EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Time resolved SAXS for controlled mineralization protocols of calcium phosphate and calcium carbonate in presence of fetuin-A.	**Experiment number**: MA-5094
Beamline: ID 02	**Date of experiment**: from: 24-September-2021 to: 27-September-2021	**Date of report**: 12-09-2023
Shifts: 9	**Local contact(s)**: **Lauren Matthews**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

[1] YOUNAS Daniyal*

[1] HAGEBERG Ingvild Uri *

[2] CHUSHKIN Yuriy*

[1] CHATTOPADHYAY Basab

[1] BREIBY Dag Werner

[3] GIBAUD Alain*

[4] UCAR Seniz

[5] LUND Reidar

[2] MATTHEWS Lauren*

[1] Department of Physics, Norwegian University of Science and Technology (NTNU), Høgskoleringen 5, 7491 Trondheim, Norway

[2] *ESRF, The European Synchrotron, 71 avenue des Martyrs, 38043 Grenoble Cedex 09, France*

[3] IMMM, Le Mans University, Bld O. Messiaen, 72085 Le Mans, Cedex 9, France

[4] *Department of Chemical Engineering, Norwegian University of Science and Technology, Norway*

[5] *Department of Chemistry, University of Oslo, Problemveien 11, 0313 Oslo, Norway*

Report:

Time resolved small angle X-ray scattering (TR-SAXS) experiments were carried out to study the reaction kinetics on early stages of crystallization which can give insights on early onsets of nucleation. The proposed experiments focussed on the time-resolved SAXS measurement to study formation of calcium carbonate ($CaCO_3$) in presence of different additives such as salts, proteins and polyelectrolytes. It is expected that the experiment will provide insights into the earliest stages of nucleation of $CaCO_3$, and information regarding mineralisation protocols with time resolution of milliseconds will be obtained. It was also proposed to study calcium phosphate formation mechanism, but we prioritised investigating $CaCO_3$ formation as it linked directly to an upcoming beamtime at ID10 (MA-5103) where we studied the different morphologies and crystal forms of $CaCO_3$ arising due to the presence of these additives.

During the experiment we performed TR-SAXS measurements for a series of concentrations ranging from 2mM up to 36mM of precursors to precipitate calcium carbonate in presence of several additives for example fetuin-A, polystyrene sulfonate (PSS), sodium chloride (NaCl) and poly aspartic acid (PAA) at temperatures of 25, 35 and 45°C.

Two different data- collection procedures were employed:

1. Precipitation in cell flow system: Pumps a bulk solution of reacting precursors through a tube in front of beam to perform measurements.
2. Precipitation in stopped flow system: Pumps separate precursors into a mixing chamber in microliter quantities that react while passing through the beam.

Cell flow datasets were analysed to study structural evolution of $CaCO_3$ for 3 different concentrations. In presence of PSS formation of bigger particles was observed on decreasing concentrations of PSS. Hurd-Flower model fitting was performed to trace the size evolution of particles, which also led to discovery of more than one sized population in the solutions which is also reported in literature. Results were published in *J. Appl. Cryst.* **56**, 2023, 1114–1124. Abstract of the publication is given below along with some representative results.

Abstract of *J. Appl. Cryst.* 56, 2023, 1114–1124:

"The formation of calcium carbonate ($CaCO_3$) nanoparticles (NPs) in the presence of polystyrene sulfonate (PSS) as an additive was examined by time resolved small-angle X-ray scattering (SAXS) in a flow system that mimics experimental conditions used at home facilities where the precipitation can be achieved in a beaker. The experiments were carried out at low concentrations to remain in the dilute regime. A model-independent analysis was performed using the Porod invariant which defines the scale factor, leaving only the distribution of radii as the adjustable parameter. The presence of the PSS additive strongly retards the precipitation of $CaCO_3$ NPs. The formation of NPs reaches a state of equilibrium after a few minutes. Here, it is shown that the concentration of precursors at a fixed PSS concentration plays a key role in determining the size of the NPs obtained. A full analysis of the SAXS patterns was carried out using the Hurd–Flower model to account for the weaker intensity decay than the classical Porod behaviour. The temporal evolution of the particle radii was determined. Wide-angle X-ray scattering experiments carried out simultaneously show that the particles formed have the structure of vaterite with growth consistent with the evolution of the Porod invariant."

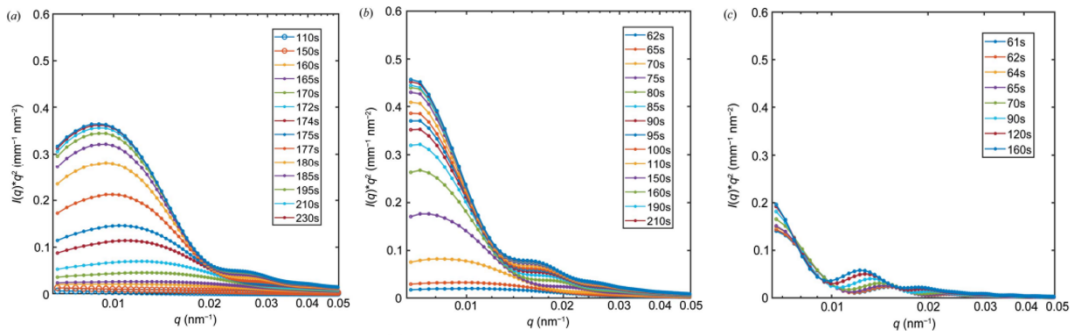

Figure 1: 1D SAXS profiles displayed as Kratky plots during the CaCO3 precipitation, for precursor concentrations of (a) c = 9.0 mM, (b) c = 7.0 mM and (c) c = 4.5 mM

Data on stopped flow experiments are being analysed and a representative example is presented below as Kratky plots exhibiting change in reaction kinetics on increased amount of NaCl additives in 18mM of Na_2CO_3 and $CaCl_2$ each as precursors.

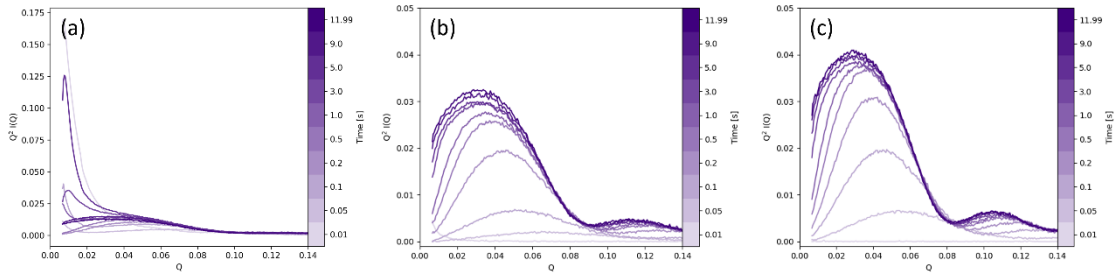

Figure 2: 1D SAXS profiles with stopped flow setup displayed as Kratky plots during the $CaCO_3$ precipitation in presence of (a) 50mM (b) 150mM and (c) 250mM NaCl

The studies on stopped flow precipitation shown in Figure 2 are being analysed. The study on CaCO3 precipitation in presence of PSS conducted with the cell flow setup has been published :

1. Gibaud, A., Younas, D., Matthews, L., Narayanan, T., Longkaew, K., Hageberg, I. U., Chushkin, Y., Breiby, D. W. & Chattopadhyay, B. (2023). *Insights into the precipitation kinetics of CaCO3 particles in the presence of polystyrene sulfonate using in situ small-angle X-ray scattering*. J. Appl. Cryst. 56, 1114–1124.

Beamline Configuration:

Energy: 12.23 keV
Wavelength: 0.101 Å
Detector: Eiger-4M. 2070 x 2167 pixels (horizontal × vertical), with a square pixel size of 75 µm
Sample-detector distance: 10 m