Experimental report

24/03/2024

Proposal: 9-13-1068 **Council:** 10/2022

Title: Soft nanoassemblies in different plasma types: competition between protein corona formation and nanoparticles disassembling

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: squalene-cyclosporin A nanoparticles
mouse plasma
rat plasma

Instrument	Requested days	Allocated days	From	To
D22	2	2	12/04/2023	14/04/2023

Abstract:

Despite undeniable promising results in in vitro and animal experiments, the behavior of nanodrugs in the human body is yet still poorly understood. Indeed, carrying pharmacodynamics studies in a living body is very complex; moreover, the translation from animal tests to human assessments is far from straightforward. We have developed an in vitro methodology enabling to clarify the interactions between nanoparticles and the components of the biological fluids and we now wish to tackle the effect of different kinds of plasma (i.e. from different animal species and from healthy vs sick animals) to try to pinpoint the microscopical specificities leading to different cellular and tissular responses. Our study will focus on the fate of soft lipidic nano-assemblies (NAs) aimed at treating heart ischemia in plasma of two species of rodents mostly used in preclinical trials (i.e. mouse and rat). Our aim is twofold: a) clarifying the competition between the disassembly of NAs and the formation of a corona around them and b) to assess the specificity of different types of plasma on these two phenomenas.

Experimental Report / Experiment 9-13-1068 / D22

Soft nano-assemblies in different plasmas: competition between protein corona formation and nanoparticles disassembling

12/04/2023-14/04/2023

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10.5291/ILL-DATA.9-13-1068

The aim of this experiment was to study the modifications undergone by two types of nanoparticular prodrug suspensions after incubation in the presence of blood plasmas collected from both healthy and sick rodents. Indeed, the plasma composition has been demonstrated to be different depending on the health state of the animal, hence leading to possibly different interactions with nanoparticles put in their presence.

a) Nanoparticles characterization.

The SANS patterns arising from the two suspensions of nanoparticles are displayed in Figure 1 (left panel). Both exhibit a Guinier plateau ($q < 0.007$ Å^{-1}) that enables to determine an average size; indeed the curves could be fitted using the scattering arising from a lognormal distribution of polydisperse spheres. The corresponding distributions are displayed in the right panel of Figure 1. The first NPs, named SQCsA-SS31 (top panel, blue trace), have an average diameter of 30 nm, while the second, SQCsA (bottom panel, red trace) have an average diameter of 100 nm. The exponential decays in the q-range [0.008-0.05 Å^{-1}] (aka Porod region) are close to q^{-4}, indicating a sharp interface between the nanoparticles and the solvent. However, the SQCsA SANS curve also exhibits an inflexion at 0.01 Å^{-1}. We have frequently observed this feature in the past without having reached a definite conclusion as to its interpretation. For example, it could be linked to the presence of lipid bilayers surrounding the particles. Finally, in the wider q range ($q > 0.08$) no structural peak is detected, suggesting a low inner organization of the nanoparticles. These observations will be further confronted to cryoTEM observations that will enrich the interpretation of the patterns.

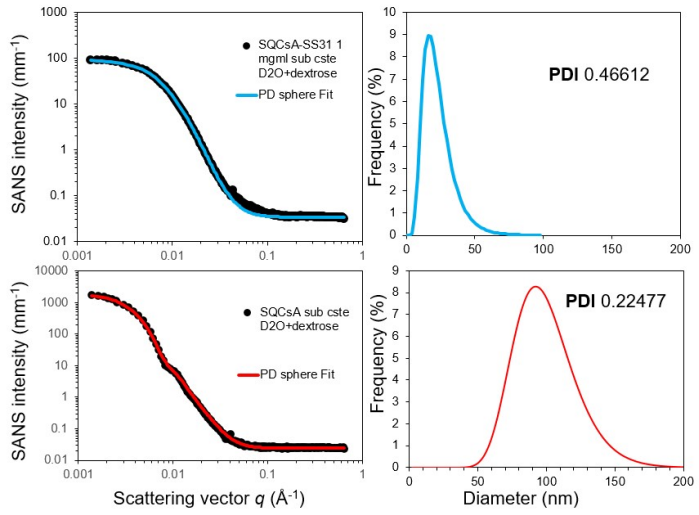

Figure 1 : SANS characterization of the two types of nanoparticles investigated. Dots = experimental data, straight lines = corresponding log-normal distribution of nanoparticle diameters.

b) Plasma characterization

For this experiment, the use of plasma was chosen over serum because it is theoretically closer to the physiological conditions than serum. However, as **Figure 2** shows it, contrary to the serum scattering, the plasma scattering doesn't reach a plateau at low *q*, probably because of the presence of large aggregates and fibrinogen in the solution. This continuously increasing scattering will make the decomposition of the scattering signal more complex, notably in the presence of NPs.

Of note, the scattering patterns of the two plasma types differ very little from each other.

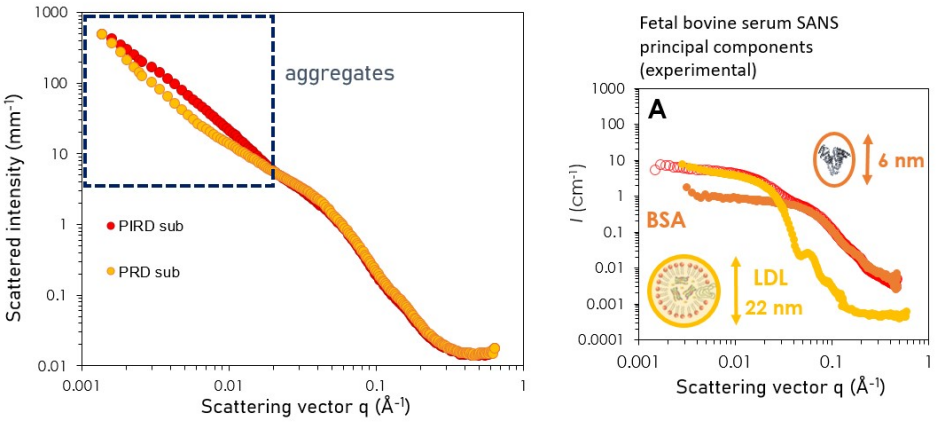

Figure 2 : Left: SANS characterization of the two plasmas (dialyzed against D_2O) used in this experiment. PIRD stand for Plasma of Ischemic Rat and PR for Plasme of (healthy) rat. Right: SANS curve of serum, LDL and BSA, the latter two being the main component of serum (curves taken from previous ILL experiments).

c) NPs-plasma mixtures characterization and component separation

Figure 3 & Figure 4 display two examples of comparisons between the SANS patterns of the bare NPs (blue symbols), of the full NPs+plasma mixture (red symbols), of the supernatant after centrifugation of said full mixtures (orange symbols) and finally of the pellet redispersed in plain D_2O (yellow symbols). In both figures, the signal of proteins appearing in the 0.02-0.1 Å^{-1} *q*-range in the full mixture and the supernatant SANS patterns is clearly removed in the redispersed pellet, suggesting the free proteins have indeed been removed from the solutions. However, the SANS patterns of the redispersed pellets is not entirely similar to those of the bare NPs, suggesting they have been modified in the presence of the plasma. In the **Figure 3** the signal at low angles is increasing (just as in the full mixture and supernatant), indicating large residual objects remain in the mixtures. In the case of **Figure 4**, the pattern tending towards a Guinier plateau is retained, but the signal in the 0.02-0.1 Å^{-1} *q*-range is slightly different ; this could be either ascribed to proteins adsorbed to the NPs or to a modification of the NPs feature appearing in this range (see **section a**). In both cases the background level and intensity in the redispersed pellet is different from the other samples because of the redispersion in plain D_2O and no conclusion can be drawn without a thorough renormalization.

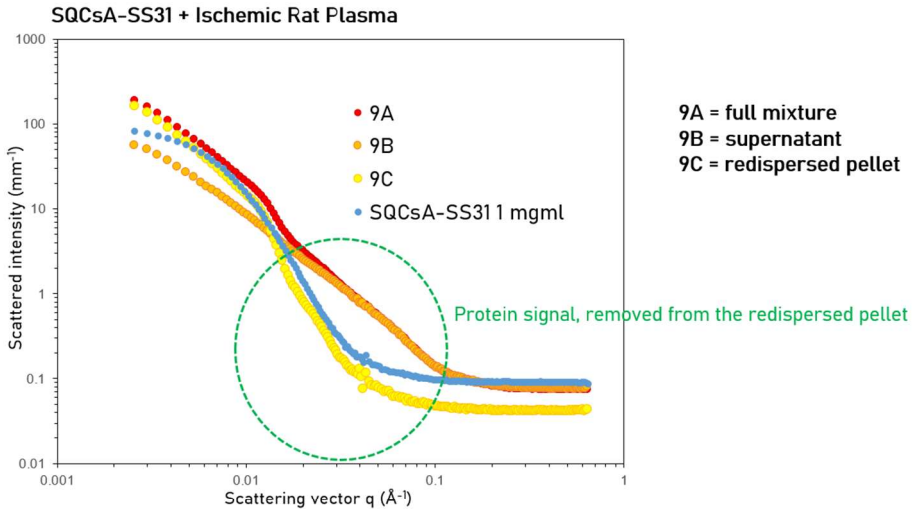

Figure 3 : comparison of the SANS patterns of bare SQCsA-SS31 NPs (blue symbols), full NPs-plasma of ischemic rat mixture (red symbols), supernatant obtained after centrifugation of the full mixture (orange symbols) and D_2O-redispersed pellet (yellow symbols)

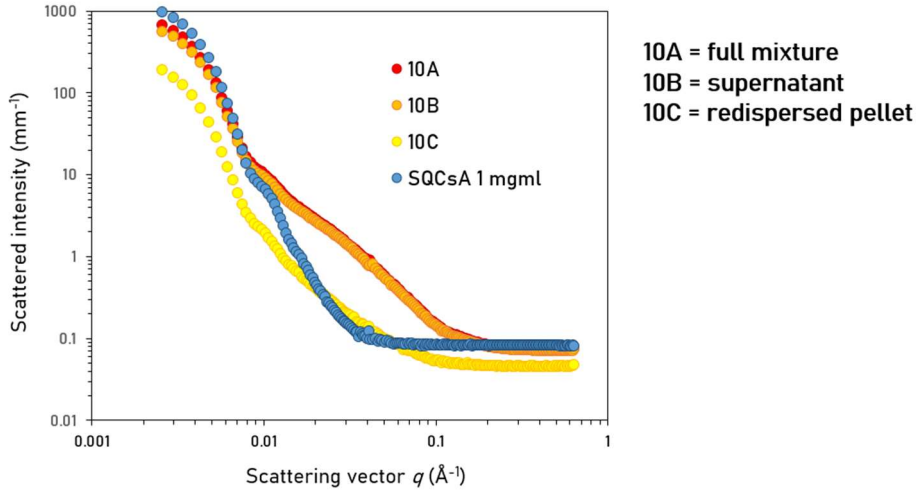

Figure 4 : comparison of the SANS patterns of bare SQCsA NPs (blue symbols), full NPs-plasma of ischemic rat mixture (red symbols), supernatant obtained after centrifugation of the full mixture (orange symbols) and D_2O-redispersed pellet (yellow symbols)

d) Temporary conclusions

The results presented here validate our separation protocol; other data were obtained with other NPs:plasma combinations for comparison. However, it seems the resulting curves differ only slightly and drawing conclusions would require finer analyses. Additionally, full plasmas are probably too complex solutions to draw detailed conclusion; we probably need to simplify our experimental model by using sera instead of plasmas and only one or two proteins at a time. A new proposal has been submitted in this spirit.