Experimental Report

Proposal: **9-10-1266** **Council:** 4/2012

Title: Kinetic formation of squalenoïde nanomedecine

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: squalenic acid
D2O
ethanol
acetone

Instrument	Req. Days	All. Days	From	To
D22	2	2	31/10/2012	02/11/2012

Abstract:

Recently, it has been shown that nanoparticles made of biological active principle are more efficient than the molecule itself. The linkage between a nucleoside analogue (anti cancer activity) to a squalene leads to nanoparticles formation with an average size of 100nm. This increases the therapeutic index and the efficiency of the administration. Up to day, for these new systems, the control of size and polydispersity with nanoprecipitation is not optimized and the mechanism of the nanoparticle formation not fully understood.

The aim of this proposal is to obtain the time resolved small neutron scattering signal from squalene derivative nanoparticles in D2O during their formation. A non toxic model (squalenic acid) with similar behavior will be used. The mixing is ensured by a home made millifluidic set-up with a continuous flow after the mixer allowing to access second time resolution by coupling space and time. Different concentrations and different solvent ratio will be studied to identify the key parameters that control the final size and polydispersity. The experimental results will be modeled by nucleation and growth theory to elucidate the underlined mechanism

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Date: 31.10.2012 to 02.11.2012
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Nucleoside analogues are a class of therapeutic agents with significant anticancer or antiviral properties. Recently, Couvreur et al[1] have observed an increase of their therapeutic index when the nucleoside is coupled to an acyclic isoprenoid chain of squalene. This new compound is able to form spherical self-assembling system from the well-known nanoprecipitation method. The principle consists of two steps 1) the addition of an ethanol solution of the nucleoside (not soluble in water) into water 2) the evaporation of ethanol to obtain the final solution of nanoparticles. The major drawback of this very promising strategy is the lack of control in size and polydispersity of the obtained nanoparticles, synthesis being optimized after trial and error tests. The aim of this project is to elucidate the mechanism of the formation of the nanoparticles to obtain at the end a protocol for shape and size control. To attain this objective, we took benefit of the H/D contrast in SANS to fully characterize the squalene derivated nanoparticles. It was observed from Dynamic light scattering (DLS) experiments that the squalene derivated nanoparticles have a wide size distribution (between 100 and 400 nm). We thus aimed to perform the measurement at lowest possible q value that can be achieved by the instrument. The SANS experiments were carried out at D22 spectrometer from 31.10.2012 to 02.11.2012.

Two different systems were examined during this experiment. They are- squalenic acid nanoparticles (SG) and deoxycytidine squalene nanoparticles (CS). The particle formation of squalenic acid and deoxycytidine squalene were studied just after mixing of squalene derivatives in ethanol and water and also after the organic solvent evaporation. The final states corresponding to the organic solvent evaporated state of nanoparticles were prepared at LIONS laboratory, CEA Saclay and the intermediate states (nanoparticles with ethanol) were prepared at Chemistry laboratory, ILL during the measurements. The evaporated samples were prepared before the visit to ILL, either a few days before or a week before. Three collimations were used at 1.15nm of wavelength. Most of the SG samples were examined at Small Angles (**SA- 0.014 to 0.31nm^{-1}**) and Medium Angles (**MA- 0.063 to 1.03nm^{-1}**) (5m/col 8m), whereas the CS series were also examined at Large Angles (**LA- 0.21 to 3.3nm^{-1}**).

The kinetic measurement by direct mixing in the flow through cell could not be tried. One possibility would have been to look for one every minute after mixing during 20 minutes (in SA for SG and SA and LA for CS) but as the diagrams at different angles were merging correctly even if they were recorded at distant times of minutes (even hours), we consider that the nanoparticle structure is acquired very rapidly and stable with time and a kinetic time resolution of one minute is not enough. It was not possible before this SANS experiment to evaluate the time resolution needed for the formation of the nanoparticle structure. Therefore, to attain the initial objective of the understanding of the mechanism, we have followed the step by step formation of the nanoparticles through the intermediate state prepared independently by drop by drop addition as described below.

The first series of measurements were done at the intermediate state, where the deoxycytidine squalene solution in ethanol was added to a fixed amount of D_2O by using the millifluidic setup[2]. The concentration of the intermediate state was varied by changing the

number of deoxycytidine squalene solution drops in D_2O. We observed that the intermediate stages were stable with time. A basic but important result is that the diagrams at different angles were merging correctly, which means that the system did evolve with time (no long term secondary coalescence for instance). The CS samples present Bragg peaks at LA around 0.08 to $0.1A^{-1}$ whereas SG samples were flat (no internal structure). At LA, incoherent scattering increases since there is more and more hydrogen (both in the ethanol and in the CS and SG molecules). Total H/D ratio presence in different samples and the chemical composition of different samples were calculated from the incoherent background.

The formation of Bragg peaks could be detected above a threshold concentration. Above, the Bragg peak positions were almost constant with increasing concentrations. The internal structure of the particles can be determined from the Bragg peaks. The nanoassemblies were first detected growing at SA with increasing the concentration. The Guinier regime is clearly visible at lower concentration. It is shifted to lower q with increasing the number of deoxycytidine squalene drops and finally disappeared at higher concentration. The specific surface and particle size has been calculated from the Porod region and the radius of gyration (R_g) from the Guinier regime. In the next step, the organic solvent was evaporated from some of the intermediate samples. There was an evolution of the structure when one extracts the ethanol. However, once evaporated, the peak positions were almost constant at different concentrations. The second Bragg peak completely disappeared after the solvent evaporation. The particle size obtained at different concentrations were nearly the same. The peaks are moving to larger distances telling that in the intermediate state, the nanoparticles are swollen by ethanol. SANS results of intermediate states and final states are shown in Figure 1 and the complete list of intermediate samples and obtained results is given in Table 1.

Table 1: List of intermediate samples investigated by SANS.

NP	Initial Conc. in Ethanol (g/mL)	Conc. intermediate state (mg/mL)	First Bragg peak position ($Å^{-1}$)	Second Bragg Peak position ($Å^{-1}$)	Particle Size (nm)
Intermediate state					
1	**6.38**	**0.64**	**0.086**	**-**	**182**
2	**6.38**	**0.96**	**0.091**	**0.061**	**260**
3	**6.38**	**1.28**	**0.088**	**0.058**	**430**
4	**6.38**	**1.91**	**0.083**	**0.060**	**480**
5	**6.38**	**2.55**	**0.083**	**0.058**	**570**

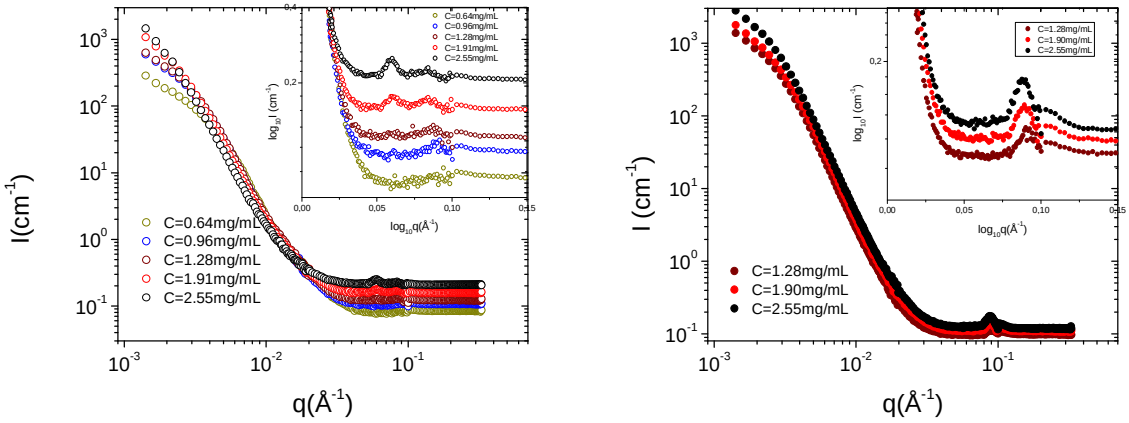

Figure 1a) SANS results of the intermediate state, obtained by drop by drop addition **b)** SANS results of the final state obtained by drop by drop addition.

A paper is under preparation with these unique results.

References:

[1] Couvreur P. et al, *Small* **2008**, 4, 247-253.
[2] Han J. et al, *Langmuir* **(2012)**, 28, 15966-15974.