Magnetic Liposomes and Boron Entrapping for Biomedical Applications – Time Resolved Neutron Scattering TR-SANS and Electron Microscopy TR-EM

ECNS2003 , K40,
Montpellier 3.-6.9. 2003

Thomas Nawroth [1a], Monika Rusp [1b], Roland May [2]

www.mpsd.de

Membrane Protein Structure Dynamics (MPSD) project

1) TU Muenchen, Physics Department, a)E17, b)E22, James-Franck-Str., D-85748 Garching, Germany
2) Institut Laue Langevin ILL, Large Scale Structure Group, Avenue des Martyrs, F-38042 Grenoble CEDEX, France

Liposomes – hollow Nanoparticles

Liposomes are **biocompatible hollow Nano-particles** covered by a lipid bilayer. They can be used as carriers for material entrapped inside the lumen and at the surface in cell-biological and medical applications [1].

Liposome applications can be impro-ved, if the liposome can be detected or mani-pulated by **magnetic forces** [2]. In this case the liposomes can either be dragged or deposited in a region of interest, e.g. a tissue or tumor. A further application is enhanced magnetic imaging using NMR / MRI techniques. This requires liposomes ex-hibiting a strong macros-copic para-mag-ne-tic moment, i.e. **magnetic liposomes**.

A further bio-functionalization of liposomes for regio-selectivity or enzymatic activity can be achieved by insertion of membrane proteins, which retains the entrappered material, if the "detergent assisted reconstitution into preformed liposomes" is applied [3]. **Size, structure and development / dynamics** can be analyzed by neutron small angle scattering SANS of liposome solutions [4] (online) and electron microscopy EM (off-line), especially with time resolved methods (TR-SANS, TR-EM).

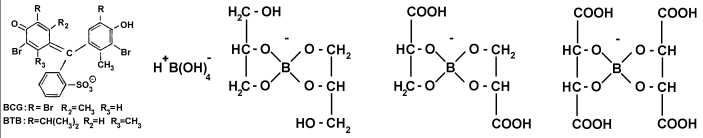
For **biomedical** applications additional **material** can be **entrapped inside** the lumen of the liposomes [1]; Dyes, drugs, metal ions, e.g. Gadolinium, Platinum, Iodine or Boron compounds can be included. In the latter case specific liposomes can be used for local cancer radiotherapy using Neutron capture in the ^{10}B (N,?) ^{7}Li reaction.

Entrapped compounds :

Dye **Boronate**

Sulfo-Phtaleins — **BCG, BTB**

Water soluble Boron compounds : **Borate** | **Bis-Glycero-Borate (BGB)** | **Boro-Bis-Glycerate (BBG)** | **Boro-Bis-Tartrate (BBT)**



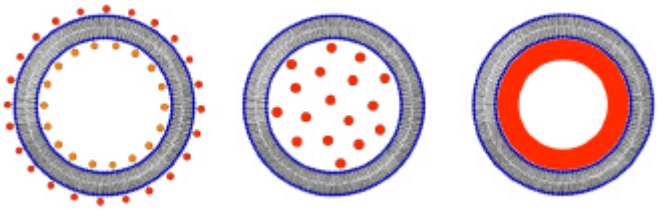
The compounds, which were entrapped into magnetic liposomes in the current dye and Neutron scattering study, are water soluble. Due to the hydrophilic charged structure, they cannot escape the liposome lumen.

With the sulfo-Phtaleins Bromo-Tymol-Blue (BTB) and Bromo-Cresol-Green (BCG) the proton flow across the lipid membrane is tracked. A 1:1 mixture of these indicators is a virtual "super-dye" of 4 pH units working range.

The Boronate-esters BGB, BBG and BBT [5c] are stabilized by Polyol binding. Magnetic liposomes of 250 nm size, bearing a double wall (5 nm lipid and 6 nm iron-oxide) enclose an inner volume of $7.15 \cdot 10^{-15}$ l / particle. Entrapping of these Boron compounds yields **10^8 – 10^9 Boron atoms / liposome** (c_{Boron} = 25 mM – 0.25 M BGB) .

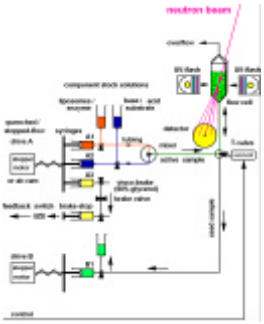
Structure principles of Metallo–liposomes

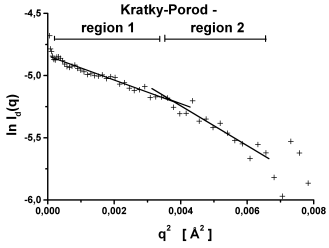
a) b) c)



Metallo-liposomes can bear the metal supplying magnetic properties as well as specific radiation interaction, in three structures: a) metal-lipid liposomes, e.g. Me-DTPA-DMPE or Me -DTPA - StearylAmide, bearing the metal inside and outside (different metals possible); as used for ASAXS at ESRF-ID1 and DESY [1], b) liposomes entrapping metal-oxide nanoparticles ($Me_xO_y)_n$ or metal-chelate (DTPA-Gd, -Sm, -Fe, -Ho, -Dy, or cis-Pt, and c) metal-oxide shell liposomes bearing a double wall structure : lipid (outside) and metal-oxide (inside). For biomedical applications the metal Me is Iron or Gadolinium: Fe-chelate, or Gd-chelate (DTPA-lipid [1]), or Fe–oxide; e.g. ?-Fe_2O_3

Time resolved Neutron scattering TR-SANS

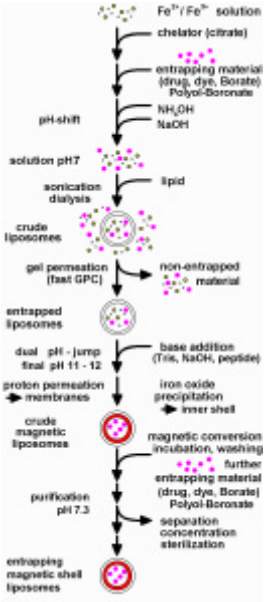




Setup for time resolved Neutron small angle scattering TR-SANS at ILL-D22. The crude liposomes (from fast GPC) with entrapped iron chelate and Boronate are subjected to a pH-jump by fast mixing with a stopped-flow device [5b]. The structure film is collected with logarithmic time scale (5.3 % time increase / frame).

Neutron scattering of crude magnetic liposomes from 10 g/l SbPC (purified Soy bean Phospholipids, mainly DiLinoleylPhosphatidylCholine) after a pH-jump at proton permeation equilibrium (30 min) as Kratky-Porod plot. The straigth lines indicate layer thickness of d_1 = 4.81 ? 0.08 nm and d_2 = 5.94 ? 0.25 nm.

Preparation of entrapping magnetic shell liposomes



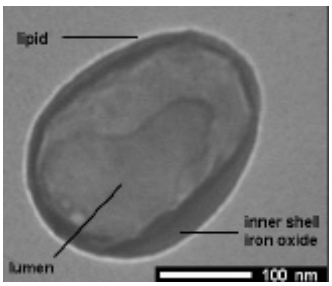
The magnetic shell liposomes with entrapped material (drug, dye, Borate, Polyol-Boronate) are obtained in a sequence of preparation steps including a pH-shift, ultra-sonication, and a dual pH-jump.

In the current study, the magnetic liposomes were prepared by a novel method from stabilized iron-complex solutions and biogenic phospholipids (DOPC, DMPC, Soybean lecithin SbPC) using a pH-shift/ pH-jump procedure and analyzed by time resolved neutron small angle scattering TR-SANS and electron microscopy [5].

During the preparation ions, dye or Boron compounds were entrapped inside the liposomes in parallel to iron oxide. This enables optical detection, as well as later biomedical applications with Neutron capture and rheological experiments with magnetic tweezers, i.e. estimation of forces at membranes.

The iron oxide shell inside the liposomes precipitates during the final alkali-jump and subsequent slow proton permeation across the lipid membranes.

Electron Microscopy



Unstained electron micrograph of a magnetic liposome from Soy bean Phosphatidylcholine SbPC. The iron oxide shell (dark) is attached inside the lipid layer (light) (original magnification 21,000x; Philips CM100, CCD). The dimensions of this individual particle are 288 x 215 nm, size s = 252 nm; outer lipid layer 4.2 nm. The inner iron oxide shell has an apparent thickness of 6.0 nm.

Conclusions

- The formation of the liposomes and the internal iron oxide structure was observed by TR-SANS and EM .

- The iron oxide was obtained as shell at the inner surface of the lipid layer. Thus our magnetic liposomes can be depicted as "magnetic shell liposomes".

- Entrapping for targetting applications was examinated with Boronates. The magnetic shell liposomes revealed a size of 100-400 nm, as required for applications in vivo [5]. For a 0.25 M solution in 250 nm liposomes the entrapping rate was 10^9/ liposome (giga-bor entrapping).

References

[1] C. Oja, P. Tardi, M.P. Redelmeier, P.R. Cullis, *Biochim. Biophys. Acta* **2000**, 1468, 31.
[2] Y.R. Chemia, H.L. Grossman, Y. Poon, R. McDermott, R. Stevens, M.D. Alper, J. Clarke, *PNAS* **2000**, 97, 14268.
[3] H.-J. Freisleben, K.Zwicker, P. Jezek, G. John, A. Bettin-Bo-gutzki, K. Ring, T. Nawroth, *Chem.Phys.Lip.* **1995** 78, 137.
[4] T. Nawroth; et al., *Physica B* **1989** 156, 489,
[5] T. Nawroth, M. Rusp , R.P. May, a) *Physica B* **2004**, 650 S, E635; b) ILL report 8-03-413; c) ILL-report 9-10-661
[6] T. Nawroth, R. Gebhardt, K. Zwicker, G. Goerigk, DESY-HASYLAB Annual report **2000, part1**.