ESRF	**Experiment title:** **The role of lipopolymers in sterical stabilization of phospholipid liposomes**	**Experiment number**: SC-3269
Beamline: ID02	**Date of experiment**: from: 29.08.2011. to: 02.09. 2011.	**Date of report**: 14.03.2012.
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Report:

Sterically stabilized liposomes (or vesicles with other word, SSLs or SSVs), are the most commonly used drug delivery vehicles in nanomedicine. Their long circulation time in vivo is assured by the incorporation of lipopolymers into the liposomal constituents, which serve the stabilization. In our experiment at ID02 we intended to study the structural features of liposomes prepared with different kind of lipopolymers and the effect of their relative molar ratio on the overal structure of liposomes.

The pharmacologically most relevant lipopolymer in SSLs is poly(ethylene glycol) (PEG) covalently attached to phosphoethanolamine (mPEG2000-DSPE or DSPE-PEG2000). Describing the electron density profile of the bilayer with the superposition of Gaussian functions, the contribution of the PEG layers to the total electron density was identified by model fitting of the SAXS curves of the investigated systems. The changes in the thickness of the PEG layer as well as the distribution of the PEG chains among the outer and inner leaflets of the bilayers were followed by changing the molar ratio of the PEG-lipid and the molar weight of the PEG molecule. These result from our last beamtime were published in Chemistry and Physics of Lipids.[1]

We obtained asymmetric distribution of the PEG-lipids for the lipid composition identical to Doxil, the first approved liposomal drug (i.e. HSPC[2]:Cholesterol:DSPE-PEG2000[3] = 3:1:1 by weight), and symmetric for the same composition but containing a PEG-lipid with halved molar weight of the PEG group. The distribution of the PEG-lipids is also asymmetric for the sample with doubled, and symmetric with halved molar ratio of DSPE-PEG2000.

[1] Z. Varga, A. Wacha, U. Vainio, J. Gummel, A. Bóta, Characterization of the PEG layer of sterically stabilized liposomes: a SAXS study, Chem. Phys. Lipids, In press corrected proof, 2012.
[2] hydrogenated soy-lechitin
[3] 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)

SAXS curves of the investigated samples, and the resulting electron density distributions are shown in Fig. 1 and Fig. 2, repsectively. Detailes of the fitting procedures and results can be found in Ref. 1.

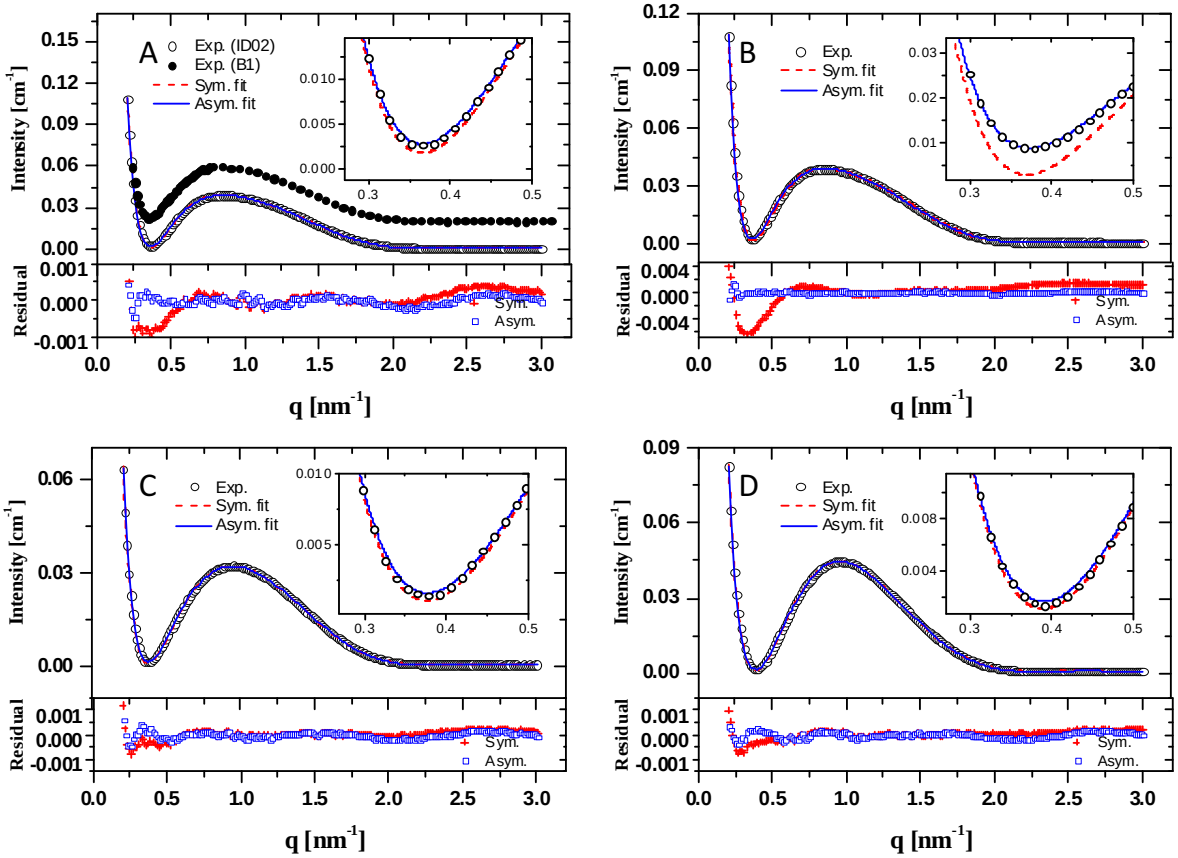

Fig. 1 Measured SAXS curves corresponding to the liposome composition in weight ratio of HSPC:DSPE-PEG2k:Cholesterol 3:1:1 (SSL-1: A), 3:2:1 (SSL-2: B), 3:0.5:1 (SSL-0.5: C) and HSPC:DMPE-PEG1k :Cholesterol 3:0.6:1 (SSL-PEG1k: D). The best model fits using the symmetric (dashed red line) and asymmetric (solid blue line) models are also shown in the figure. The insets show the q-region of 0.3 – 0.5 nm^{-1}, where there are a significant differences between the two models for the SSL-1 and SSL-2 samples (A and B, respectively). The reproducibility of the measurements is demonstrated in A (SSL-1 sample), where the measured curves from the B1 and ID02 beamlines are shown (the curve from B1 is shifted for better visibility).

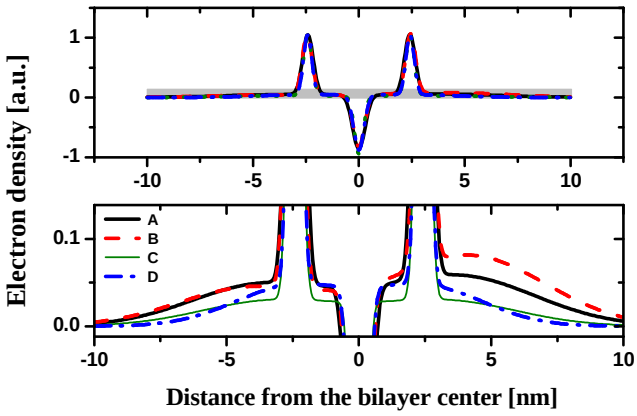

Fig. 2 The reconstructed electron density profiles of the systems shown in Fig. 1 using the model fitting procedures described in Ref. 1.

We have also investigated the bilayer structure of the liposomes stabilized by distearoyl-PEG2000 (a neutral PEG-lipid) and hyaluronic acid covalently attached to extruded phosphocholine/phosphoethanolamine liposomes. The differences in the form factors of the bilayers can be followed on the scattering curves, the detailed analysis of which is under way.

Beside the bilayer structure, the size and size distribution of the whole liposomes can also be investigated based on our SAXS measurements. Fig. 3/A shows the SAXS curves of SSL systems (HSPC/Chol./DSPE-PEG2000 = 3:1:1 by weight; in PBS[4] pH 7.4 buffer) prepared by extrusion through polycarbonate filters with pore size of 100, 80, and 50 nm.

[4] phosphate buffered saline: 10 mM phosphate, 150 mM NaCl

First, we have calculated the size of the liposomes from the Guinier-radius obtained from the curves assuming a spherical shell model. We got 109.7, 95.3, and 86.8nm for the above mentined samples, which is slightly larger than the pore size of the used membranes. The latter is due to the deformation of the liposomes during the extrusion. These sizes are in good agreement with the values from dynamic light scattering (105.7, 94.5, and 84.7 nm, respectively), and from freeze-fracture combined transmission electron microscopy. Supplemented by a full q-range model fitting procedure these investigations will provide a comprehensive study on the size of these pharmacologically relevant liposomes[5]. The measurement of the SAXS curves of SSL and its unPEGylated form were also measured in the temperature range of 20 – 60 °C, which revealed an inrease in the overal size and change in the bilayer structure of the liposomes.

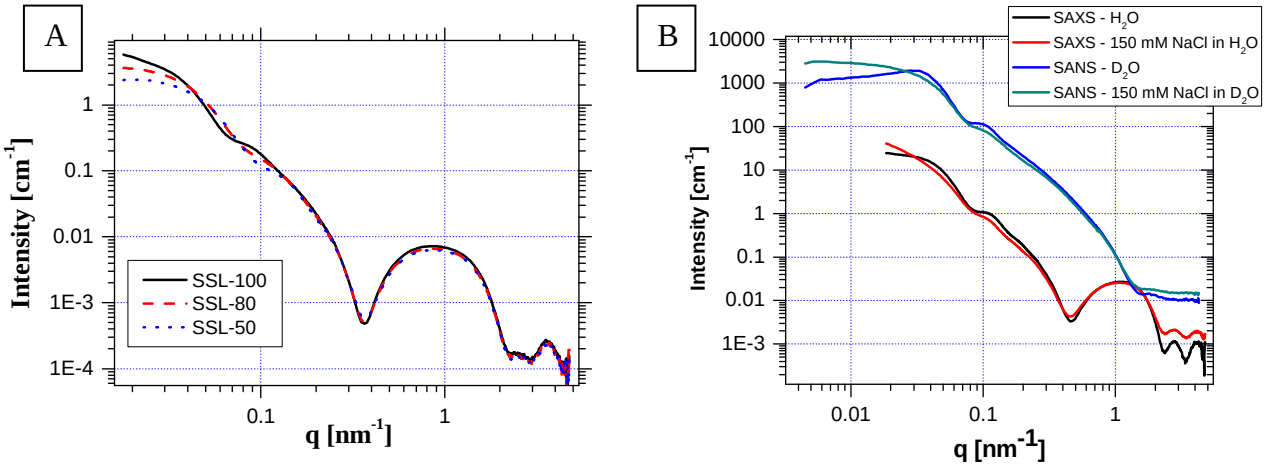

Fig. 3 (A) SAXS curves of SSL systems extruded through polycarbonate filter with pore size of 50, 80, and 100 nm (SSL-50, SSL-80, and SSL-100, respectively). (B) SAXS (from ID02) and SANS (from D11 at ILL) curves of extruded DPPC/DSPE-PEG2000 liposomes in water (D_2O in the case of SANS) and in 150 mM NaCl solution.

Fig. 3/B shows the scattering curves of DPPC[6]/DSPE-PEG2000 = 100 :5 (mol/mol) systems prepared in MQwater and in 150 mM NaCl solution, extruded using membranes with 100 nm pore size. For comparison, the SANS curves of the same systems (but prepared in D_2O), measured at the D11 beamline of ILL, are also shown in the Figure. The salt free systems exhibit a clear correlation peek due to the charged character of the SSLs and it vanishes upon increassing the salt concentration hence decreasing the screening length. It is also notable, that the available q-range in the case of SAXS cannot resolve unambiguously this phenomena, so either the use of USAXS or SANS could reveal the effective interactions between SSLs. Nevertheless, these results underline our previous observation that SSLs in physiological media can be treated as independenet particles.

Time resolved SAXS measurements on the interaction of SSL with bovine serum albumin (BSA), and the osmotic shrinkage of the liposomes systems were also studied. Concerning the interaction with BSA, we found no pronaunced sign of binding of the albumin to the liposomes what is in line with the purpose of PEGylation, i.e. to prevent protein absorption in vivo.

We also studied the osmotic shrinkage of liposomes by using samples prepared in 10 mM TRIS-HCl pH 7.4 buffer, and mixed with 300 mM NaCl solution (in the same buffer) in one to one volume ratio with the stopped flow device available at the beamline. We have observed changes in the SAXS curves that can be related to alterations in the shape of the liposomes (presumably deformation to ellipsoids) as well as in their bilayer structure in the time scale of several seconds (work in preparation). To our best knowledge, these results are unique in the field, and will considerably improve our knowledge about the structure and stability of SSL systems.

[5] Z. Varga, A. Wacha, J. Gummel, T. Kremmer, A. Bóta, A comprehensive study of the size of sterically stabilized liposmes, in preparation.
[6] 1,2-dipalmitoyl-sn-glycero-3-phosphocholine