ESRF	**Experiment title:** Structure and stability of artificial oxygen carriers by USAXS	**Experiment number**: SC-5247
Beamline: ID02	**Date of experiment**: from: 25.01.2022 to: 28.01.2022	**Date of report**:
Shifts: 9	**Local contact(s)**: Dr. Michael Sztucki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

This experiment was performed remotely. Samples have been sent to ESRF, and data collection was performed by Michael Sztucki, while Martin Schroer was present via online conference system. Due to the cancellation of another beamtime, we received 9 shifts of beamtime.

In this experiment, we determined the structure and stability of concentrated albumin-derived artificial oxygen carriers (A-AOC) in aqueous suspension, by combined ultra-small angle X-ray scattering (USAXS) and SAXS measurements. Utilizing the versatility of ESRF's ID02 beamline (variable sample-detector-distance of up to 31m; high photon flux), structural information from the micron to the nanometre range in aqueous environment have been accessible from medical relevant A-AOC suspensions over a broad concentration range.

In detail, data collection on aqueuous suspensions of A-AOCs was performed at several sample-detector distances (1 m; 8 m; 31 m), yielding in total an accessible q-range from 0.002 nm^{-1} to 7.5 nm^{-1} (covering a length scale range from 0.8 to 3000 nm).
Scattering data were collected of A-AOCs from different synthesis approaches: Ultra-sound-assisted and microfluidizer-assisted [1-5]. For each, sample concentrations from 20 to 0.2 wt% were studied at different temperatures (5°C to 65°C).
Fig. 1 displays representative USAXS profiles for samples from two different synthesis approaches at selected temperatures. Specific differences of the A-AOCs are detectable: The structure and size of microfluidizer-based particles is stable for the whole temperature range covered, while gelation/aggregation between the particles is reflected from the increase at very small angles. In contrast, A-AOCs from the ultra-sound synthesis are not temperature-stable, but decrease in size.

Noteworthy, the contribution of unbound albumin is clearly visible from Fig. 1b), indicating the sensitivity of the method for studying complex physiological sample systems over a wide length scale range.

Analysis of the USAXS profiles yields access to the particle size distribution (Fig. 2). These data allow for the first time the correct size determination of the A-AOCs in aqueous solution, thus, in their medically relevant environment, as conventional characterization methods lack resolution or probe samples under non-physiological conditions.

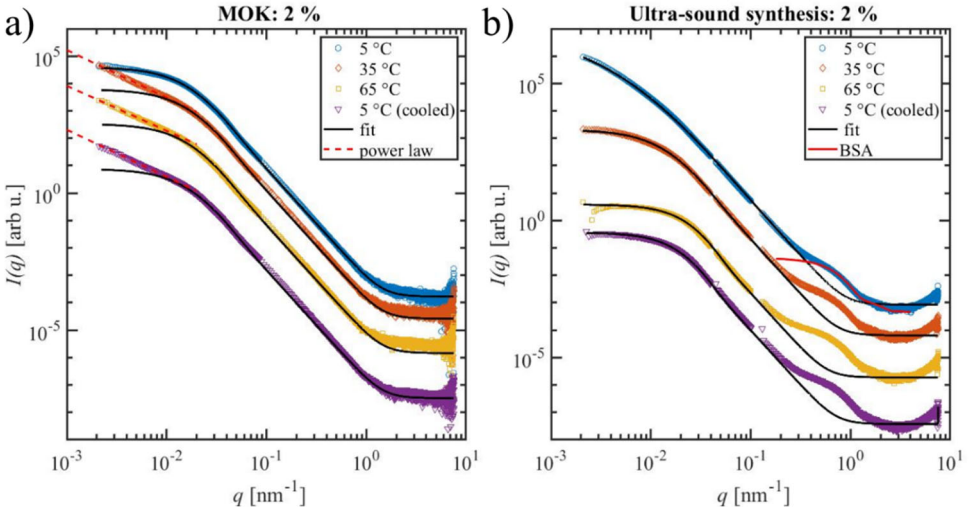

Figure 1: USAXS profiles, merged from measurements at different sample-detector distances. A-AOCs a) from microfluider (MOK)- and b) from ultra-sound based synthesis, collected at different temperatures.

As demonstrated, our USAXS measurements yielded access into both the ultrastructure and the stability of A-AOCs. Clearly, our different synthesis approaches lead to particle of different sizes and stability, as probed over a wide concentration and temperature range. Based on the (ongoing) data analysis, structure-stability correlations will be determined. These findings will promote the fine-tuning of our AOC-synthesis approaches.

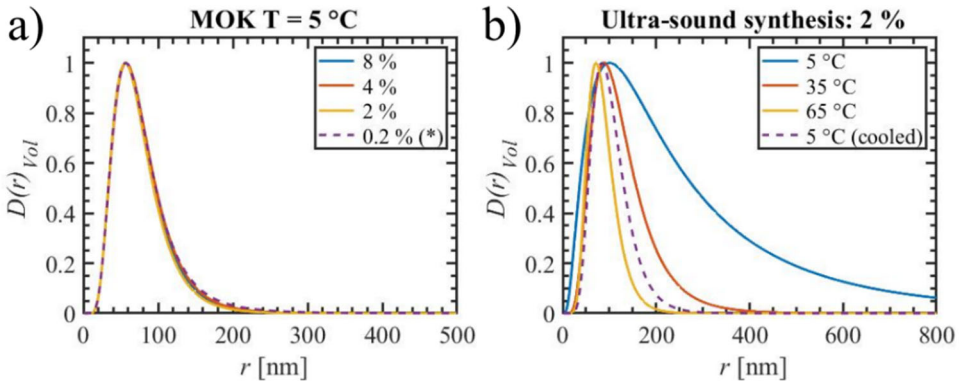

Figure 2: Size distribution determined for A-AOCs from two syntheses approaches: a) microfluidizer-assisted at different dilutions; b) ultrasound-assisted at different temperatures).

We plan publish the experimental USAXS results within upcoming articles describing in detail the synthesis, structure, and stability of A-AOCs. The data will be also presented at the XVIII SAS2022 conference in Campinas and at Europhysiology 2022 in Copenhagen.

References

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