EXPERIMENTAL REPORT

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TITLE Localisation of iron deposition inside magnetic liposomes by time-resolved Neutron scattering TR-SANS after pH-change

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Liposomes are biocompatible hollow nanoparticles, which are established for DNA, dye, Boron or drug transport in research and clinical applications. While several groups work with metal bearing liposomes, we found a novel method of manipulating liposomes by enclosure of a magnetic iron oxide shell inside: magnetic shell liposomes (see preparation details in ILL-report 9-10-661). In this first study by time-resolved neutron sacttering and electron microscopy the structure-stability limits during the most critical preparation step, the iron oxide shell deposition during a large pH-jump with iron-chelate entrapped neutral liposomes, were estimated. The lipids were purified soy-bean phospholipids (SBL, 40% lecithin) and DMPC (Di-Myristoyl-Phosphatidyl-Choline). The pH-jump was varied between pH10.5 and 13 by stopped-flow mixing of liposomes and bases during observation by TR-SANS at the D22 instrument with an initial resolution of 1 s (5,3% log. increase/ frame) for up to 2 h and electron microscopy (SANS samples and pilote experiments, TR-EM).
Results: As shown in fig.1-4, the value of the pH-jump turned out to be very critical: A low pH-jump (7 $\Rightarrow$ 10.5 and below) yielded a delayed and weak iron oxide deposition, while a large pH-jump (7 $\Rightarrow$ 12.5 and more) and high ionic strength destroyed the liposomes rapidly (fig.2,3). In a small range of the pH-jump (7 $\Rightarrow$ 11 -12) the iron deposition was complete in the preserved liposomes, which during preparation of magnetic liposomes are subsequently neutralized. At pH11 the lipid SBL (fig.1) was less base-stable as compared to DMPC (fig. 4). The shell-structure of the magnetic liposomes was obtained from Kratky-Porod plots of SANS. Liposomes with correct pH-jump (= fig.1 after 1800s, fig.4) had a break in the plot (fig.5), which indicates a double shell (d = 4,81±0.08 & 5.94±0.25 nm). This was directly demonstrated by electron microscopy (fig.6, unstained iron image). **Conlusions:** Magnetic liposomes are obtained in a narrow pH-jump range, some improvements are possible.
References: T. Nawroth, M. Rusp, R.P. May a) European Neutron Scattering Conference (2003) proceedings, K3 & K40; b) GDCH conference Munich (2003) proceedings, c) Physica B (2004), in press

Figures: **(1)** Experiment N° 8-03-413 **(2)**

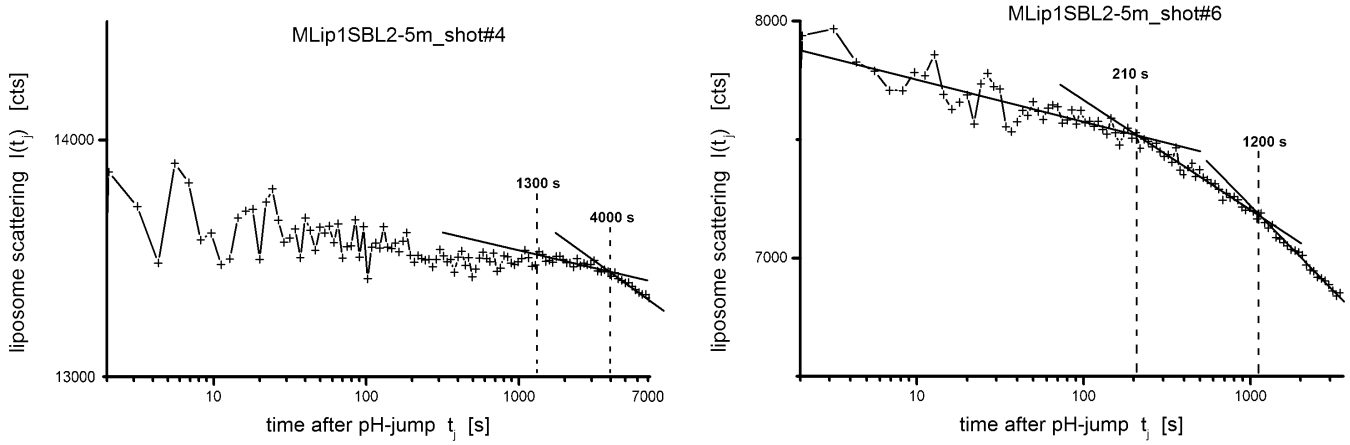

Fig.1) During time resolved neutron scattering of liposomes from SBL (10g/l, H_2O) after a moderate pH-jump to pH10.5 by stopped-flow mixing (1:1) with 1 M Tris-KCl buffer the iron oxide deposition takes 1300s, the liposomes decompose after 1h (4000s); while **(Fig.2)** after a large pH-jump to pH13 with 0.5M NaOH-buffer, decomposition starts at 210-1200s.

(3) **(4)**

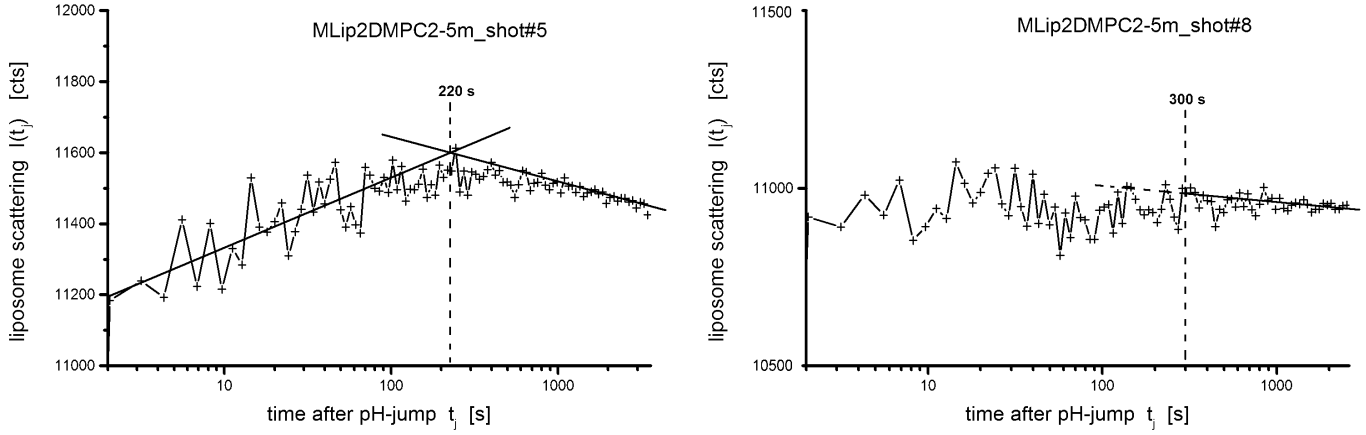

Fig.3) During time resolved neutron scattering of liposomes from DMPC (10g/l, H_2O) after a strong pH-jump to pH13 by stopped-flow mixing (1:1) with 0.5 M NaOH-KCl buffer the iron oxide deposits within 220s, the liposomes decompose after t_j > 600s.; while **(Fig.4)** after a moderate pH-jump to pH11.5 with 0.05 M NaOH- buffer, the liposomes are stable for > 2000s, the iron oxide shell deposits during 300 s.

Fig. 5) SANS of magnetic liposomes (SBL) 30 min after a pH-jump with 1M Tris to pH10.5

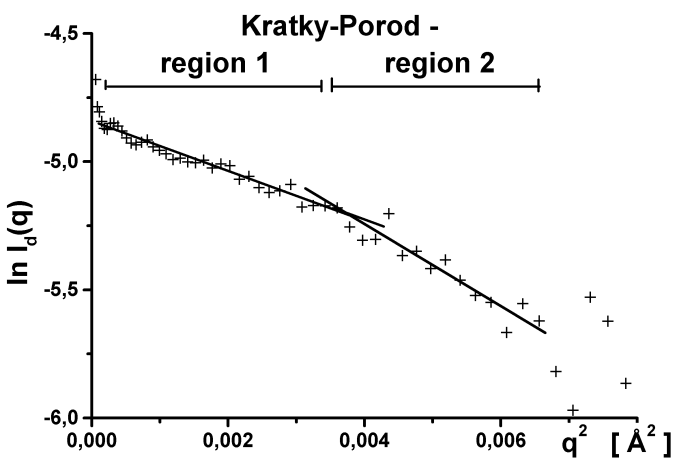

Fig.6) Electron micrograph of a magnetic liposome showing iron and lipid shells