



## Experiment Report Form



	<b>Experiment title:</b> Hybrid Poly Ion complexes as Colloidal MRI Contrast Agents	<b>Experiment number:</b> SC-4606
<b>Beamline:</b> ID02	<b>Date of experiment:</b> from: 26/01/2018 to: 29/01/2018	<b>Date of report:</b> 08/09/2023
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**Report:**

**Scientific Background**

Hybrid polyion complexes (HPICs) result from the mixing of double hydrophilic block copolymers and oppositely charged polyvalent metal ions.<sup>1,2</sup> The driving force for their formation is the coulombic interactions between polyelectrolyte and metal ions together with the entropic gain from the release of the counterions (and bounded water molecules). We recently proposed HPICs containing Gd<sup>3+</sup> ions as new probes for Magnetic Resonance Imaging as they showed high stability over a large range of pH and ionic strengths and relaxivity values higher than standard molecular complexes.<sup>1</sup> This approach of mixing of ions and polymers within micellar architectures opens many opportunities to develop new families of HPICs for future biological applications. In this context, we were interested on HPICs based on Ga<sup>3+</sup> ions for positron emission tomography. In particular, as block copolymer, we used poly(ethylene oxide)-b-poly(acrylic acid) (PEO-PAA) with molecular weight 5kDa and 3.2kDa respectively. Different amounts of Ga<sup>3+</sup> salt (gallium nitrate) were added in

order to progressively counterbalance the polyelectrolyte charge and the charge ratio was defined as  $\rho = 3.[\text{Ga}^{3+}]/[\text{PAA}]$ . Their hydrodynamic diameter and their stability to pH could be characterized by Static and Dynamic light scattering.

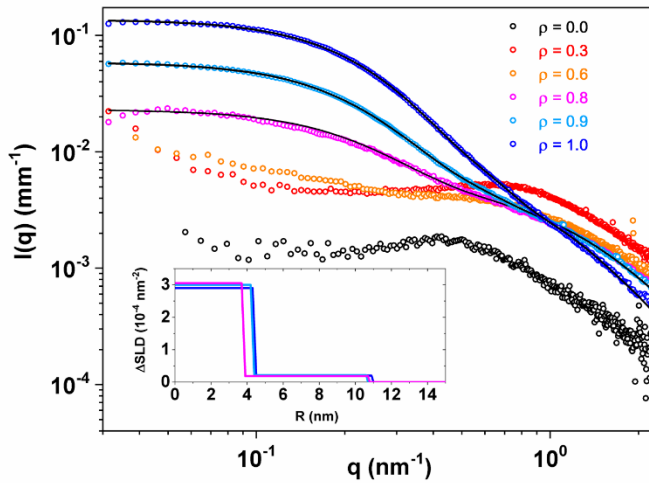
Therefore, with this experiment we aimed at **investigating the structural evolution as a function of the composition and pH together with the ion distribution** inside the self-assemblies thanks to Small Angle X-ray Scattering and Anomalous Small Angle X-ray Scattering.

## Results and Discussion

We performed experiments with a sample to detector distance of 1m in order to cover a  $q$ -range,  $2 \times 10^{-2} < q < 2 \text{ nm}^{-1}$  where  $q$  is the scattering wave vector defined as  $q = (4\pi/\lambda) \sin \vartheta/2$ ,  $\lambda$  being the wavelength ( $\lambda \sim 1.21 \text{ \AA}$ ) and  $\vartheta$  the scattering angle. Measurements were performed in a flow through quartz capillary (diameter of the order of 1.8 mm) which allows a reliable background subtraction and the control of radiation damage that might be induced by the x-ray exposure.

Experiments were performed on HPIC solution prepared at different  $\rho = 3.[\text{Ga}^{3+}]/[\text{AA}]$  in the range from 0.3 to 1. The concentration of  $\text{Ga}^{3+}$  was kept constant ( $c = 10 \text{ mM}$ ) while varying the PEO<sub>5k</sub>-PAA<sub>3.2k</sub> content (up to 10 wt%) pH was adjusted to  $7.0 \pm 0.2$ . For experiments on the effect of pH, it was adjusted by dropwise addition of NaOH (1 M) or HCl (1 M).

SAXS patterns were collected by tuning the x-ray energy close to the Gallium-K-edge ( $E=10371.5 \text{ eV}$ ) at  $\Delta E = -117, -47, -27, -11, -3, -1, +1, +13$  and  $+53 \text{ eV}$ .



**Figure 1.** Evolution of the SAXS scattering signal at fixed energy (10254 eV) as a function of the ratio  $\rho$ , for pH 7.2 and a fixed concentration of  $\text{Ga}^{3+}$  of 10 mM. Solid lines: best fitting according to eq. (2) and (3) of the SAXS scattering signal of Ga HPICs solution with  $\rho$  from 0.8 to 1. For clarity, only 10% of the experimental points are plotted. **Insert:** The electron density profiles ( $\Delta\text{SLD}$ ) for the core-shell particle deduced from the best fittings

Firstly, SAXS patterns at a fixed energy on the mixtures of  $\text{Ga}^{3+}$  and PEO-PAA polymer at five different ratios  $\rho$  (Figure 1), allow to elucidate the conformation of the nano-objects. As already said, the concentration of  $\text{Ga}^{3+}$  was kept constant ( $c=10 \text{ mM}$ ) while varying the PEO-PAA content. Electrostatic interactions are dominant until a ratio  $\rho$  of 0.8. Approaching the electroneutrality, the electrostatic repulsions are fully screened by the presence of  $\text{Ga}^{3+}$  counterions and nano-object with an average radius of gyration  $R_g$  of 7.5 nm are then formed. The values of  $R_g$  listed in **Table 1** were derived performing the Guinier analysis of the low- $q$  region of the data set according to eq. (1):

$$I(q) = I_0 \exp\left(\frac{-R_g^2}{3} q^2\right) \quad (1)$$

Where  $R_g$  is the radius of gyration allowing estimating the overall size of the particles [1].

The SAXS intensity profiles for  $R \geq 0.8$  were modelled using the form factor of polydisperse spherical core-shell particles  $P(q)_{CS}$ , assuming  $S(q) \approx 1$ .

$$P(q)_{CS} = \left[ V_c(\rho_c - \rho_s) \frac{\sin(qR_c) - qR_c \cos(qR_c)}{(qR_c)^3} + V_s(\rho_s - \rho_m) \frac{\sin(qR_e) - qR_e \cos(qR_e)}{(qR_e)^3} \right]^2 \quad (2)$$

$V_c$  and  $V_s$  indicate the volumes of core and shell,  $R_c$  the radius of the core,  $R_e$  is the external radius, and  $\rho_c$ ,  $\rho_s$ ,  $\rho_m$  are the scattering length densities of the core (PAA), shell (PEO) and solvent. An additional contribution describing the spatial fluctuations of the polymer chains in the shell was also added to model the high- $q$  behaviour of the scattering data (Ornstein-Zernike term) [2]. Therefore, the total intensity  $I(q)$  is described as:

$$I(q) = V^2 \Delta\rho^2 P(q)_{CS} + I(q)_{OZ} \quad (3)$$

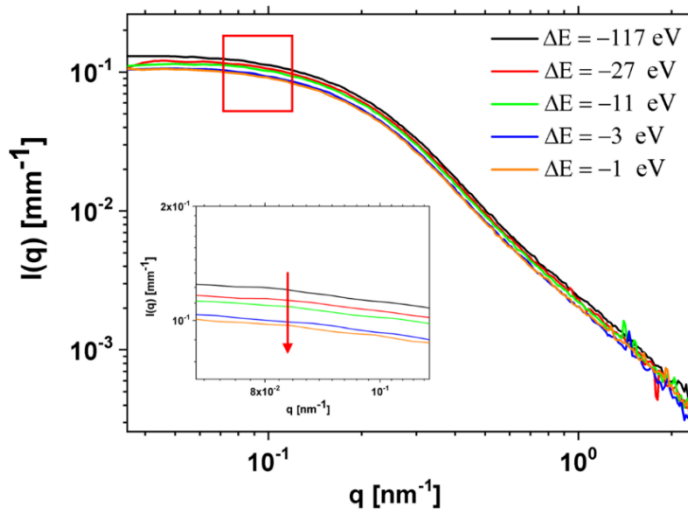
$$\text{where } I(q)_{OZ} = \frac{I_0}{(1+\xi^2 q^2)^d}$$

and  $d$  was fixed to -1.6 as expected for swollen polymer chains in good solvent.

**Table I.** List of structural parameters from the modelling of the SAXS intensity profiles at different values of  $R$  according to **eq (2) and (3)**. The values of the radius of gyration ( $R_g$ ) are also shown.

Parameters [nm]	$R=1$	$R=0.9$	$R=0.8$
$R_c$	4.3±0.9	4±1	4±1
$R_e$	11	11	10.8
$\xi$	0.94	0.69	0.63
$R_g$	7.5±0.4	7.5±0.4	-

In **Table I** the structural parameters from the fitting are listed. The scattering length of the core,  $\rho_c$ , was calculated according to stoichiometry for the different ratios and used as fixed parameters for the fitting: 0.001245 nm<sup>-2</sup> ( $R=0.8$ ), 0.00124 nm<sup>-2</sup> ( $R=0.9$ ) and 0.001235 nm<sup>-2</sup> ( $R=1$ ). The measured scattering was found to mainly originate from the core of the nano-objects, since the PEO chains of the corona show low X-ray contrast.



**Figure 2.** Scattered intensity versus the scattering wave vector  $q$ , as a function of the incident energy (HPIC solution with  $\rho = 1$  and pH 6.8). **Insert.** Zoom of a selected region. The arrow is a guide for eyes. Only five energies are shown for the sake of clarity, corresponding to the difference  $\Delta E$  to the absorption K-edge of Ga<sup>3+</sup> counterions: -117 eV, -27 eV, -11 eV, -3 eV and -1 eV.

In **Figure 2**, the measured intensities for different values of the incident energy are reported for a Ga HPIC solution with  $\rho = 1$ . The intensity varies as function of the incident energy approaching the Ga<sup>3+</sup> threshold. Such energy dependence of the scattering intensity allows extracting information on the gallium ion localization within the solution of HPICs. [3-6]

The contrast variation in ASAXS is given by the energy dependence of the atomic scattering factor  $\Delta f(Z, E)$ , where  $Z$  is the atomic number (for Ga<sup>3+</sup>  $Z=31e^-$ ) and  $E$  is the incident x-ray energy.

$$\Delta f(E) = \Delta f_0 + f'(E) + if''(E) \quad (4)$$

where the non-resonant term  $f_0$  is the atomic number of the element,  $f'$  and  $f''$  are the real and imaginary part of  $f$  as a function of the incident x-ray energy  $E$ .

As a result, the energy dependence of the radial scattering length density profile  $\Delta\rho_{SL}$  can be calculated as:

$$\Delta\rho_{SL}(r,E) = \nu(r) r_e \Delta f(E) = \Delta\rho_{SL}^0(r) + \nu(r) r_e [f'(E) + if''(E)] \quad (5)$$

with  $\nu(r)$  being the spatial distribution of the anomalous scattering units (i.e. Ga ions),  $r_e$  the classical electron radius and  $\Delta\rho_{SL}^0(r)$  the non-resonant scattering length density profile (i.e. the usual contrast well below the absorption edge).

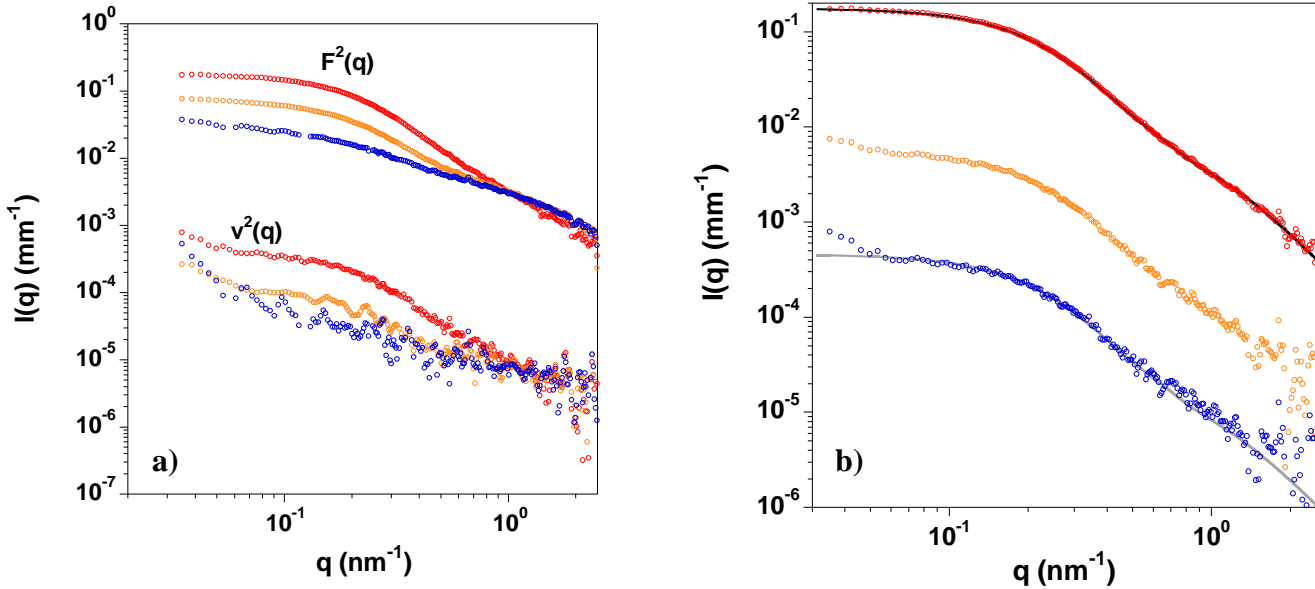
Therefore, the total scattering intensity  $I(q, E)$  can be expressed as follows:

$$I(q, E) = F_0^2(q) + 2f'(E)F_0(q)\nu(q) + [f'(E)^2 + f''(E)^2\nu^2(q)] \quad (6)$$

where the term  $F_0^2(q)$  denotes the non-resonant intensity, depending on the total electron density of the material,  $\nu^2(q)$  is the energy dependent resonant term which denotes the counterion ( $\text{Ga}^{3+}$ ) self-contribution and  $F_0(q)\nu(q)$  is the cross-term, containing information about both the resonant and non-resonant terms.

Firstly, decomposition of the ASAXS signal from a Ga HPIC solution ( $\rho=1$  at pH 6.8) was obtained according to eq. (6). [3-6]

The individual contributions of the non-resonant (spherical nano-objects) and the resonant ( $\text{Ga}^{3+}$  counterions) terms are represented in **Figure 3a** for different ratios  $\rho$ . The resonant term  $\nu^2(q)$ , originating from the localized  $\text{Ga}^{3+}$  counterions, gets weaker with decreasing  $\rho$ . Simultaneously, SAXS presents a characteristic electrostatic peak in the intensity patterns for  $\rho < 0.8$ . Therefore,  $\text{Ga}^{3+}$  counterions are not localized anymore and may diffuse freely in the solution without any particular structural organisation. The counterion condensation is detected only once the nano-objects are structured.



**Figure 3.** (a) Effect of the ratio  $\rho$  on the ASAXS scattering curves decomposed on the  $F_0^2(q)$  term (top of the figure) and the  $\nu^2(q)$  term (bottom). In red,  $\rho = 1$ . In orange,  $\rho = 0.9$ . In blue,  $\rho = 0.8$ . (b) Decomposition of the measured ASAXS intensity according to eq. (6), for a Ga HPIC solution ( $\rho = 1$  at pH 6.8). In red (upper curve), the  $F_0^2(q)$  term. In orange (middle curve), the  $F_0(q)\nu(q)$  term. In blue (lower curve), the  $\nu^2(q)$  term. The solid lines represent the best fitting to eq. (3). It has been scaled down (grey line) as guide to the eye to the intensity of the  $\nu^2(q)$  term, to illustrate the similarity in the  $q$  dependence of the  $F_0^2(q)$  and the  $\nu^2(q)$  term.

Interestingly, as showed in **Figure 3b** in the case of  $\rho = 1$ , the term  $F_0^2(q)$  (i.e. the non-resonant intensity due mainly to the polymer) and the term  $\nu^2(q)$  (i.e., the energy dependent resonant term related to the gallium) have similar  $q$  dependence. Indeed, the model of eq. (3) can be used to describe (Figure 3) the counterion spatial distribution with the same structural parameters reported in Table I. This indicates that the spatial distribution of both the copolymers and the counterions within the HPICs have the same dependence on the radial distance  $r$ . Strong correlation is therefore presented between the counterions and the PAA chains present in the core of the particles.

Based on these qualitative results by decomposition, as the partial intensities according to eq. (6) show a similar intensity distribution for the energy independent SAXS term and the contribution originating from the

counterions, the model used for the fitting of ASAXS curves assumes the counterions distribution to be arranged in a similar core-shell structure as PEO<sub>5k</sub>-PAA<sub>3.2k</sub>. Moreover, by analogy with the work of Dingenouts, et al.,[3] an Ornstein-Zernike term (OZ) was added to simulate the high- $q$  tail contribution of the density fluctuation not only in the distribution of PEO/PAA but also in the counterions distribution. The OZ term in the latter case scales according to the energy dependent contrast variation of the Ga<sup>3+</sup> counterions.

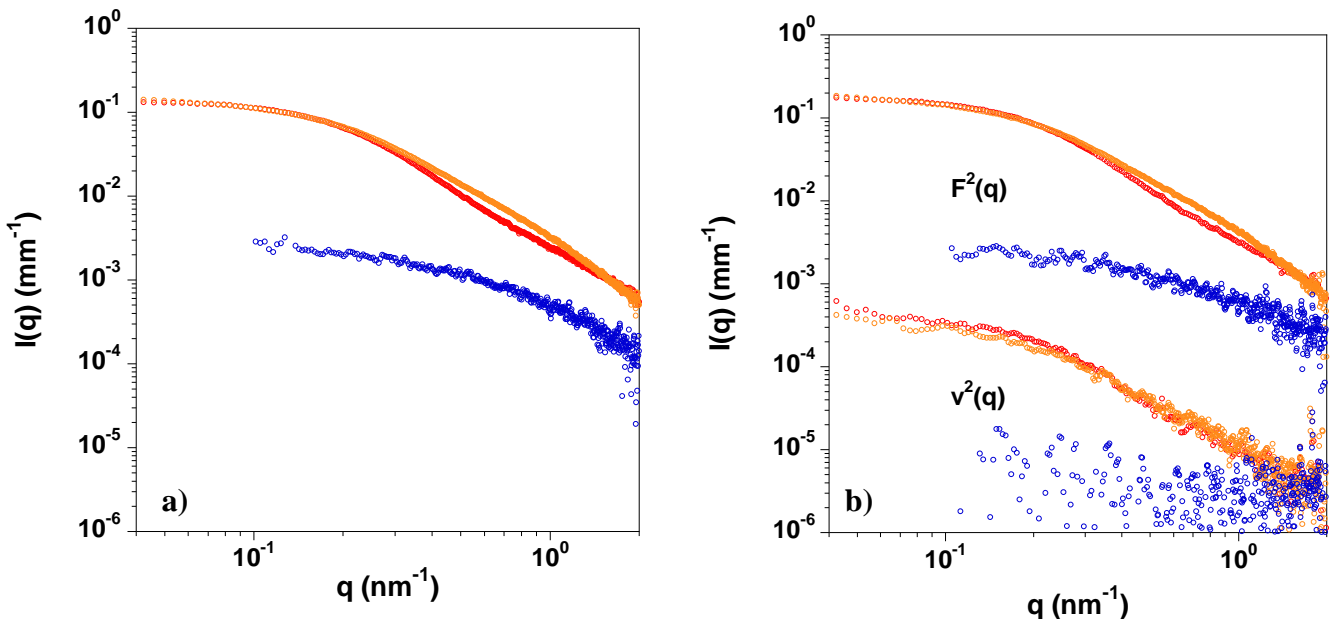
Therefore, the total ASAXS intensity  $I(q, E)$  is described as:

$$I(q, E) = P(q)_{cs} + I(q)_{OZ} + P(q, E)_{cs, Ga} + I(q, E)_{OZ, Ga} \quad (7)$$

$P(q)_{cs}$  uses the relative scattering length density differences of PAA, PEO and solvent according to eq. (2), whereas  $P(q, \Delta E)_{cs, Ga}$  uses in the same equation the radial scattering length density profile based on the spatial distribution of Ga ions according eq. (5). It should be emphasized, that all contributions in eq. (7) use the same unique set of structural parameters derived already from the SAXS analysis for fitting the scattering curves recorded at different energies. Only the scattering contrast of the Ga ions varies according to eq. (4) and the density of Ga ions is the only fitting parameter.

The simultaneous fitting of the ASAXS datasets recorded at several energies allowed then to derive quantitative information about the distribution of the Ga ions. The relative change of the scattered intensity at the different energies directly depends on the number of the bound ions and allows to determine the density of the gallium ions,  $d_{Ga}$ , in the system without further assumptions and estimate the aggregation number.  $d_{Ga}$  was found around  $5 \pm 1$  ions per nm<sup>3</sup> (i.e. ca 1700 ions per HPIC). This is slightly higher than the previous estimation (3.4 ions per nm<sup>3</sup> see above). Assuming a DP<sub>n</sub> of 44 for the PAA block and 3 carboxylate functions per gallium, this leads to an evaluation of the aggregation number of the HPICs around 120 and most probably in the 60-180 range. The large error originated from the 20% of error on the value of  $R_c$ .

According to the ASAXS modelling, Ga ions are concentrated in the core. However, a small fraction of counterions (ca 7%) is also present at the interface and along the PEO chains. It should be noted that the presence of Ga<sup>3+</sup> ions in the PEO shell may simply be related to a slight excess of ions compared to the electroneutrality. This is related to the somewhat poor accuracy of the ratio  $\rho$  due to the slight polydispersity of the copolymer. One can safely conclude that, at  $\rho = 1$ , the nano-objects present a core rich in PAA polyions. The counterion distribution in the core and shell shows density fluctuations, i.e. an organization into ion-rich/ion-poor regions (“blobs”), the nature of which, however, cannot be determined from the fitted Ornstein-Zernike term. The presence of an Ornstein-Zernike contribution in the SAXS signal (non-resonant term) also indicates the presence of PAA rich (or poor) zones in the core and of PEO respectively in the shell.



**Figure 4:** (a) SAXS scattering signal of Ga HPICs solution for a Ga HPIC solution ( $\rho = 1$ ) at various pHs. (b) Decomposition of the measured ASAXS intensity according to eq. (5) ( $F_0^2(q)$  term top curves, and  $v^2(q)$  term bottom curves) for a Ga HPIC solution ( $\rho = 1$ ) at various pHs. In red, pH=6.8. In orange, pH=4. In blue, pH=2.

Finally, structural investigations by ASAXS were also carried as a function of a decreasing pH from 6.8 to 2. De-aggregation is observed for values of  $\text{pH} < 4$  with a transition from globule to coil structure (**Figure 4a**). The value of the radius of gyration drops from 7.5 to 2.7 nm, the latter corresponding to the average size of the block copolymer coil. Counterion condensation is observed for pH 6.8 and 4.0, as shown in **Figure 4b**. Again, the same dependence on the radial distance  $r$  is observed for the counterions and the polyanion at the different pHs. At pH 2, counterions appear to be free.

## **Conclusions**

This experiment allowed us to have a clear picture of the self-assemblies formed the addition of gallium ions to a PEO<sub>5k</sub>-PAA<sub>3.2k</sub> block copolymer solution, both the size and the morphology.

Indeed, these nano-objects start to form only when the charge ratio,  $\rho$ , between the positive gallium ions and the negative PAA polyanions is higher than 0.6-0.7. At charge stoichiometry ( $\rho = 1$ ), all gallium ions and polymers form electroneutral HPICs. These nano-objects present a core-shell structure with a very low polydispersity in size. The core contains gallium interacting with the carboxylate functions and the shell is made of PEO. The core-shell structure has an overall radius around 11 nm with a core radius close to 4.5 nm. Roughly speaking, each HPIC contains around 100 polymers and 1400 gallium ions. These studies demonstrate, in particular, the utility of ASAXS for tracking inorganic ions in the solution or within the nano-objects and for corroborating data obtained with other techniques. This experiment gave rise to a publication [7].

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