ESRF	**Experiment title:** ***Divalent and monovalent ion distributions in Hyaluronic acid using anomalous Small Angle X-ray Scattering***	**Experiment number**: 02 01 675
Beamline: BM2	**Date of experiment**: from: 07-09-2005 to: 11-09-2005	**Date of report**: 07-08-2006
Shifts:12	**Local contact(s)**:Cyrille Rochas	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Erik Geissler LSP Université J. Fourier de Grenoble**

***Ann-Marie Hecht LSP Université J. Fourier de Grenoble**

***Cyrille Rochas LSP Université J. Fourier de Grenoble**

***Ferenc Horkay National Institutes of Health, Bethesda, MD USA.**

Report:
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The distribution of mono-and divalent ions in solutions of high molecular mass hyaluronic acid (HA) containing either sodium or rubidium chloride and varying concentrations of calcium or strontium chloride in near-physiological conditions is studied by SANS and anomalous SAXS. Both SANS and SAXS reveal a continuous increase in the scattering intensity at low q with increasing divalent ion concentration, while at high q the scattering curves converge.
In the absence of divalent ions, the shape of the anomalous scattering amplitude from the monovalent ions is consistent with the distribution calculated from the Poisson-Boltzmann equation. In monovalent - divalent ion mixtures, however, the divalent counter-ions occupy the immediate neighbourhood of the charged polyanion. Above a given concentration their anomalous scattering signal saturates. As the divalent ion concentration increases, both the diameter and the amplitude of the monovalent ion cloud decrease. Even in a large excess of divalent ions, ion exchange is incomplete.

Figure 1 : measured differences in scattering intensity $\Delta I(q,E_1,E_2)= I(q,E_1)- I(q,E_2)$, normalized by $[I(q,E_1)]^{1/2}$ for a 4% hyaluronic acid solution in 100 mM RbCl containing differing amounts of calcium ions.

Figure 2 : difference in scattering intensity $\Delta I(q,E_1,E_2)= I(q,E_1)- I(q,E_2)$, normalized by $[I(q,E_1)]^{1/2}$ for a 4% hyaluronic acid solution in 100 mM NaCl and 100 mM $SrCl_2$.

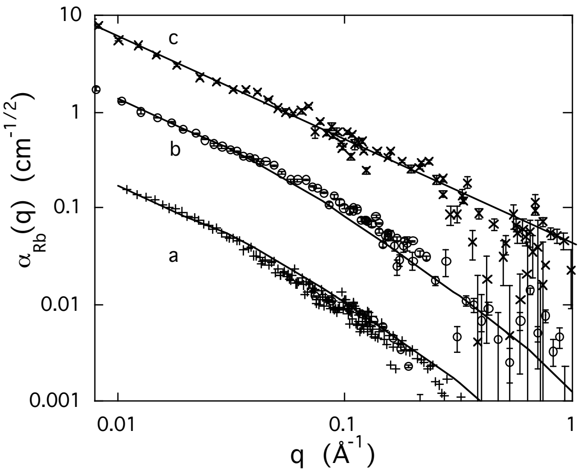

Figure 1

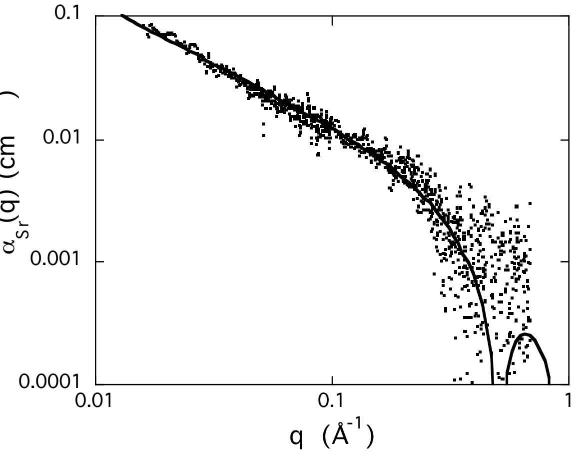

Figure 2

Figure 1 Plot of normalized SAXS intensity difference response $\alpha_{Rb}(q)= \Delta I(q,E_1,E_2)/[I(q, E_1)]^{1/2}$ at E_1=14.9, E_2= 15.196 keV from RbHA solutions containing a) 0 mM $CaCl_2$ (+), b) 50 mM $CaCl_2$ (O) and c) 100 mM $CaCl_2$ (×). Successive data sets are shifted upward by one decade. Continuous lines in (a) and (b) are the solution of the Poisson-Boltzmann equation for monovalent counter-ions. In (c), the straight line is a guide for the eye. To reduce scatter the data in the figure are binned, the vertical bars displayed being the standard errors of the regrouping procedure.

Figure 2 Plot of $\alpha_{Sr}(q)= \Delta I(q, E_1,E_2)/[I(q, E_1)]^{1/2}$ with E_1=15.8 keV, E_2 = 16.193 for sample NaHASr100. Continuous curve is the fit to the function $(1/q)|J_1(qR)/qR|$, where R, the radius of cross-section of the Sr^{++} ion cloud is 7.0 Å.