Analysis of Supercooled Smectic Nanoparticles by Asymmetrical Flow Field-Flow Fractionation

Introduction

• Supercooled smectic nanoparticles are under investigation as carrier systems for poorly water-soluble drugs [1,2].

• In dependence on the type and concentration of the stabilizer(s), these dispersions are complex systems where different colloidal species like vesicles formed by excess stabilizer(s) may coexist with the smectic nanoparticles [1].

• Asymmetrical flow field-flow fractionation (AFFF) combined with multi-angle laser light scattering (MALLS) is a promising method for the analysis of such complex colloidal formulations due to its versatility, broad separation range (a few nm up to about 1 μm) and the possibility to obtain homogeneous sample fractions. In addition to the size determination, semi-preparative AFFF was employed to obtain a defined fraction of smectic lipid nanoparticles which were then used to evaluate the feasibility of the preparation of PEGylated smectic lipid nanoparticles by a simple post-insertion process [4].

Experimental Setup

Asymmetrical Flow Field-Flow Fractionation (AFFF)

An Eclipse separation system (Wyatt) equipped with a trapezoidal channel (height 350 μm, length 265 μm, longest width 21 and 46 mm for the analytical and semi-preparative channel, respectively) and coupled with a MALLS (Dawn EOS [Wyatt]) and a diode detector (Shimadzu) was used.

A polyethylene sulfone membrane (MWCO 50,000) served as accumulation wall and bi-distilled, sterile (pore size 0.1 μm) water preserved with 0.02 % sodium azide was used as carrier liquid and for sample dilution.

100 μl of the samples were injected during focusing (focus flow 2 ml/min) and eluted under varying cross flow (CF) conditions and a detector flow of 1 ml/min.

Data were analysed by the Astra software version 4.90.

Photon Correlation Spectroscopy (PCS)

HPPS (Malvern), 4 measurements with 12-14 runs to 10 s.

Results

• AFFF/MALLS measurements of the original dispersion of smectic nanoparticles (I and II) clearly indicate the presence of a distinct fraction of small colloidal structures (note the strong RI-detector signal around 15 mm and the bimodal size distribution).

• The small-size fraction probably represents mainly vesicles formed by the excess of the phospholipid which have been detected earlier by electron microscopy [1].

• The use of a semi-preparative channel (III) allows fractionation of higher concentrated samples. Even injection of the original dispersion (injected mass CN about 5 mg) did not result in a channel overload.

• Sample fractions, collected between 30 and 45 mm were combined and concentrated by centrifugation/ultrafiltration (Vivaspin 20, MWCO 100 kDa, Sartorius; 17 mm; 25 °C, 3000 s). The CN concentration of the purified nanoparticle dispersion was about 1 mg/ml as estimated by the SEC chromatography.

• The purified sample had a monomodal size distribution and a high single homogeneity was also indicated by PCS (IV and V).

• By the application of an initial high and constant CF of 3 ml/min, the free micelles could accurately be separated from the lipid nanoparticles and quantified.

• There was only a minor decrease of the determined mass of free micelles after incubation with the lipid nanoparticles with a bit lower content of free micelles in the sample incubated at 90 °C (VIII).

• The elution profile of the nanoparticles was somewhat altered after incubation but the size distribution remained almost unchanged (IX). No differences in the content of the free micelles and the size distribution of the lipid nanoparticle were found in measurements 1 and 6 days after starting incubation.

Summary and Conclusion

• Highly homogeneous smectic lipid nanoparticles could be obtained by semi-preparative AFFF and interactions of DSPE-PEG micelles and the lipid nanoparticles could thus be studied without a potential interference by additional small colloidal structures (e.g. vesicles) which have been detected earlier in the electron microscope and which could also be found in the original dispersion by AFFF/MALLS.

• Most of the micelles remained intact after incubation with the lipid nanoparticles indicating that this post-insertion process in the evaluated CN/DSPE-PEG ratio was rather ineffective.

In our ongoing work, collected sample fractions shall be analyzed to verify the presence of DSPE-PEG in the fraction of lipid nanoparticles and incubation conditions shall be optimized.

References


Elution conditions: Constant CF of 3 ml/min for 20 min, CF decreasing from 3 to 0.5 ml/min within 3 min.

Inubation Study

Incubation conditions: Constant CF of 3 ml/min for 15 min, CF decreasing from 3 to 0.5 ml/min within 5 min, CF decreasing from 0.5 to 0 ml/min within 20 min. 5 % ethanol without initial concentration CF and starting the first CF gradient from 2 ml/min. Incubated samples were diluted 1:5 v/v.

Elution profiles and size distributions of the original dispersion of smectic nanoparticles (I and II) and after incubation with lipid nanoparticles (III). MALLS analysis is not possible at high sample concentration.

Elution profile and size distribution of the purified nanoparticles after incubation with lipid nanoparticles shows no differences in the concentration of the free micelles and the size distribution of the lipid nanoparticles.