

One of the interesting systems addressed in the proposal was the ganglioside GM3 in aqueous solution, with different counterions.

Ganglioside GM3 (Gal -(NeuAc)-Glc-Cer) is a charged glycosphingolipid which aggregates in bilayers when dissolved in water on a large scale of temperature and concentration. We performed SAXS and WAXS measurements over three decades of concentration (0.1% - 15%) in the temperature range 15°- 60°C to study bilayer microstructure. We investigated three different counter-ion GM3 salts, namely GM3-Na, GM3- Ca and GM3-Rb, in a condition of no added salt.

The incident radiation wavelength has been set to 1.2 Å. For SAXS measurements the detector was displaced off-axis and two sample-to-detector distances were chosen, 1.5 m and 6.5 m, in order to cover a q-range extended from 0.003 Å⁻¹ to 0.42 Å⁻¹. WAXS experiments were performed on a q-range from 0.5Å⁻¹ to 4Å⁻¹. Due to the high flux, possible sample radiation damage had to be considered and tested. Sets of 4 repeated measurements were then performed with 0.5 sec irradiation times, and then summed up after comparison.

The shape of I(q) spectra of the concentration series of GM3 solutions, confirm that the local geometry of the membrane bilayer is conserved passing from 0.1% b.w. up to the most concentrated solution at 15% b.w. for all the GM3 salts and for all temperatures. Fig 1 shows the concentration series of GM3-Na at 60°C temperature. The form factor of a model bilayer with a two steps electron density profile is suitable to fit the experimental SAXS spectra.

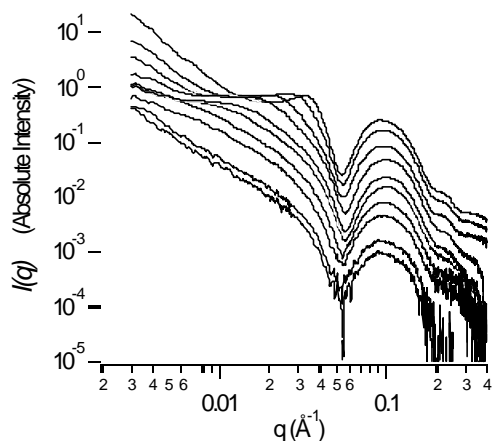


Fig. 1. Scattering intensity at 60°C temperature of GM3 Na salt solutions of increasing concentration, namely 0.1%, 0.2%, 0.4%, 0.57%, 0.8%, 1.7%, 3.5%, 5%, 10%, 15% by weight. Exposure time was 0.5 sec and two spectra taken with the detector placed at 1.5 m and 6.5 m, respectively, were matched to give the reported $I(q)$.

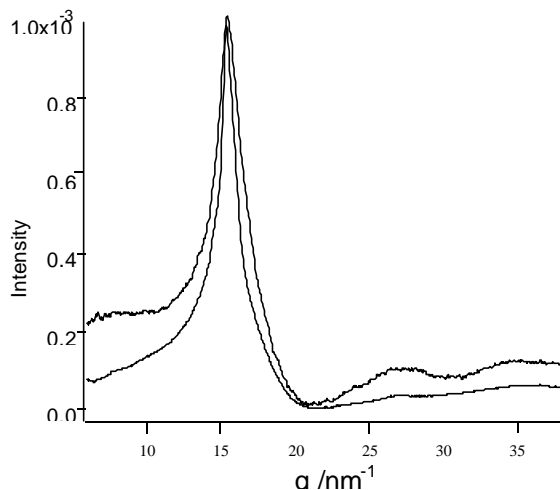


Fig.2. WAXS spectra obtained on bilayers of GM3 (15%, upper line) and of DMPC (60%, lower line) at 15°C. Intensities are rescaled to the same concentration, 15% bw.

This “locally lamellar” structure of the GM3 aggregate is confirmed by parallel WAXS measurements. The WAXS spectrum obtained for the 15% GM3 solution at 15°C can be seen in Figure 2 compared with a spectrum obtained for a 60% DMPC solution at the same temperature, that is in the L_{α} lamellar phase with ordered hydrophobic chains. Intensities are rescaled to the same hydrophobic electron concentration. The DMPC spectrum is well known, the huge and narrow peak at a reciprocal distance of 15.5 nm^{-1} being associated to the strong spatial correlation due to the ordering of the hydrophobic chains below the melting temperature. It can be noticed that, in front of this high intensity peak, the rest of the spectrum is very poorly structured, if even, with a very low intensity on both sides. On the other hand, the GM3 spectrum indicates the presence of a somehow broader main peak, centered around the same q -value, which is, then, not unexpected. But, contrary to DMPC, the structure of the GM3 spectrum out of this region is clearly much richer and defined. At higher q , two additional broad peaks can be identified, in q -ratios $\sqrt{3}$ and $\sqrt{5}$ with respect to the 15.5 nm^{-1} one. In the meanwhile, at lower q , the scattered intensity does not drop to very low values. The structure factor is also determined over the entire range of concentration and for the three different counter-ion solutions.

Results demonstrate strong influence of counter-ion in the absence of screening “buffer”. What actually showed a peculiar behavior was the rubidium GM3 salt. It soon appears that for all the five investigated temperature up to 60°C at the highest concentration of 15% sharp peaks rises over the usual $I(q)$ curves. In the case of the 25°C temperature these sharp peaks are present starting from the 5% b.w. concentration, as can be seen in Fig. 3. The ordered hexagonal symmetry peaks are well appreciated up to 0.1 \AA^{-1} . The pattern of peak sequence exactly satisfy an hexagonal symmetry. On increasing concentration the peak position shift to lower q . It is moreover evident that the sharp peaks of this structured phase rise over the usual broad $S(q)$ peaks of the normal liquid dispersion of GM3 aggregates present in the case of the Na and Ca counter-ion salts.

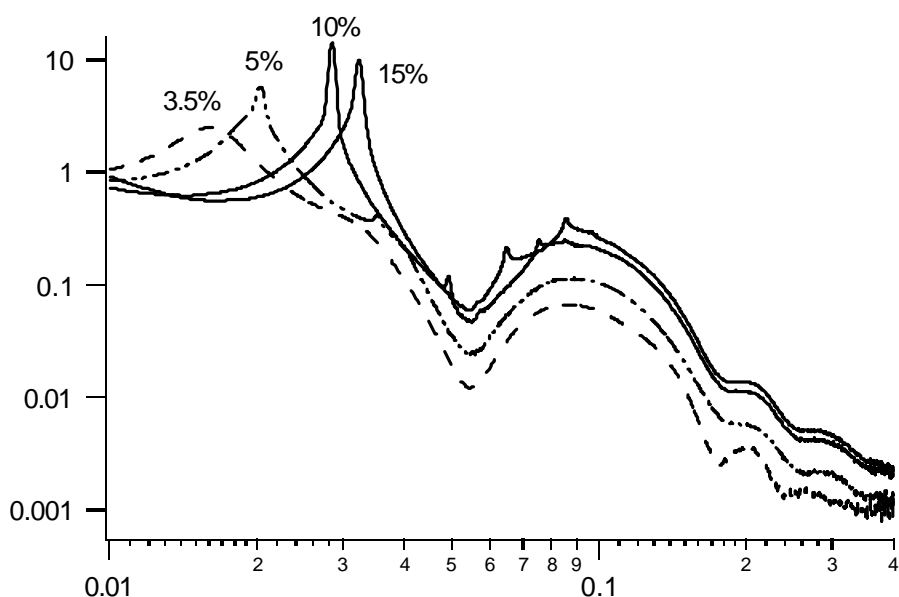


Fig 3. $S(q)$ obtained for GM3-Rb at 25°C for high concentrations. $S(q)$ first peaks are evident. Sharp peaks sequence of ordered exagonal phase is clearly observed at 5%, 10% and 15% bw concentrations.