

# Comparison of CHAPSO and DHPC – Edge-Activators – in Nanodiscs, a SANS Study

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Bicellar mixtures composed of long- and short- chain lipids have served as model biomimetic membranes for the structural study of membrane associated proteins [1-6]. They have many advantages over the traditional detergent-based substrates for stabilizing membrane proteins. First, the planar bilayered region provides a native environment for membrane proteins. Moreover, they are easy to prepare and magnetically alignable – suitable for nuclear magnetic resonance (NMR) study. It has been widely accepted that the edge-activated molecules prefer to locate themselves at the rim of defects on the bilayer forming nanodiscs or perforated lamellae to minimize the curvature energy. While one of the most common short-chain lipids used for bicellar mixture is dihexanoyl phosphatidylcholine (DHPC), another detergent molecule, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), which is often used for stabilizing the membrane proteins and thus is presumably more biologically relevant than DHPC, demonstrates a similar function as DHPC. We have identified the spontaneous structural diagrams of a variety of dimyristoyl phosphatidylcholine (DMPC)/DHPC mixtures using small angle neutron scattering (SANS) [7-9]. However, the structures of DMPC/CHAPSO bicellar mixtures, to the best of our knowledge, have not yet been resolved. Here, SANS is applied on the DMPC/CHAPSO system to investigate their spontaneous morphologies of samples with various lipid concentrations ( $C_{lp}$ ) and temperatures. The effect of the edge-activated molecules on the structural diagram is compared between the DMPC/CHAPSO and DMPC/DHPC systems.

The SANS measurements were performed at N5 spectrometer. The scattered intensity,  $I$ , was collected as a function of scattering vector,  $q$  defined as

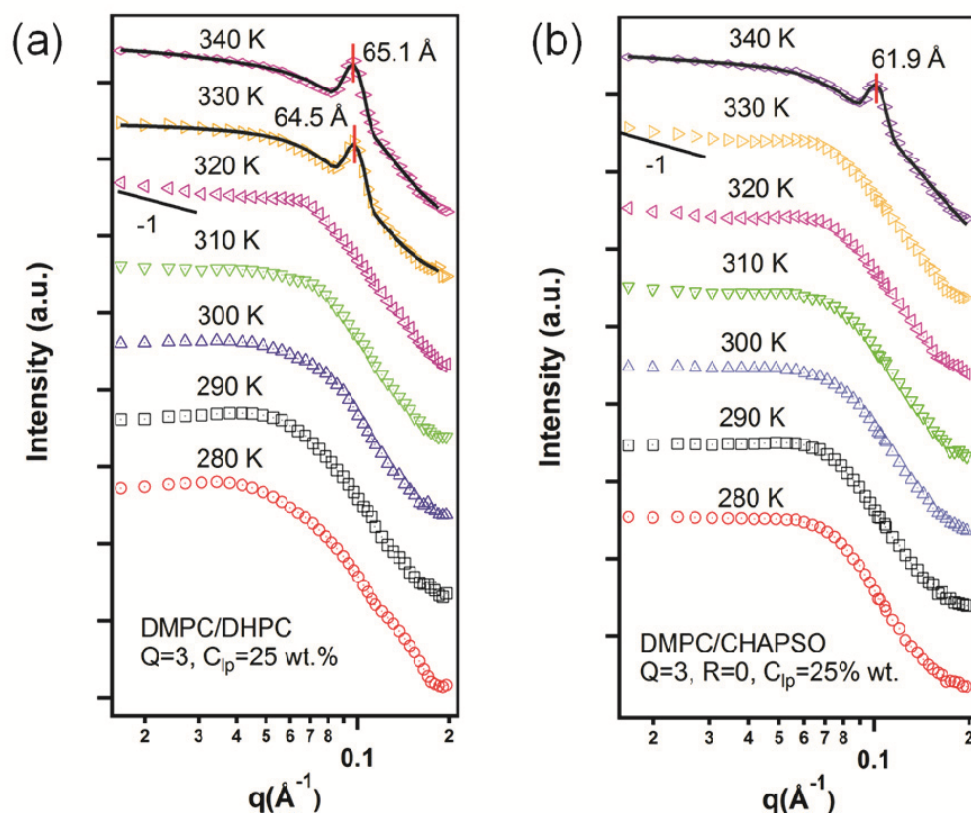
$q = \frac{4\pi}{\lambda} \sin \frac{\theta}{2}$ , where  $\lambda$  and  $\theta$  are the neutron wavelength and scattering angle, respectively. In order to cover a  $q$  range between 0.006 and 0.2  $\text{\AA}^{-1}$ , three different wavelengths of 1.55, 2.37 and 3.99  $\text{\AA}$  were used. The incident beam was focused by a multi-channel focusing collimator and another de-smearing collimator was installed to reduce the vertical divergence on the detector side. A detailed description of the optical configuration and instrumental resolution can be found elsewhere [10].

Figure 1 shows the SANS profiles of 25 wt.% DMPC/CHAPSO and DMPC/DHPC mixture with increased temperature. The SANS patterns and corresponding morphologies of DMPC/DHPC as a function of temperature have been reported elsewhere. It has been reported that at 25 wt.% discoidal bicelles form at low temperatures (lower than the melting transition temperature,  $T_M$  of DMPC  $\sim$  300K). The bicelles (a low- $q$  plateau) then slowly transform into ribbons (a  $q^{-1}$  dependence) and then sharply into multi-lamellar vesicles (MLVs), indicating an invariant Bragg peak at  $q \sim 0.1 \text{\AA}^{-1}$  upon dilution as  $T > 320\text{K}$  [8-9]. The current SANS result of DMPC/DHPC in Fig 1 (a) is consistent with that in the previous study [9]. Like DMPC/DHPC, DMPC/CHAPSO also forms bicellar discs at low  $T$ , and MLVs at high  $T$  ( $\geq 340 \text{K}$ ). However, there are subtle differences between the two systems. First, the formation of MLVs in the case of DMPC/DHPC occurs at  $T = 330\text{K}$ , which is 10 K lower than DMPC/CHAPSO, indicating DMPC/DHPC bicelles/ribbons are less stable as that in DMPC/CHAPSO mixture.

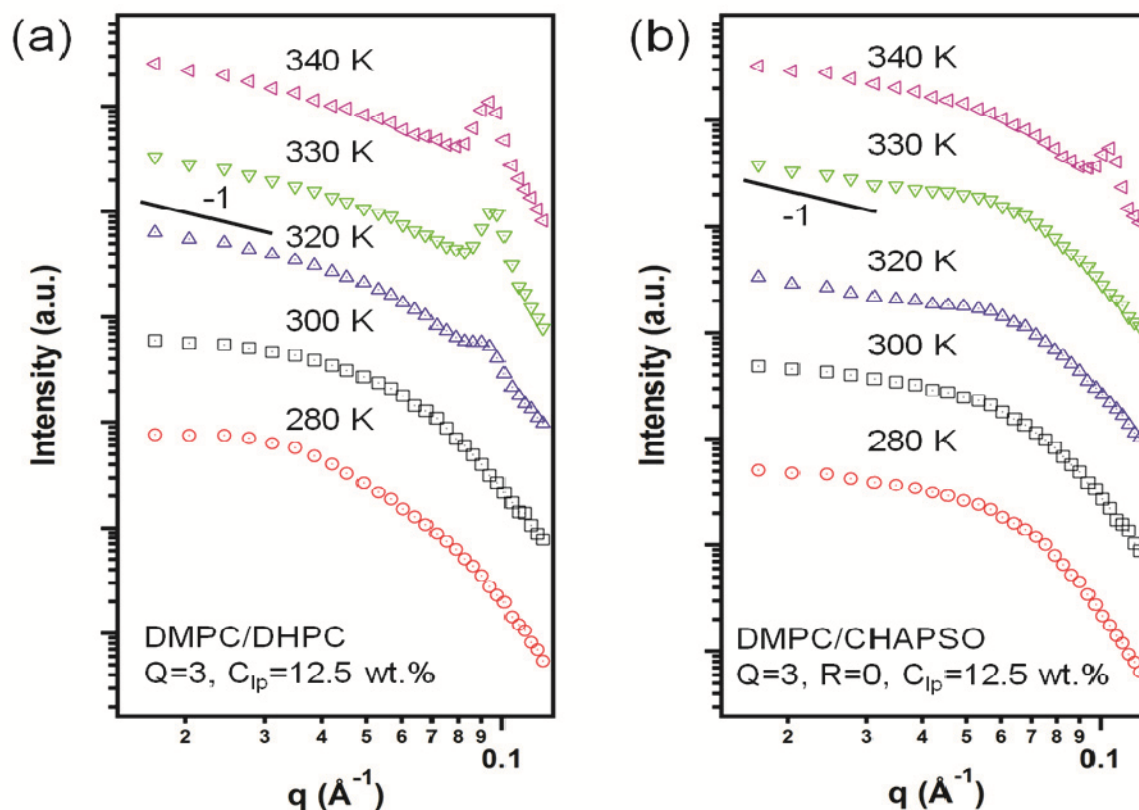
Fig 2 shows a similar structural transformation from ribbon to MLVs in both cases of DMPC/DHPC and DMPC/CHAPSO at a lower  $C_{lp}$  (12.5 wt.%). Nevertheless, the difference in the transition temperature is even greater  $\sim 20\text{K}$  (320K for DMPC/DHPC and 340K for

DMPC/CHAPSO). Data (not shown here) of further diluted samples (e.g., 2.5 wt.%) indicate that the low-T ribbons/bicelles are not stable in the DMPC/DHPC mixtures but remain attainable in the case of DMPC/CHAPSO. The experimental result indicates the edge-activator molecule (i.e., DHPC and CHAPSO) plays an important role in controlling morphologies of the bicellar mixtures. This is presumably attributed to the

miscibility between the edge-activator and long-chain lipid. Since DHPC and DMPC have a similar molecular structure, their miscibility is expected to be higher than that of CHAPSO and DMPC, leading to the morphology with less edges or defects. The study provides a fundamental insight to the structural transformation in this self-assembly process.



**Fig. 1** SANS data of (a) DMPC/DHPC and (b) DMPC/CHAPSO with  $C_{ip}=25$  wt.% at different temperatures of 280K (circles), 290K(squares), 300K (tip-up triangles), 310K (tip-down triangles), 320K (tip-left triangles), 330K (tip-right triangles), 340K (daimonds). The curves are best ift results and red lines represent the peak position.



**Fig. 2** SANS data of (a) DMPC/DHPC and (b) DMPC/CHAPSO mixtures with a  $C_{lp}$  of 12.5 wt.% at different temperatures of 280K (circles), 300K(squares), 320K (tip-up triangles), 330K (tip-down triangles) and 340K (tip-left triangles).

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