REPORT 16-02-13: *ARTIFICIAL MEMBRANES AND COLLAGEN DIFFRACTION FOR BIOMEDICAL APPLICATIONS*

The aim of this proposal was to characterize different membrane and micelle structures, such as liposomes, saponin micelles, and lipid bicelles for biomedical applications and drug delivery. Liposomes[1] and bicelles[2] had been previously studied by SAXS, so these studies were focused on the influence of the preparation method in the final structure of lipid vesicles and concentration variation on the formation of bicelles, intended to be a step further in the knowledge of these structures and their applications. Otherwise, saponins are natural surfactants still lacking a deep characterization[3]. In addition, skin samples were analyzed in order to detect the difference of the SAXS patterns of healthy and melanoma affected collagen, as a way to trigger the possibility of cancer detection via SAXS, following the protocol established for breast cancer samples by M. Fernández et al.[4]

The liposomes were formed by phosphatidylcholine (PC): multilamellar vesicles (MLV) were obtained by hydration of a lipid film; some MLV were extruded through polycarbonate membranes with different pore sizes to obtain homogeneous populations of large unilamellar vesicles (LUV); others were sonicated, or processed in a Microfluidizer (pressure cycles) to obtain oligolamellar vesicles (OLV).

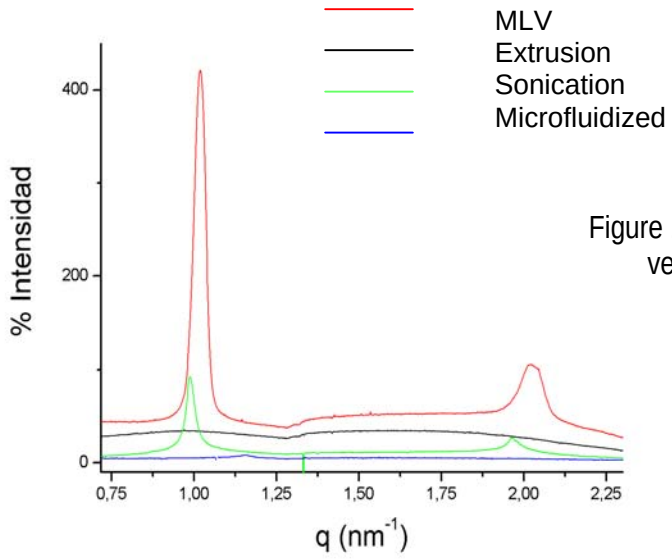

Figure 1: Diffraction peaks corresponding to vesicles prepared by different methods

The results are shown in the Figure 1. MLV and sonicated liposomes presented more intense peaks than extruded and microfluidized ones, as a consequence of a higher number of concentric layers in the liposome structure. Also, sonicated liposomes resulted mainly in oligolamellar structure instead of unilamellar. We can observe slight differences in d-spacings as a function of

preparation method, being smaller for microfluidized liposomes than for the others. It could mean that some lipid hydrocarbon layers are compressed by the pressure applied[5].

In a second task, bicellar systems were formed of dimyristoyl and dihexanoyl PC (DMPC and DHPC) at different molar ratios ([DMPC]/[DHPC]=3,5 or 2), and final lipid concentrations (15 and 20% w/w). As the path of the scattered beam has a long way in the air, a huge air scattering is affecting the curve (Figure 2), which prevented us from a reliable solvent subtraction and analysis. Furthermore, the accessible range truncated the curve in the middle of the scattering peak, making it impossible to get information on the form factor of the bilcelles. Also, the control of temperature, essential to avoid lamellar to bicellar or micellar transitions in these systems, was not available at the station. Thus we cannot be 100% sure of having only bicelles.

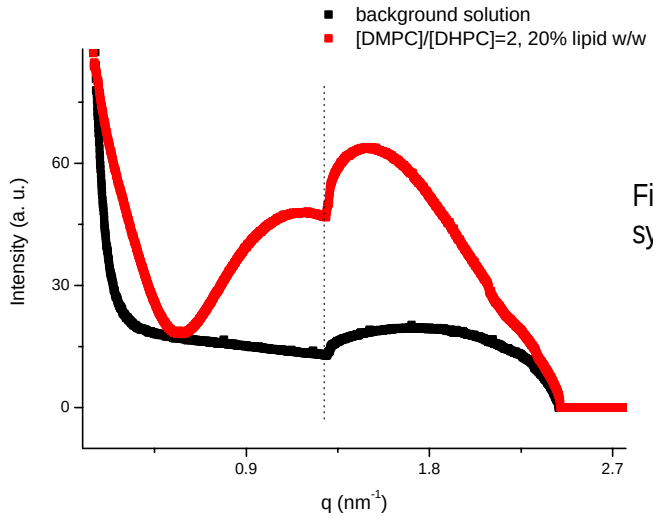

Figure 2: Diffraction pattern of bicellar system and background solution

Our third aim was to determine the differences of collagen in healthy versus melanoma affected skin, which can be seen in Figure 3 as significant changes between both skins. This preliminary result is similar to those obtained by M. Fernández in the studies of collagen presents on breast cancer tissue[3]. From these measurements, detailed analysis of these SAXS curves will be performed to achieve full understanding of the structure of the melanoma affected collagen.

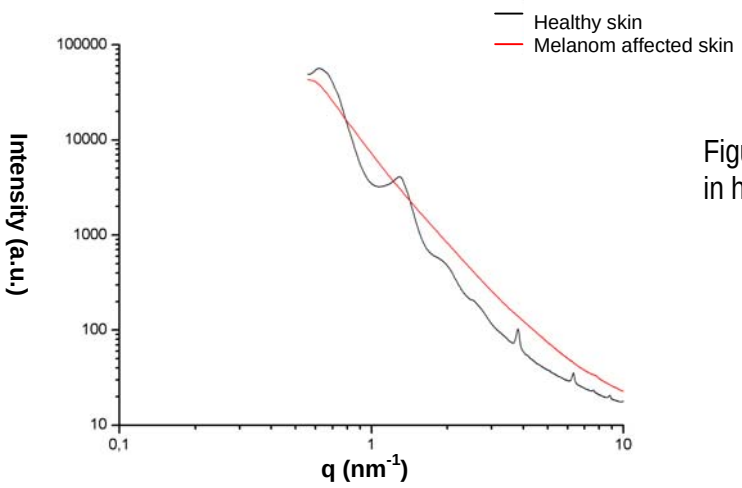

Figure 3: Diffraction curves of collagen in healthy and melanoma skin

Finally, the experiments on Quillaja bark saponin showed for the first time that the system exhibits enough electron density difference to be studied by SAXS. However, detailed analysis of the curves was not possible due to the air scattering mentioned above. Figure 4 illustrates the problem and the need of set-up improvements to get good quality results.

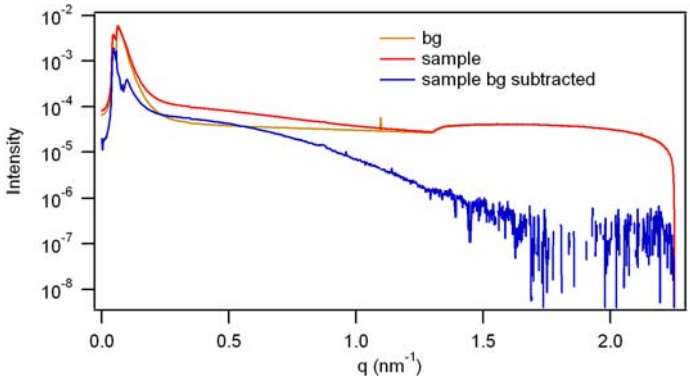

Figure 4: Scattering curve of a simple containing 8.4 wt% of QBS in water. Measured sample, background (BG) solution and BG correct curve.

[1] M. Cócera et al., Langmuir 2004, 20, 3074-9.
[2] S. Mitra and S. Dungan, J.Agric.Food Chem. 2001, 49, 384-94.
[3] M. Fernández, Thesis, Helsinki (Finland) 2006
[4] N. Hauet et al., Biophys. J. 2003, 84, 3123-7