



	<b>Experiment title: Determining the Kinetics of Structural Changes During the Formation of Interpolyelectrolyte Complexes (IPECs) by Stopped-Flow SAXS Experiments</b>	<b>Experiment number:</b> SC-5309
<b>Beamline:</b> ID02	<b>Date of experiment:</b> from: 04/11/2022 to: 07/11/2022	<b>Date of report:</b> 01/03/2023
<b>Shifts:</b> 9	<b>Local contact(s):</b> Lauren Matthews	<i>Received at ESRF:</i>
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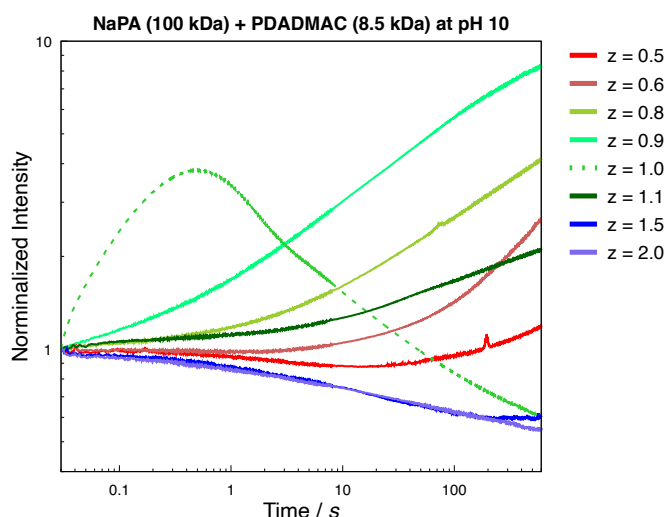
### Report:

The goal of this experimental session was to study formation and ageing processes taking place upon mixing the oppositely charged polyelectrolytes poly(diallyldimethylammonium chloride) (PDADMAC) and sodium polyacrylate (NaPA) by time-resolved SAXS (TR-SAXS) using a stopped-flow SAXS set-up. In mixtures of oppositely charged polyelectrolytes one typically observes formation of interpolyelectrolyte complexes (IPECs), whose size and structure can vary largely and depending on the type of polyelectrolytes (homopolymer/copolymer), their Mw, the mixing ratio, and the solvent quality (e.g. the salt concentration). Concerning a static point of view, the resulting phase behaviour and structures have been studied intensely for many years and in general are rather well understood, even if sometimes it is not easy to ascertain thermodynamic equilibrium and long-time metastable structures may be observed that depend on the pathway of their formation. Typically, the initial reaction between the oppositely charged polymers is fast, but the rate of subsequent structural transformations and their precise evolution is rarely investigated and understood, despite its high importance for a deeper understanding of IPECs, and this in particular for their potential applications in the field of delivery.

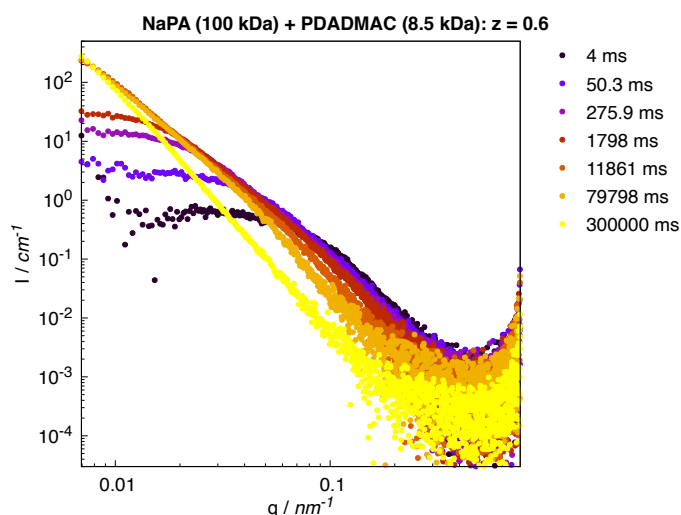
In our experiments concerning this report, we varied systematically the charge mixing ratio  $z = [-]/[+]$  and the length/Mw of the polyacid component NaPA, in order to see how these system parameters determine the structural evolution of the formed complexes. In addition, we explored the effect of hydrophobic modification of NaPA (hmPAA) by having increasingly higher amounts of hydrophobic ester chains (dodecyl) in the PA. By doing so, we gained a deeper understanding of the structure formation in such IPECs and will be able to better judge how the formed structures depend on the kinetics of formation. For the purpose of our interest in tracking the formation and ageing kinetics of such formed complexes, we studied IPEC formation with PDADMAC of

8.5 kDa and NaPA of 5.1 and 100 kDa (aqueous solutions). All mixing series were investigated at pH 10 (full charging of PA) for nominal charge ratios  $z$  of 0.5, 0.6, 0.8, 1.0, 1.5 and 2.0. Further on, we studied mixtures of 8.5 kDa PDADMAC with hm-NaPA of  $\sim 15$  kDa that contain 10, 20 and 40 mol% of dodecyl side chains at pH 10 and for all the  $z$  values given above. All measurements were performed at 25 °C, for a fixed nominal total charge concentration of 8 mM ( $\sim 0.8 - 2.0$  g/L, depending on  $z$ -ratio) and each measurement series was followed for 5 mins with at least 5 repetitions per mixing experiment. All stock solutions were degassed by sonication in an ultra-sonic bath for  $\sim 20$  min before use.

For following the structural changes with a sufficient time-resolution and fast data acquisition ID02, equipped with the SAXS: Eiger2-4M detector, was the ideally suited instrument. To cover a relevant  $q$ -range of  $0.005 - 1$  (or 3)  $\text{nm}^{-1}$  we measure at S-D distances of 2.5, 10 and 30.7 m at a wavelength of 0.1 nm (energy: 12230 eV) at a given high flux of  $1.93 \cdot 10^{13}$  ph/s. Efficient mixing was assured by use of a SFM-4000 instrument (BioLogic) equipped with a Berger Ball-mixer and a hard-stop for a precise flow control (flow speed: 4.665 mL/s). The observation head of the instrument was equipped with a quartz capillary with a thickness of 1 mm in diameter. Regular cleaning of the cuvette was performed by flushing the capillary with a 2 % Hellmanex solution, followed by intense flushing with MilliQ water.

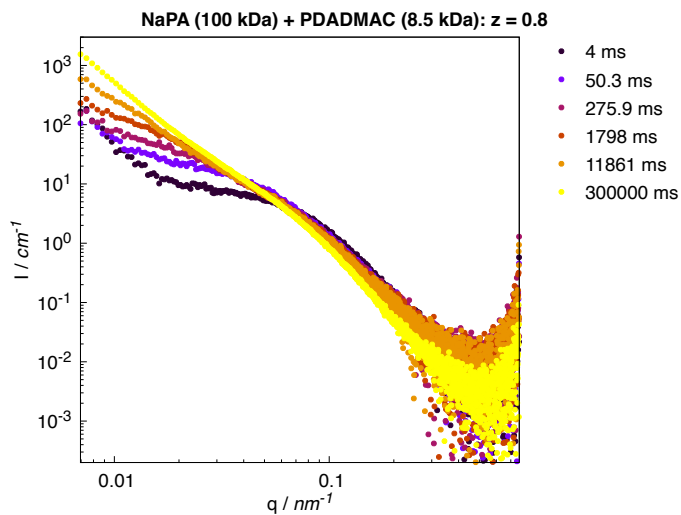


**Fig. 1:** Stopped-flow light scattering data from 100 kDa NaPA + 8.5 kDa PDADMAC for various  $z$ -ratios (pH 10). Growth regime for  $z > 0.5$  till stoichiometric mixing ( $z = 1.0$ ), relaxation into long-term stable IPECs for  $z > 1.2$ .

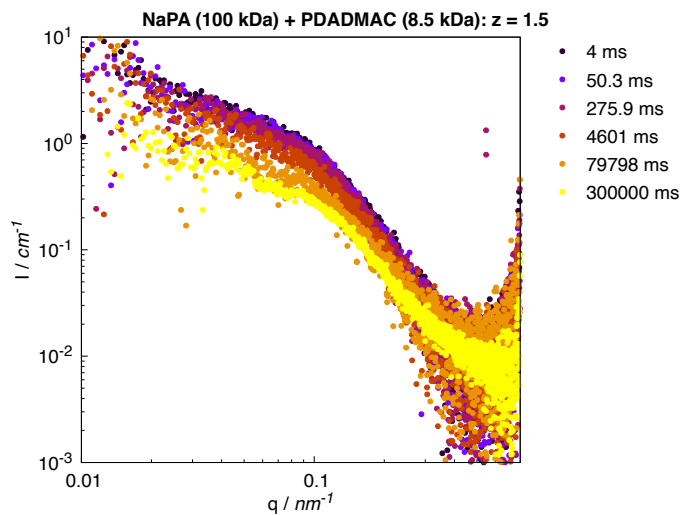


**Fig. 2:** TR-SAXS data from 100 kDa NaPA + 8.5 kDa PDADMAC for  $z = 0.6$  at pH 10. Curves from selected frames show the growth of initial particles into larger structures (excess of smaller chain component).

In general, we were able to follow the structural evolution of IPECs over a wide range of mixing ratios for all measured combinations via TR-SAXS. Here are just some examples given from a selected series for the situation of mixing two linear homopolymers. Initial complexes formed fast within the dead time of the mixing experiment ( $< 4$  ms) as indicated by the difference in intensities for samples after mixing compared to the pure stock solutions of the polyelectrolytes. A comparison of the intensity evolution within the Guinier regime from performed SAXS measurements with stopped-flow light scattering measurements (Fig. 1), previously conducted in our labs, shows a good agreement in observed kinetic trends addressing the good reproducibility in terms of mixing quality for these experiments. Here, e.g., mixing experiments of 100 kDa NaPa with 8.5 kDa PDADMAC, presenting a larger asymmetry in chain length, show for  $z < 1.0$  (excess of PDADMAC) growth kinetics into larger structures (Fig. 2 and Fig. 3) over a wide range in  $z$ -ratios. A marked difference for  $z = 0.6$  (Fig. 2) and  $z = 0.8$  (Fig. 3) is apparent in the mid- $q$  region from both plots. For mixtures reaching towards stoichiometric conditions ( $z = 1$ ), the phase behaviour shifts into a biphasic region via precipitation, where IPECs have more dense and compact structures.



**Fig. 3:** TR-SAXS data from 100 kDa NaPA + 8.5 kDa PDADMAC for  $z = 0.8$  at pH 10. Curves from selected frames represent the evolution of initial particles into more dense and compact structures with continuous growth (precipitation regime).



**Fig. 4:** TR-SAXS data from 100 kDa NaPA + 8.5 kDa PDADMAC for  $z = 1.5$  at pH 10. Curves from selected frames show the relaxation of initially formed structures into stable IPECs (excess of NaPA with larger chain length asymmetry).

The observed phase behaviour is switched when NaPA is in excess, as in the case for  $z = 1.5$  (Fig. 4). The monophasic phase behaviour of equilibrated IPECs is reflected by a decay in  $I_0$  with the formation from larger, rather fuzzy-like structures into more core-shell-like structures, becoming more obvious when having a closer look at larger  $q$ .