Experimental report

15/09/2016

Proposal: 9-10-1485 **Council:** 4/2016

Title: Squalene Nanomedecine: solvent effect in controlling size and internal structure

Research area: Chemistry

This proposal is a new proposal

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Samples: solvent acetone
solvent D2O
solvent DMSO
Gemcitabine-Squalene C36H53F2N3O5
Deoxycytidine-Squalene C36H55N3O5
Solvent ethanol

Instrument	Requested days	Allocated days	From	To
D22	0	0		
D33	2	2	29/06/2016	01/07/2016
D16	3	3	12/07/2016	15/07/2016

Abstract:

Due to the quick adaptation of microorganisms to chemicals and the shrinking range of drugs available, medicine try to find a way to avoid drug resistance and treatment side effects.
In 2016 Couvreur et al. discovered a new prodrug molecule, the Squalene-Gemcitabine, able to self-assemble with liquid crystal structure via squalenoylisation: the nucleoside-like is linked with amide bond to a biocompatible fatty acid the squalenic acid. The prodrug is nanoprecipitated via ouzo effect (solvent-antisolvent mixing). Saha et al. have shown that the nature of the solvent plays a key role in structure stability and nanoparticle size.
With a SANS comprehensive characterization, we would like to understand the role of the solvent (polarity, viscosity and water coefficient diffusion) on the nanoformation, to further better control the internal structure and the polydispersity of the particles. Two solvents (acetone and DMSO) and 2 molecules (Squalene-Gemcitabine, Squalene-Deoxycytidine) will be used in different concentrations and ratios.

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Aims of the experiment and results:

The aim of the experiment was to investigate the role of the solvent on the formation of the deoxycytidine squalene (Sqdc) and Gemcitabine squalene (SqGem) nanoparticles (NPs) prepared from acetone or DMSO at different ratio and concentrations. The squalenoyl derivatives were first dissolved in the organic solvent then added drop by drop in 1 mL of deuterated water. A syringe pump controlled the flow rate (30µl/s) of the organic solution addition into water under high stirring (1000rpm). The solutions were studied before and after the evaporation/dialysis of the organic solvent (respectively named initial states and final states) for different squalenoyl concentrations (controlled by the number of added drops). The allocated time on D33 was 2 days, the q-range from 2 10^{-3} to 3 10^{-1} $Å^{-1}$ was covered with 3 configurations: Small q (13Å, 12 m) Medium q (6Å, 12 m) Large q (6Å, 2 m). We measured 43 samples during the run for the three configurations.
The allocated time on D16 was 3 days, we cover the q range from 0.06 to 0.5 $Å^{-1}$ with 1 configuration at 4.55 $Å^{-1}$, detector distance 100 cm and detector angle 12°.

The axes we focus on during this run:

- The SqDC nanoparticles formation in DMSO (initial and final states)
- The SqGem nanoparticles formation in DMSO (initial and final states)
- Effect of the addition of dextrose on intermediate and final state. The aim was to identify if the increase of stability with dextrose is not accompanied by a modification of the size distribution
- Effect of deuterated versus hydrogenated solvent in the nanoprecipitation process
- Effect of the addition of an extra non-ionic surfactant (pluronic F127)

I) D33 first results: Effect of solvent

Two examples of SANS results obtained with DSMO are presented in fig 1 and 2.

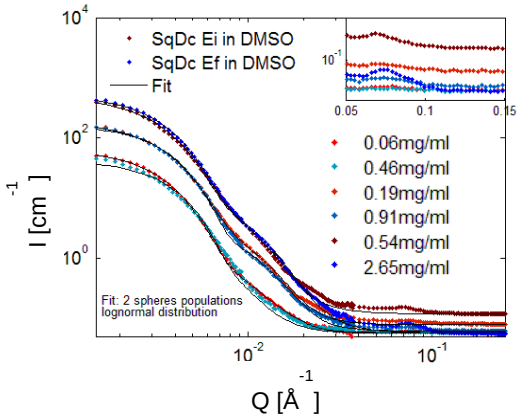


Fig 1: SANS curves of SqDc nanoparticles solutions for different SqDc concentrations for initial states (in red) and final states (in blue) of the nanoprecipitation process with DMSO organic solvent. Continuous lines are fitting from two log-norm spherical populations.

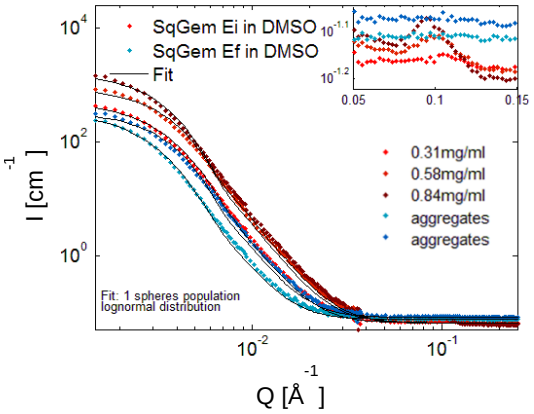


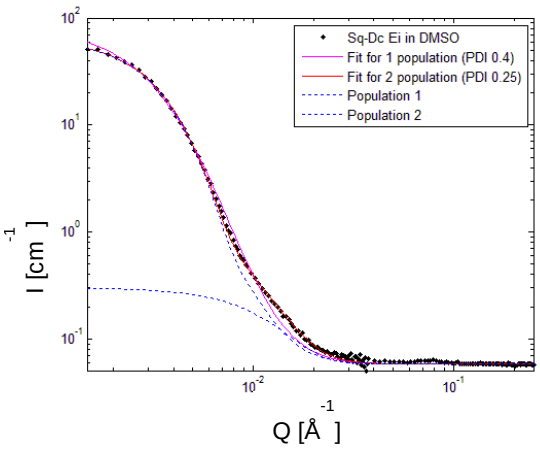
Fig 2: SANS curves of SqGem nanoparticles solutions for different SqGem for initial states (in red) and final states (in blue) of the nanoprecipitation process with DMSO organic solvent. Continuous lines are fitting from two log-norm spherical populations.

For the SqDc samples nanoprecipitated with DMSO, first conclusions can be extracted from fig 1. The nanoparticles are relatively monodisperse (indicated by the presence of oscillations), and the amount or size of nanoparticles is increasing with the number of added drops (the intensities increase with concentrations). The signals in both initial and final states are dependent on the squalenoyl concentration. Contrary to the nanoprecipitation obtained with ethanol and acetone, when DSMO is used, the final and initial states are similar. An internal structure is visible with a Bragg peak at 0.0715 $Å^{-1}$, whose intensity is increasing with squalenoyl concentration.

For the SqGem series (fig 2), difficulties arise from the low stability of the compound that aggregates rapidly during the run. With DMSO, the SqGem nanoparticles are more polydisperse than when they are prepared from éthanol[2] and may contains aggregates. An internal structure is visible with a Bragg peak around 0.09$Å^{-1}$ like the one obtained when nanoparticles are prepared from ethanol.

Treatment of SANS scatterind curves:

The intensity of Squalenoylated Deoxycitidine curves were fitted with two populations of polydisperse spheres using the SASview software.



Fixed parameters for the fit

$\rho_{solvant}$ (Å^{-2})	6.25 10^{-6}
ρ_{SqDc} (Å^{-2})	1.24 10^{-6}
Polydispersity (log normal)	0.25
background	0.058

Free parameters for the fit

Population 1 :	
Radius R1 (Å)	478
Scale ϕ1 (volume fraction of Sq-Dc)	2.5 10^{-5}
Population 2 :	
Radius R2 (Å)	126
Scale ϕ2 (volume fraction of Sq-Dc)	4.9 10^{-6}

Fig 3: Example of data fitting for aninitial state of SqDc NPs prepared in D_2O/DMSO at 0.06mg/ml of SqDc. Comparison of the one population fit (pink continuous line) and the two populations fit (red continuous line) with the contribution of each population to the intensity (bleu dashed lines).

We can clearly see on Fig 1 that the fit is enhanced when 2 populations are taken into account. The presence of a population of smaller size has also been observed by cryo-TEM experiments on one of the samples analysed on D33. Complementary cryo-TEM analyses are in progress.

As the particles are porous, one needs to take into account the swelling by the solvent and its impact on the SLD of the particle. We calculate the swelling of the nanoparticles by comparing the volume fraction issued from the composition known from the sample preparation to the volume fraction obtained from SANS data analysis. This procedure is described in the supplementary material of our former article.[2]

From the fitting procedure, we can extract the variation of the radius, the density number of nanoparticles and the swelling by the solvent. The results from this analysis are presented in fig 4 to 6 for SqDc formed from DMSO solutions. The swelling behaviour is similar to the one obtained for the ethanol case. In the concentration range of this study, we observe an increase number of particles with a constant radius in agreement with a nucleation phase. This behaviour is opposite to the ethanol case.

Experimental results:

From experimental results obtained for the different cases (ethanol[2], acetone (not shown here), DMSO) we want to propose the key to control the size distribution for squalenoyl nanodrugs.

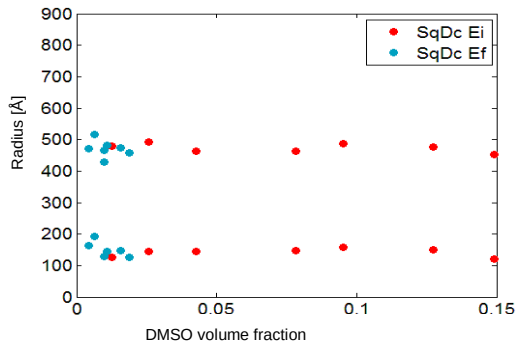


Fig 4: Radius of the two populations for initial and final states of SqDc nanoparticles prepared with DMSO as a function of solvent concentration.

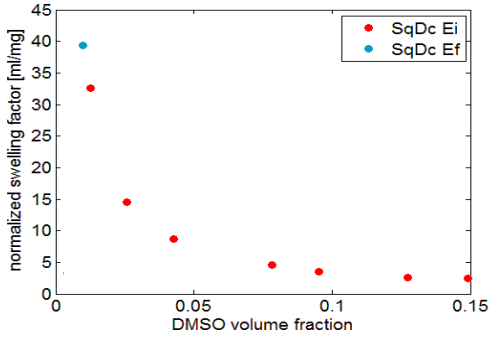


Fig 5: Decrease of the swelling factor with increase of external organic solvent content.

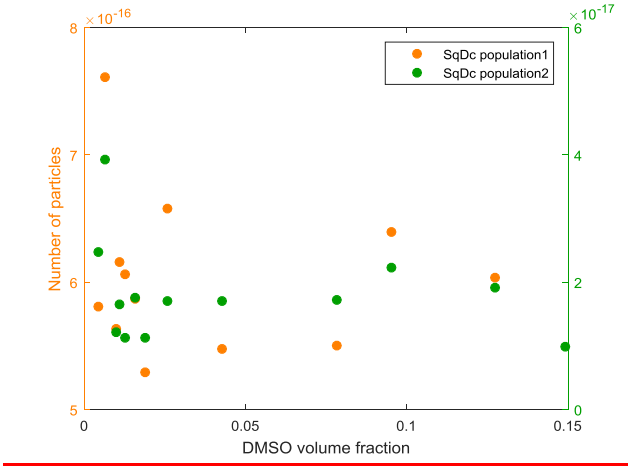


Fig 6: Evolution of the number of particles of SqDc for initial and final states (nanoparticles prepared in DMSO).

To measure solely the impact of the modification of solvent with added droplets, the concentration of NPs was normalized by the concentration of squalenoyl derivatives (in mg/ml). The obtained number density normalized to a common concentration of 1 mg/ml is plotted in fig 6. The points are rather dispersed, but if we look at the intermediate states, the tendency is clearly a constant normalized density number. This means that for each added droplet, a nucleation is obtained. Contrary to the ethanol case, no aggregation is observed with increasing DMSO content in the beginning of the nanoprecipitation. The tendency obtained with DMSO is retrieved with acetone. But for acetone a strong influence of the temperature on the polydispersity was observed (tested on one case).

The choice of DMSO and acetone as a solvent rise questions To answer we would like to nanoprecipitate SqDc particles, with a forth solvent, for example THF or isopropane.

Main conclusions and perspectives:

- We obtain the variation of size distribution and swelling for SqDC from DMSO.
- We obtain the variation of size distribution and swelling for SqGem from DMSO, but on a smaller concentration range.
- The tendency observed with acetone and DMSO is opposite to the ethanol case and a complementary study on a forth solvent is needed to conclude on the process.
- We observed a strong effect of the temperature used during the nanoprecipitation process. This point need complementary analysis and will be the subject of a continuous proposal in this field.

II) Results from D16

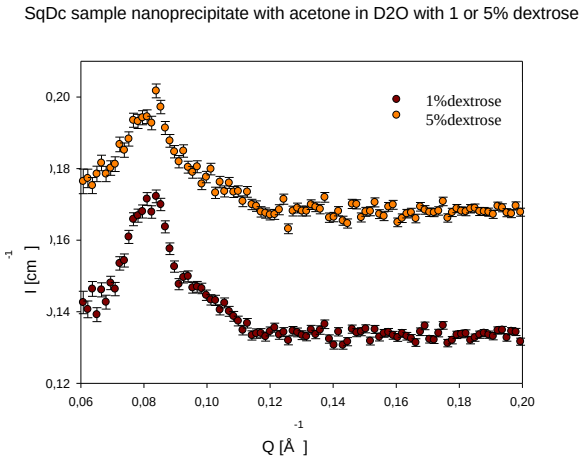


Fig 7 : SqDc nanoparticle nanoprecipitated with acetone with 1% or 5% dextrose

In D16, we tried to obtain a larger resolution for the Bragg peaks indexation, but this was not possible. We do not obtain higher resolution to identify the nature of the internal structure of the nanoparticles.
Fig 7 presents two examples of SqDC nanoparticles measured on D16. With and without Dextrose in the solution, we are not able to discriminated on any impact on the crystal.

From D16, we observe some variation of the peak position assumed to be due to the aging of the sample, but only X-ray on synchrotron source allow the Bragg peaks discrimination (Data of ID02 not shown). It is thus difficult to compare two series if they are not prepared in the same time scale (preparation and aging).

References:

1. Couvreur, P. et al. Squalenoyl Nanomedicines as Potential Therapeutics. Nano Lett. 6, 2544–2548 (2006).
2. Saha, D. et al. The role of solvent swelling in the self-assembly of squalene based nanomedicines. Soft Matter 11, 4173-4179 (2015).