Experimental report

29/01/2021

Proposal: 9-11-1931 **Council:** 4/2019

Title: Self-assembled stimuli-responsive polymersomes loaded with doxorubicin for tumor drug delivery

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: doxorubicine in H2O/D2O
poly([N-(2-hydroxypropyl)] methacrylamide)-bpoly[4-(aminophenylboronic pinacol ester)methacrylate] in D2O+H2O
poly([N-(2-hydroxypropyl)] methacrylamide)-b-poly[2-(2-(4-isopropylphenyl)aceta mido)ethyl methacrylate] in D2O/H2O
poly([N-(2-hydroxypropyl)] methacrylamide)-bpoly[4-(ethyl-phenylboronic pinacol ester)methacrylate] in D2O/H2O
poly([N-(2-hydroxypropyl)] methacrylamide)-b-poly[2-(diisopropylamino)ethyl methacrylate] in D2O/H2O

Instrument	Requested days	Allocated days	From	To
D11	2	2	30/01/2020	01/02/2020

Abstract:

The main aim of this proposal is to determine the influence of the pH on doxorubicin (DOX) partition between the aqueous phase and the hydrophobic membrane of the vesicle during the polymersomes preparation by microfluidic nanoprecipiation which is of fundamental importance on the control of the DOX loading process into polymersomes.

The aim of the experiment was to analyse samples of stimuli-responsive polymersomes loaded with doxorubicin to gain a better understanding of their inner structural behaviour with intention to control their drug release and biological properties under physiological conditions. To this end we wanted to carry out small-angle neutron scattering experiments on our samples to evaluate the core radius and volume of our polymersomes, as well as ascertain their shell structure.

Our pH and ROS-responsive block copolymer polymersomes based on poly([N-(2-hydroxypropyl)]methacrylamide) such as poly([N-(2-hydroxypropyl)]methacrylamide)-*b*-poly[4-(aminophenylboronic pinacol ester)methacrylate] (PHPMA-ROS) were prepared by microfluidic self-assembly while using solutions of several polymer concentrations, an array of solvent-to-antisolvent combinations and doxorubicin loading conditions.

We carried out the measurements at 25°C which is the same temperature we used for previous experiments performed on these samples to obtain their primary analysis (eg. DLS/SLS carried out at the room temperature). We used circular quartz cells with 1 mm pathlength to maximize the utilized cross-section of incident neutron beam and exposed volume of sample.

We used neutron wavelength of 6 Å. The acquisition of the scattering data was carried out at detector distances of 1.5 m, 8 m and 20 m. We measured all samples at 8 m detector distance and decided on additional measurements at different distances based on the shape of obtained scattering curve to maximize the utilization of allotted experimental time as well as obtain all the important information small-angle neutron scattering of our samples could provide.

The pH and ROS-responsive polymersomes were measured in pure heavy water and also in different mixtures of heavy and light water in order to index match the particles core or a layer of the shell.

As part of our research we attempted to observe the influence of the concentration of reactive oxygen species on the degradation of the prepared ROS-responsive nanoparticles. 10 mM of H_2O_2 were added to the polymer samples which were then measured several times at later times to observe the degradation. The time sensitive measurements would not be possible without the high beam intensity and fast configuration changes possible and available at D11.

Based on an analysis of obtained data we concluded our polymersomes can mostly be described as spherical core-shell structures (which is in agreement with dynamic light scattering data and TEM imaging) that have three layers with different thicknesses and scattering length densities enclosing a uniform core with layers in the shells in contact with the aqueous medium being hydrophilic and a hydrophobic layer between them. The hydrophilic layers seem to be swollen with solvent and accompanying salts.

The structure of the shell seems to be more complex since we observe deviations of various fitting functions in the large q domains, as demonstrated on **Figure 1**.

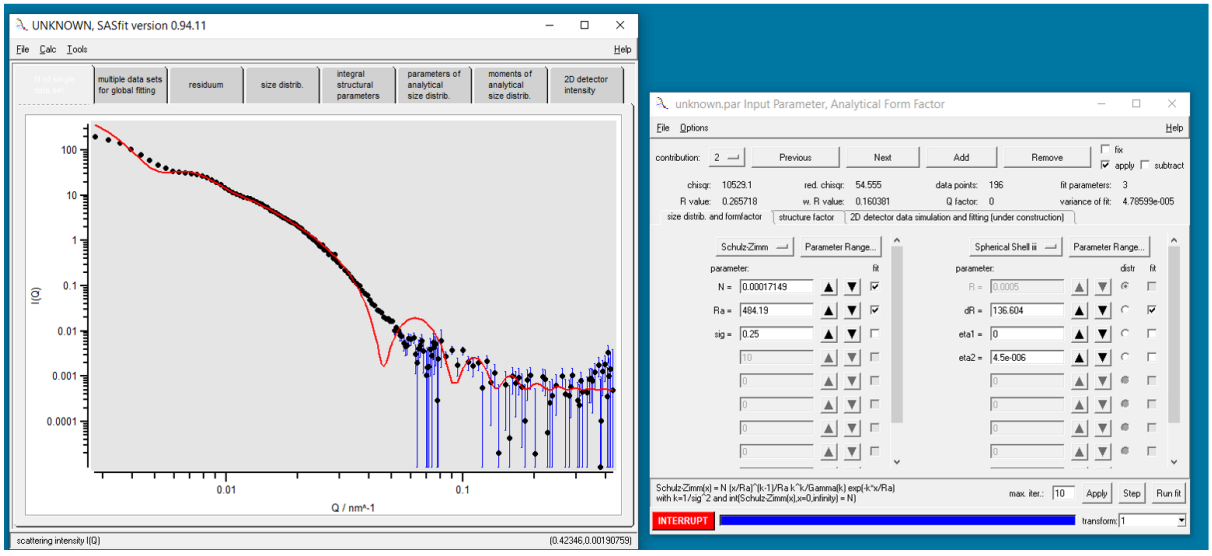

Figure 1. Sasfit analysis of sample PHPMA-ROS

Data analysis was also performed using pair distance distribution functions (PDDF) from which profiles of scattering length density can also be derived using the PCG software package (O. Glatter 2012). **Figure 2** shows a comparison of the PDDF(r) for a sample of PHPMA-ROS with doxorubicin (DOX) in D_2O (blue data) and in a mixture of D_2O and H_2O (red data). Using the mixed solvent should enhance the visibility and thus the location of DOX. From the profile of PDDF it can be concluded that the scattering object has a vesicular structure and that the DOX is preferentially located in the shell region of the object. It has to be noted though that the PDDF distribution covers a rather wide range of r indicating that the shell may be thicker than what would correspond to a regular vesicular

structure. This may be due to the presence of additional low molecular weight components in the system that are required to create the polymersomes in this system or it could be ascribed to a rather wide distribution of sizes of polymersomes, which was also suggested by the fitting.

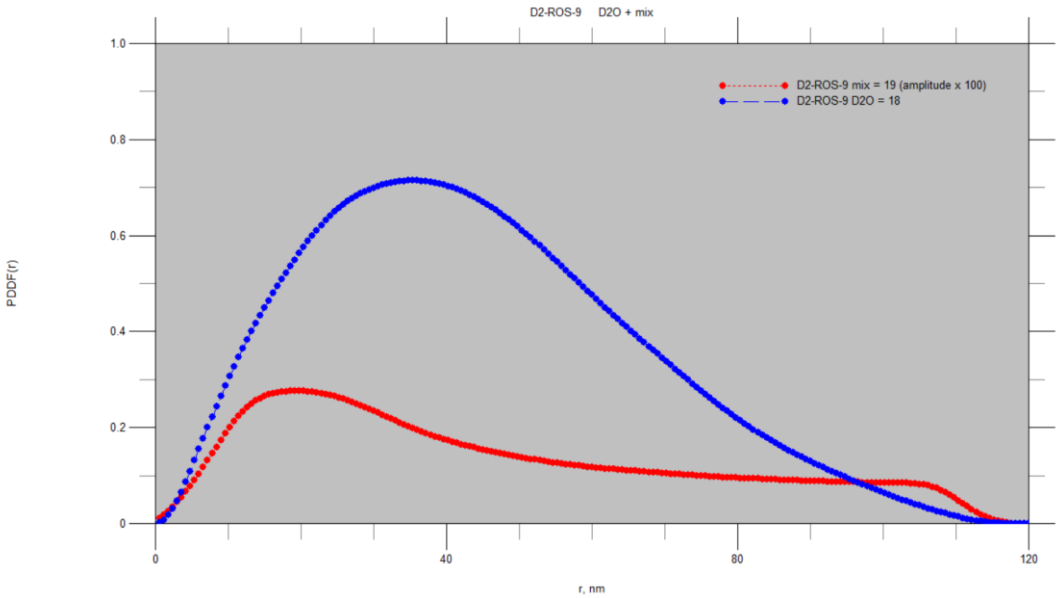

Figure 2. Pair distance distribution function for sample PHPMA-ROS in D_2O (blue data) and index matching mixture of D_2O and H_2O (red data)

Figure 3 shows the calculated profile of scattering length density for a typical sample. An intriguing feature is the negative values of SLD in the central region of the object ($r < 10$ nm) for a polymer dissolved in pure D_2O. An explanation may be proposed related to the technique of sample preparation. The polymersomes are prepared in a microfluidic device using H_2O and subsequently H_2O is exchanged for D_2O by dialysis. It can be imagined that H_2O is not completely removed from the core of the object during the exchange process which may provide some information on the permeability of the shell of the polymersome.

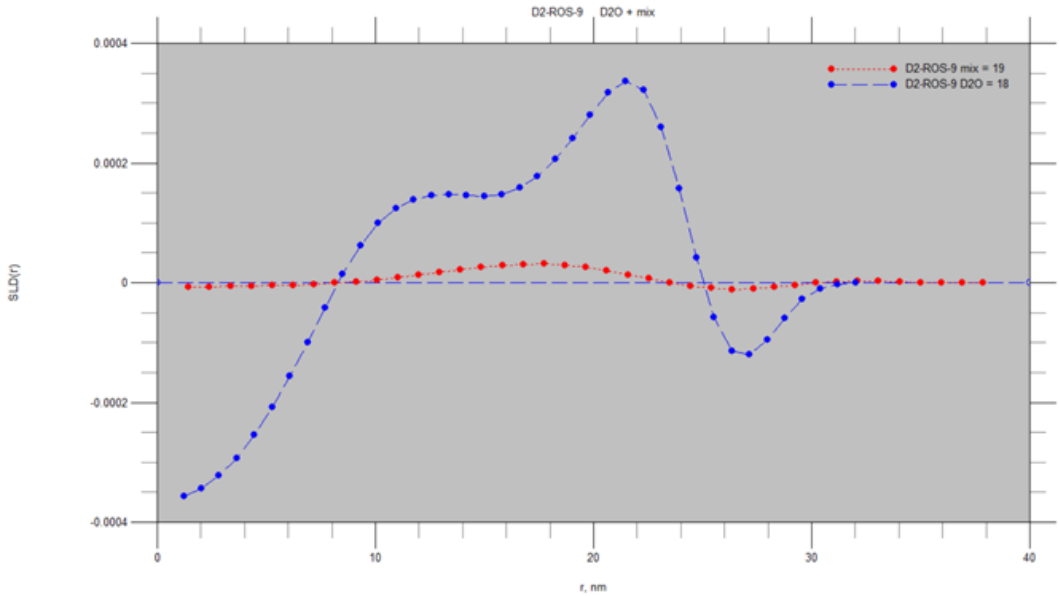

Figure 3. SLD profiles calculated from PDDF(r) using PCG software from measurements in D_2O (blue data) and index matching mixture of D_2O and H_2O (red data)

Data were also analyzed using the GNOM/DAMMIN reconstruction software with PyMOL visualization. For certain samples the results confirm hollow structures, e.g. **Figure 4.** Such results are consistent in repetitive calculations.

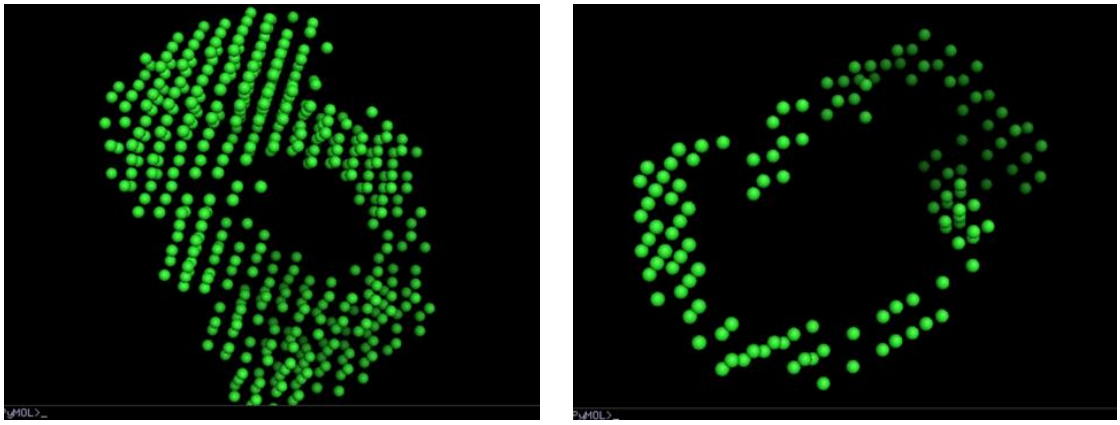

Figure 4. Examples of hollow structures obtained by the DAMMIN reconstruction

For some other samples irregular structures are obtained, e.g. **Figure 5.** This may indicate that either irregular aggregates have been produced or that the expected core-shell particles have irregular thickness of the shell and the thin parts of the shell have not been reconstructed.

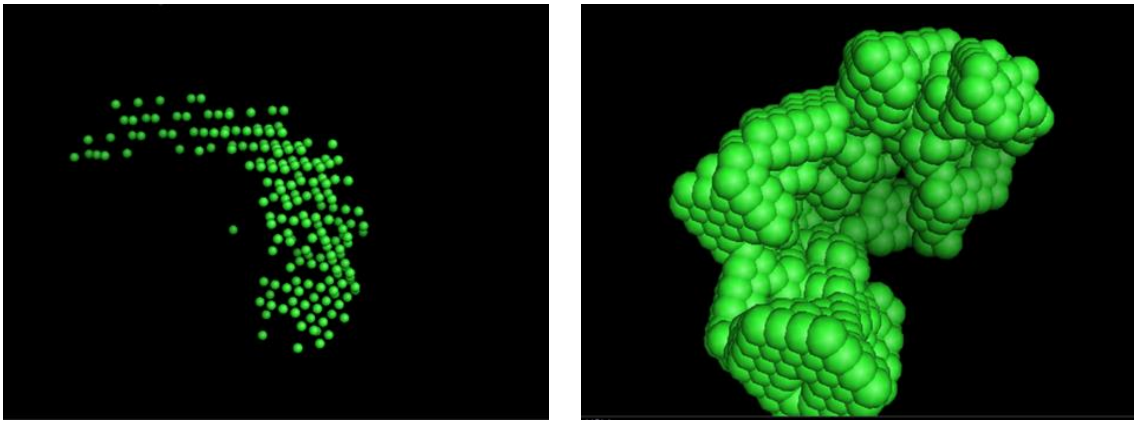

Figure 5. Examples of irregular structures obtained by the DAMMIN reconstruction (for the right image the unit sphere size was increased)

Keeping in mind the limitations of the reconstruction procedure we can still conclude that for certain samples the prepared nanoparticles were not ideal polymersomes despite the fact that preliminary test measurements by light scattering gave consistent results.

Degradation of the nanoparticles was studied as a function of time in presence of hydrogen peroxide. For a sample of PHPMA-ROS the evolution of the PDDF is shown as a function of time in **Figure 6.** From the shift of the distribution during degradation it can be concluded that smaller particles are degraded more rapidly.

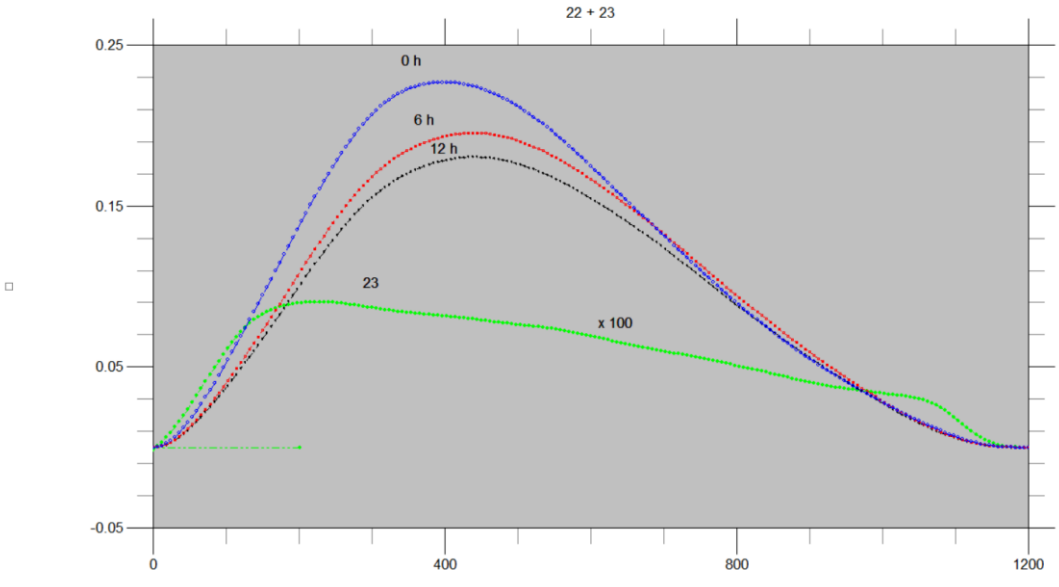

Figure 6. Time evolution of the PDDF during degradation of a PHPMA-ROS sample by H_2O_2