Experimental report

12/02/2018

Proposal: 9-13-696 **Council:** 10/2016

Title: Squalenoyl-adenosine nanoparticlesin serum albumine solutions

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Human Serum Albumin
solvent D2O
Adenosine-Squalene C37H55N5O5
Mouse serum albumin

Instrument	Requested days	Allocated days	From	To
D11	0	0		
D33	1	1	21/02/2017	22/02/2017

Abstract:

Nanodrugs are very promising to control drug delivery without burst release and fast metabolization. In this field, the squalenoyl strategy appears as very efficient to increase the pharmacological activity of chosen drugs. It consists in coupling an active drug with a squalene derivative to produce a compound able to form a spherical internally structured nanoassembly through nanoprecipitation process. Recently, the particular case of Squalenoyl-Adenosine attracted large interest for its dramatic efficacy in both experimental models of cerebral ischemia in mice and spinal cord injury in rats. The mechanism of action seems to go through a step of disassembling of the nanoparticles (NPs) as shown by DLS and FRET but a quantitative study such as the one proposed here by SANS is mandatory for providing density numbers, size distribution and internal structure evolution. the aim is to go deeper in the understanding of the specific activity of these NPs.

Experimental report:
Squalenoyl-adenosine nanoparticles in serum albumin solutions

TESTARD Fabienne; Frédéric Gobeaux; GRILLO Isabelle; GUENOUN Patrick; MARRET Elodie and RENAULT Jean Philippe. (2017). Squalenoyl-adenosine nanoparticles in serum albumine solutions. Institut Laue-Langevin (ILL) doi:10.5291/ILL-DATA.9-13-696

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Nanodrugs are very promising to control drug delivery without burst release and fast metabolization. In this field, the squalenoyl strategy appears as very efficient to increase the pharmacological activity of chosen drugs. It consists in coupling an active drug with a squalene derivative to produce a compound able to form a spherical internally structured nanoassembly through nanoprecipitation process.[1–4] Recently, the particular case of Squalenoyl-Adenosine attracted large interest for its dramatic efficiency in both experimental models of cerebral ischemia in mice and spinal cord injury in rats.[5,6] DLS and FRET experiments suggest a mechanism of action through disassembling of the nano-objects. The aim of this SANS experiment was to study more closely the interaction of Sq-Ad nanoparticles with plasma serum and specifically its main component, the serum albumin, and provide quantitative results.

Nanoparticle characterization in D_2O and deuterated buffers.

Before carrying the study itself, several controls were carried out, including assessing different batches of Sq-Ad nanoparticles in D2O, with different saline buffers of different ionic strength as well as the bovine serum albumin (BSA) and Fetal Bovine Serum (FBS).

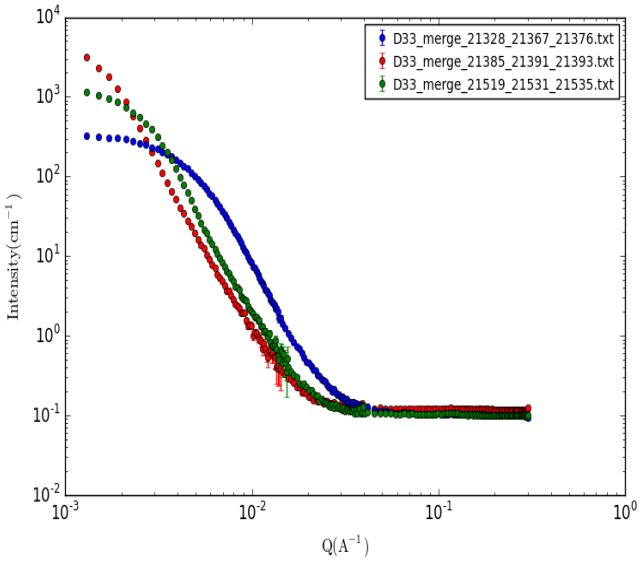

Figure 1 : SANS patterns of Sq-Ad NPs in D2O (blue dots), Sq-Ad in D2O-PBS just after mixing (green dots) and Sq-Ad in D2O-PBS two hours after mixing (red dots).

First we observe that the nanoprecipitation process is robust since nanoparticles resulting from different nanoprecitpitations (same operator) yield very similar SANS patterns. Differing backgrounds indicates various amounts of residual ethanol in the solutions (from 6.4 to 12.2%). SANS profiles of Sq-Ad nanoparticles can be fitted by lognormal distributions of spheres (Radii~30 nm; Polydispersity = 0.3). These NPs particles are moreover stable upon dilution. Nevertheless, comparison with previous data obtained from different synthesis batches show some discrepancy in term of internal structure (but not in terms of size distribution), suggesting sensitivity to impurities.

Mixing the NPs with low salt buffers (0.14 mM NaCl, pH 7.8 and 0.14 mM NaCl pH 5.7) does not lead to any change in colloid stability. However, at higher ionic strength (e.g. 140 mM), scattering at small

angles increases, indicating that NPs are no longer stable and immediately aggregate. Macroscopically, flocculation becomes visible after a dozen of hours.

Nanoparticles interactions with Fetal Bovine Serum (FBS) and Bovine Serum Albumin (BSA)

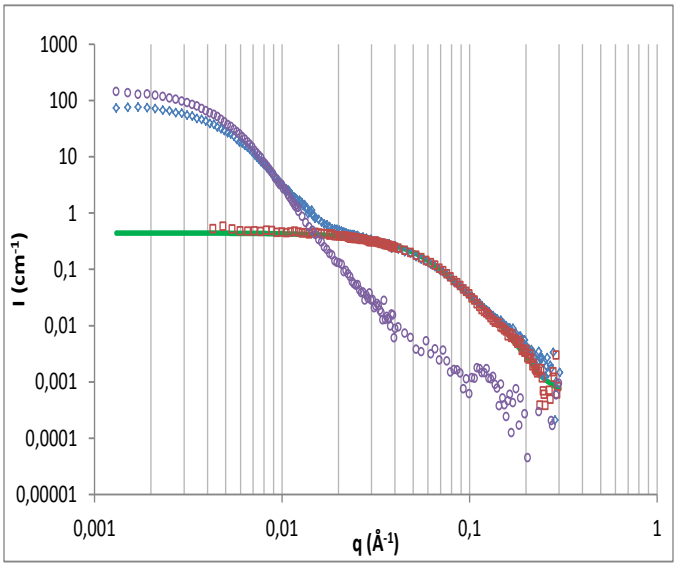

Figure 2 : SANS patterns of Sq-Ad NPs in D2O (purple dots), Sq-Ad+BSA in D2O (blue dots) and BSA in D2O (red dots).

The SANS patterns of Sq-Ad NPs in D_2O, BSA in D2O-PBS and Sq-Ad NP + BSA in D2O-PBS are shown in Figure 2. The first observation is that the presence of BSA in the D2O-PBS buffer stabilizes the NPs since no sign of aggregation are detected. On the contrary, scattered intensity is lower than that of NPs in D2O, yielding a slightly lower fitted radius, although the polydispersity remains rather high.

The SANS pattern of NPs+BSA can be fitted as the linear combination of the Sq-Ad and BSA patterns previously obtained: $I_{exp} \sim 0.7 \times I_{SqAd/D2O} + I_{BSA}$. In the case of NPs, we can hypothesize that they are eroded by the BSA that would extract Sq-Ad monomers out of them, hence resulting in a decrease in NP size. This postulated interaction between Sq-Ad molecules and BSA has been further characterized with complementary spectroscopic techniques.

Similar results have been obtained for BSA with different ionic strength solutions.

Similar tendencies have obtained with NPs mixed in FBS.

Finally, the Sq-Ad NP colloidal stability seems to extend in the 25-37°C temperature range.

Current conclusions

As it was previously shown[7], SANS is an adequate method to characterize nano-precipitated squalenoyl nanoparticles in terms of size distribution, density and, if applicable, internal structure. SANS analysis can also provide information on interactions with other solutes.

The main conclusion that can be drawn from this set of experiments is that the Sq-Ad nanoparticles are destabilized at high ionic strength because screening of charges decrease repulsive interactions and leads towards aggregation. However, in the presence of proteins (fetal bovine serum and more specifically bovine serum albumin) their colloidal stability is increased. Additionally, this stabilization comes along with a decrease in size of the nanoparticles. This can either be attributed to the erosion of the nanoparticles by monomer extraction or to solvent expulsion by osmotic pressure. Further experiments are ongoing to test these hypotheses.

These results are being further analyzed in details and have been correlated with other physical-chemical characterizations such as dynamic light scattering, small angle x-ray scattering, cryo-

electron microscopy and spectroscopic analysis and the redaction of an article reporting the full study is underway.

References

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