Experimental report

19/06/2017

Proposal: 9-10-1478 **Council:** 4/2016

Title: Perfluorocarbon-loaded polymeric nanoparticles for long-term ultrasound imaging in vivo

Research area: Chemistry

This proposal is a new proposal

Main proposer: Francesca BALDELLI BOMBELLI

Experimental team: Olga KOSHKINA
Francesca BALDELLI BOMBELLI
Ilaria TIROTTA

Local contacts: Ralf SCHWEINS

Samples: Nanoparticles

Instrument	Requested days	Allocated days	From	To
D11	2	2	23/06/2016	25/06/2016

Abstract:

Ultrasound is a very attractive and patient-friendly imaging technique, as it is easy to use, fast and non-invasive. Nevertheless, its use is severely limited by the low stability of current contrast agents, as they contain gaseous or vaporizing phase. We developed perfluorocarbon-loaded poly(lactide-co-glycolide) nanoparticles, which are suitable for long-term imaging, in a time-scale of days. However, the mechanism of contrast generation is completely unclear. As our particles are stable in regard to their size and fluorine content upon ultrasound exposure, they likely do not contain a vaporizing phase. Thus, elucidating the structure of these nanoparticles is a key to describe the mechanism of acoustic contrast generation. As SANS has been successfully applied by other researchers to study fluorinated colloids, we propose SANS to investigate the structure of the nanoparticles.

Experimental report

Experiment-Nr.: 9-10-1478

Perfluorocarbon-loaded polymeric nanoparticles for long-term ultrasound imaging in vivo

The goal of the experiment was to determine the interior structure of perfluoro-15-crown-5 ether (PFCE)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs).[1] We apply these NPs as ultrasound imaging agents for long-term ultrasound imaging. Acoustic characterization, which we performed prior SANS measurement, demonstrated an unusual behavior of these NPs in acoustic field. To explain this behavior, we carried out solid state NMR and 2D solution NMR (Heteronuclear Overhauser Enhancement and Exchange Spectroscopy (HOESY)) along with other physicochemical characterization. Both NMR techniques revealed that highly hydrophobic PFCE is in a very close contact with water, in a range of maximal few Ångström. The behavior of NPs was compared to nanocapsules, which we prepared using a different surfactant to form miniemulsion. In contrast to NPs, we could not detect the HOE effect in nanocapsules. These results indicated that the interior structure of our NPs do not have a core-shell structure and thus different other perfluorocarbon-loaded colloids, which usually have a core-shell morphology.

In our SANS experiment we measured NPs in D_2O and in H_2O/D_2O 36/64 (*v*/*v*) mixture, which matches PFCE. Additionally, we compared the structure of our NPs to several controls including PFCE-loaded core-shell nanocapsules and non-loaded PLGA NPs (schematic representation and structures of PLGA, PFCE and PVA, which was used as surfactant for synthesis of PFCE-loaded NPs end PLGA-only NPs in Figure 1 a).

Figure 1 b-d shows the form factors of PFCE-loaded nanoparticles, nanocapsules (NCs) and non-loaded PLGA NPs plotted versus the scattering vector q in both solvents. The first view at the scattering patterns of NPs and NCs reveals differences in colloidal stability between both systems. While NPs remain stable during the whole duration of the measurement, NCs display lower colloidal stability and aggregate, resulting in the increased scattering intensity at low q-region. This aggregation could possibly be a result of Ostwald ripening, as NCs contain a liquid PFCE core. Similar behavior was previously observed by NMR and DLS previously: both size of NCs and HOE increased after several hours, indicating that PFCE is leaking out of the capsules (data not shown).

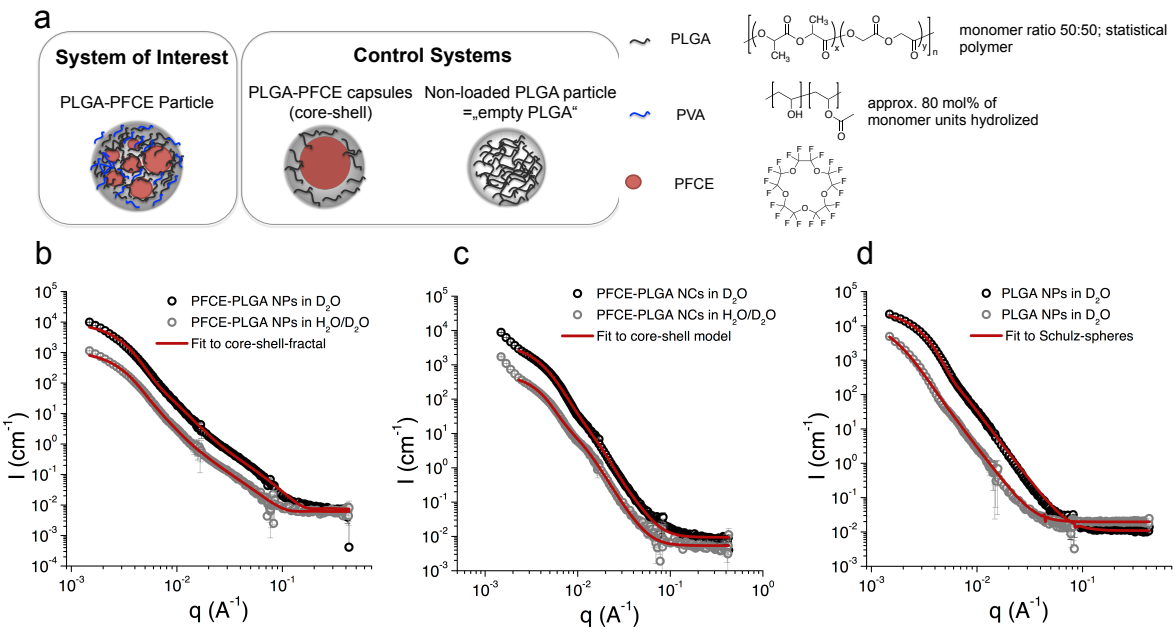

Figure 1. Schematic representation of studied particles and scattering patterns of PFCE-PLGA NPs (b), PFCE-PLGA NCs (c) and PLGA NPs (d) in D_2O and H_2O/D_2O 36:64 (*v:v*) mixture with fits to fractal core-shell, polydisperse core-shell and spheres with Schulz size distribution respectively. Modell analysis was carried out using SASview 4.1.

The model fitting of the scattering profiles is still ongoing. When low q-region is excluded from the fit the scattering patterns of NCs can be described with a core-shell model, where core and shell both have a Schulz-distribution of radius and thickness (Figure 1b). For non-loaded "empty" PLGA NPs a full sphere model with a Schulz size distribution provided reasonable results (Figure 1c). The slight deviation of the fitting model from the experimental data at high q-region could result from surface roughness or from presence of some NP-dimers at low concentration, which did not affect high q-region. Both form factors could be fitted using a simultaneous fitting approach in H_2O and H_2O/D_2O mixture (Table 1) using either radius (PLGA-NPs) or radius and thickness (NCs) as a global parameter in both solvents. Modell analysis of PFCE-loaded PLGA nanoparticles, however, turned out to be challenging. Most likely the structure consists of fractal PFCE domains, which are encapsulated in PLGA and PVA matrix. The most reasonable fit of the experimental data was obtained with a model of fractal core-shell particle.[2] This model describes a particle as a fractal aggregate from core-shell building blocks. However, this model is not optimal to describe the experimental data, resulting in high values of chi2. This deviation between model and experimental data could be because the model does not take the entanglement of polymeric chains from different fractal core-shell building blocks into account. Thus, our system could be a combination of mass-fractal core-shell with a surface fractal model. Therefore, fractal core-shell model should be extended with an additional term to describe this

interaction. Other form factor models, like core-shell sphere, full sphere or fractal sphere with mixed SLD of PLGA and PFCE could not describe the experimental data.

In summary, SANS seems to confirm our hypothesis that PFCE-loaded PLGA nanoparticles do not have a core-shell structure with liquid PFCE core. However, development of customized models is needed to finalize structural evaluation.

Table 1. Fitted and calculated parameters of nanocapsules and „empty" PLGA nanoparticles in D_2O and H_2O/D_2O 36/64 (*v:v*) mixture.

Nanocapsules in D_2O and H_2O/D_2O			**PLGA-only nanoparticles D_2O and H_2O/D_2O**		
	D_2O	**H_2O/D_2O 36/64**		**D_2O**	**H_2O/D_2O 36/64**
Scale (vol. fraction)	0.007	0.007	**Scale (vol. fraction)**	0.0077	0.0077
radius /nm	16	16 ± 0.08	**radius / nm**	63 ± 0.07	63
thickness / nm	17	17 ± 0.1	**SLD * 1e^{-6} Å^2**	2.35 ± 0.002	2.45 ± 0.002
SLD core * 1e^{-6} Å^2	3.87± 0.04	3.87e-6	**SLD solvent * 1e^{-6} Å^2**	6.36	3.87
SLD shell * 1e^{-6} Å^2	2.91e-6 ± 0.0025	2.38e-6 ± 0.0033	**Distribution of radius**	0.28	0.56
SLD solvent * 1e^{-6} Å^2	6.36e-6	3.87e-6	**background**	0.01	0.02
Distribution of radius	0.76	0.46	**Chi^2/Npts**	54	28
Distribution of thickness	0.35	0.44			
background	0.009	0.053			
Chi^2/Npts	15	9			

[1]M. Srinivas, L. J. Cruz, F. Bonetto, A. Heerschap, C. G. Figdor, I. J. M. de Vries, Biomaterials **2010**, 31, 7070.
[2]J. Texeira, J. Appl. Cryst. **1988**, 21,781-785.