lowering the membrane rigidity, as expected. In line with the aforementioned experiments, the data show marked differences between the samples lacking or containing alkanes in the membrane. In particular, the rigidity is lowered by the addition of the alkanes at lower temperatures, while the trend is inverted at 60 and 70 C with a small alkane-mediated membrane stiffening. Eicosane and squalane give results comparable to one another. A striking difference between the samples with or without alkanes was found by visual inspection of the suspensions after the temperature ramp (Figure 2c). The samples are indistinguishable from one another when prepared, but at the end of the scan the C10mix had lost its turbid appearance, and instead an interface was visible denoting a macroscopic phase separation. In contrast, the samples with alkanes were single phased and maintained turbidity, which is typical of samples containing vesicles. The results show that the alkanes significantly affect the protomembrane bending rigidity: at low temperature, the rigidity is lowered by the alkanes, while the difference is negated (and instead the rigidity is slightly higher) at high temperatures.

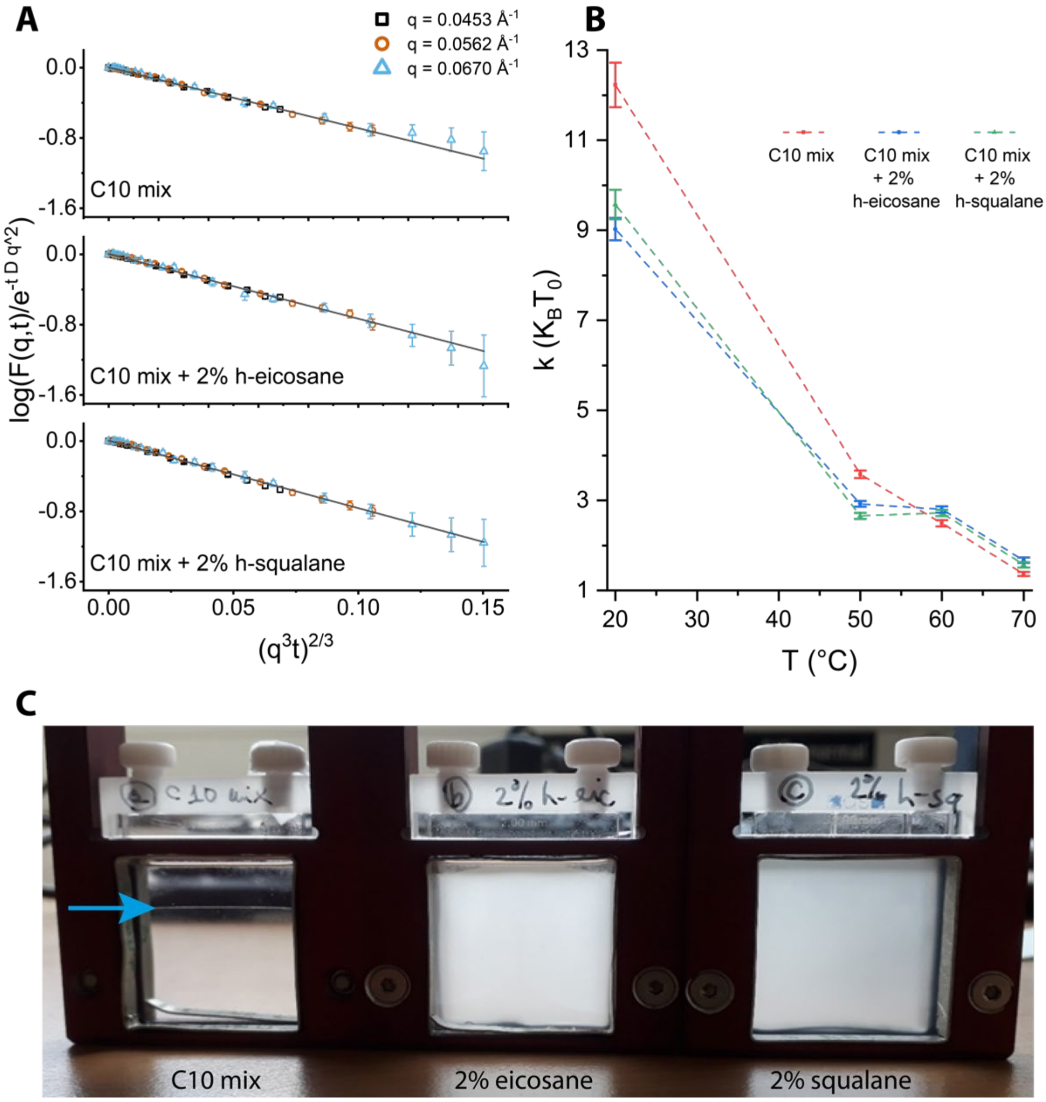

Figure 2. (A) NSE data at T = 70 C plotted in the representation log[F(q, t)/exp(Dq^{2}t)] vs. $(q^3t)^{2/3}$. This highlights the stretched exponential decay predicted by the Zilman–Granek theory [5] and additionally shows that a more complex model taking into account the lipid droplet signal is unnecessary. (B) Plot of the bending rigidity estimates as a function of the temperature. (C) The sample appearance after the NSE thermal scans. The blue arrow points to the interface between the water and lipid phases.

References

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