# Effect of Calcium Ions on the Structure of Synthetic and Biopolymer Gels

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## INTRODUCTION

Conformational changes of charged biopolymers induced by cations (e.g., calcium ions) are important in a variety of biological processes, such as muscle contraction, nerve excitation, gene transcription, and ion channel dynamics.[1,2] Our goal is to understand the mechanisms of ion association and structure formation in complex biopolymer systems from the interaction of their components. We use hydrogels as model systems for identifying fundamental chemical and physical interactions related to aggregation, self-assembly, phase transition, etc. Earlier studies of synthetic polyelectrolyte gels were not performed in a biologically realistic regime (e.g., ionic concentration, pH). We investigate the structure and osmotic properties of different polyelectrolyte gels to understand better the mechanisms of their interactions with calcium ions.

Here we report osmotic swelling pressure and small-angle neutron scattering (SANS) measurements made on three different hydrogel systems: poly(vinyl alcohol) gels, neutralized polyacrylic acid gels, and DNA gels.

## EXPERIMENTAL

**Materials.** Poly(vinyl alcohol) (PVA) gels were made by cross-linking with glutaraldehyde at pH = 1.5 in aqueous solutions.[3] For the experiments a fractionated polymer sample was used ($M_w$ =110,000). Cross-links were introduced at polymer concentration 5% (w/w), the molar ratio of monomer units to the molecules of cross-linker was 400.

Poly(acrylic acid) (PAA) gels were synthesized by free-radical copolymerization from acrylic acid monomers and the cross-linker N,N'-methylenebis(acrylamide) in aqueous solution.[4] Ammonium persulfate was used as an initiator. The concentration of the cross-linker was 0.3%. After gelation the acrylic acid units were first neutralized in NaOH and equilibrated with aqueous solutions of 40 mM NaCl. Then the concentration of $CaCl_2$ in the equilibrium solution was increased gradually up to 0.8 mM $CaCl_2$. At $CaCl_2$ concentrations higher than 1 mM a volume transition occurs in this system.[4]

DNA solutions were made from a sodium salt of DNA (Sigma, salmon testes, $M_w$ = 1.3 $10^6$) in 100 mM NaCl solution. The percentage of G-C content of this DNA is 41.2%. Solutions containing 3% DNA were cross-linked with ethylene glycol diglycidyl ether at pH 8.5 in the presence of TEMED.[5] Different amounts of calcium chloride were added to the DNA gels. The SANS measurements were made below the threshold calcium ion concentration (approximately 0.3 mM) at which volume transition takes place.

**Methods.** Swelling pressure measurements were made by equilibrating the gels with aqueous solutions of poly(vinyl pyrrolidone) ($M_n$ = 29 kDa) of known osmotic pressure.[6,7] A semi-permeable membrane was used to prevent penetration of the polymer into the network. When equilibrium was reached, the concentration of both phases was measured. This procedure gives for each gel the dependence of the osmotic swelling pressure upon the polymer volume fraction, $\varphi$.

Elastic (shear) modulus measurements were carried out on cylindrical gel samples using a TA.XT2I HR Texture Analyser (Stable Micro Systems, UK).

Small angle neutron scattering (SANS) measurements were made on the NG3 instrument[8] at the National Institute of Standards and Technology (NIST), Gaithersburg MD, and on the D22 instrument at the Institut Laue Langevin (Grenoble).

All experiments were carried out at 25 ± 0.1°C.

## RESULTS AND DISCUSSION

In general, biopolymer systems contain associations due to intermolecular interactions (e.g., hydrogen bonding). Hydrogen bonding and hydration play a central role in stabilizing the structure of biopolymer systems. First we illustrate the effect of hydrogen bonding on a PVA gel. Then we investigate the effect of charged groups on the structure and osmotic behavior of a synthetic polyelectrolyte gel (neutralized polyacrylic acid) and two biopolymers (DNA and polyglutamic acid).

**Poly(vinyl alcohol) gels.** Figure 1 shows the scattering response from a PVA hydrogel. To extend the range of wave vectors $q$, data obtained from static light scattering (SLS) are also shown, expressed in terms of the same absolute units as the SANS measurements. (For the SLS measurements the gel was rotated in order to yield an ensemble average over the speckle pattern scattered by the sample.) Two plateaus can be distinguished, one in the intermediate $q$ region and the other at low $q$. The scattering data were evaluated[9] by eq.1

$$I(q) = \Delta\rho^2 \frac{kT\varphi^2}{M_{os}} \frac{1}{1+q^2\xi^2} + \Delta\rho^2 \frac{8\pi\Xi^3 <\delta\varphi^2>}{\left(1+q^2\Xi^2\right)^2} \quad (1)$$

where $\Delta\rho^2$ is the contrast factor between the solvent and the polymer, $k$ is the Boltzmann constant, $\xi$ is the thermal correlation length, $\varphi$ is the polymer volume fraction, $M_{os}$ is the longitudinal osmotic modulus, $\Xi$ is the correlation length of the frozen-in structure and $<\delta\varphi^2>$ is the mean square amplitude of the associated concentration fluctuations.
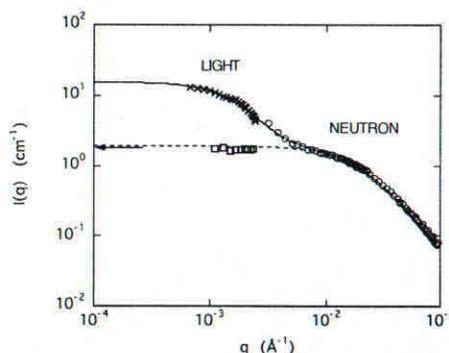


**Figure 1.** Combined SANS (circles) and SLS (crosses) data from a PVA hydrogel at polymer concentration $\varphi$ = 0.043 with cross-link density 0.25%. Correlation lengths from the fit to eq. 1 are $\xi$ = 53 Å and $\Xi$ = 425 Å. Dashed line: first term of eq. 1. Horizontal arrow: intensity estimated from macroscopic osmotic swelling pressure measurements together with the corresponding neutron scattering contrast factor. Squares: intensity of osmotic fluctuations determined by dynamic light scattering.

At high $q$ a shoulder is visible, which corresponds to the Ornstein-Zernike thermal contribution to the scattering. At lower $q$ the excess scattering can be attributed to elastic constraints, and is described by the second term of eq. 1. The dashed curve in Fig. 1 is the calculated thermal component of the fit, while the arrow at the ordinate axis gives the intensity calculated from the swelling pressure measurements and the corresponding neutron contrast factor for this system. The squares denote the intensity of temporal fluctuations measured by dynamic light scattering (DLS), expressed in the same neutron scattering units.

Within the experimental uncertainties of these three independent techniques, these results are in reasonable agreement.

**Neutralized Polyacrylic Acid Gels.** In general, neutralized polyelectrolytes in the presence of added monovalent salt are expected to behave like uncharged polymers. As the concentration of divalent ions increases monovalent counterions are replaced by divalent ions, and above a certain concentration the polyelectrolyte gels undergo a volume transition from an expanded to a collapsed state.

Figure 2 shows the SANS spectra of a PAA gel in a solution of 40 mM NaCl with two different calcium contents, one below (0.6 mM CaCl$_2$) and one above (2.0 mM) the transition (1.0 mM CaCl$_2$). Clearly the shape of the curves in Fig. 2 is qualitatively different from that of the uncharged PVA gel. The principal features of these spectra are 1) at low $q$ a power law behavior with a slope of $-3.6$, characteristic of surface scattering; 2) an intermediate region of slope approximately $-1$ and 3) at higher $q$, a shoulder, similar to that found in neutral gels. To describe this behavior, the following expression has been used[10],

$$I(q) = \Delta\rho^2 \frac{kT\varphi^2}{M_{os}} \frac{1}{\left(1+qL\right)\left(1+q^2\xi^2\right)} + Aq^{-m} \qquad (2)$$

where $L$ is the persistence length of the polymer in the semi-dilute solution, and $A$ and $m$ are constants.
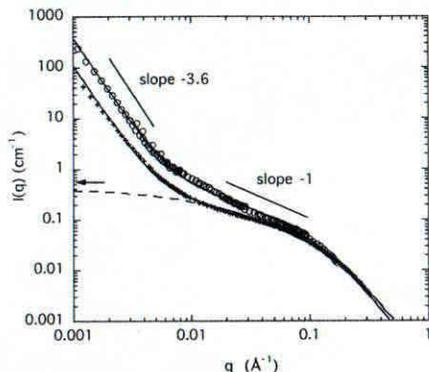


**Figure 2.** SANS intensity from neutralized poly(acrylic acid) hydrogels in 40 mM NaCl solution with two different CaCl$_2$ concentrations (+ 0.6 mM CaCl$_2$, o 2.0 mM CaCl$_2$). Lines: fit of eq. 2 to data of 0.6 mM CaCl$_2$ sample, together with the osmotic component (dashed line). Horizontal arrow: intensity estimated from macroscopic osmotic swelling pressure measurements together with the corresponding neutron scattering contrast factor.

**Comparison between the SANS Spectra of Synthetic and Biopolymers.** In Figure 3 are shown the SANS spectra of a DNA, PAA, and polyglutamic acid (PGLU) sample swollen in near physiological salt solutions. Apart from a difference in intensity, which is due to the different neutron contrast factors of these polymers, the three curves are similar. At low values of $q$ a strong increase in scattering intensity is observed, due to the presence of large aggregates. In this region the scattering intensity $I(q)$ can be described by a power law dependence of the form $I(q) \propto q^{-m}$, where $m \approx 4$ is characteristic of surfaces. In the high $q$ region a downward curvature of the intensity is visible. In DNA this feature is due to the finite radius of the double helix (ca. 1 nm).

The similarity between the scattering profiles of these different polyelectrolyte samples implies that, in spite of significant differences in chemical composition and flexibility of these molecules, the physical forces that govern the long-range correlations in these systems are the same. Over the whole range of length scales probed by SANS the

structural hierarchy is only weakly affected by the chemistry of the building blocks
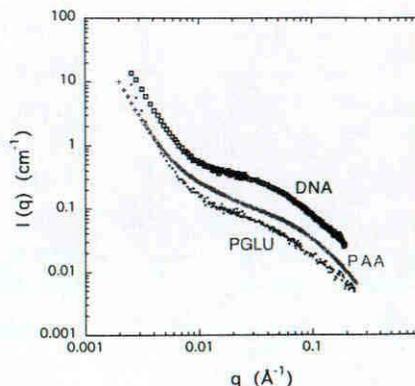


**Figure 3.** SANS spectra of neutralized polyacrylic acid (PAA), DNA, and polyglutamic acid (PGLU) samples.

## CONCLUSIONS

SANS measurements are able to detect differences in the local organization of the polymer chains in gels. In neutral gels, structural perturbations arising from the non-uniform distribution of cross-links generate excess scattering at small values of $q$. In polyvinyl alcohol hydrogels the scattering response can be described by an expression containing a thermal component and a contribution from concentration fluctuations frozen-in by the cross-links. The former can be described by an Ornstein-Zernike expression the amplitude of which is consistent with the osmotic modulus deduced from macroscopic osmotic and mechanical measurements.

The scattering response of polyelectrolyte gels is different from that of the neutral gels. At low $q$ the former systems exhibit a power law behavior characteristic of surface scattering. At intermediate values of $q$ the signal varies as $q^{-1}$, indicating that aligned regions of the polymer chain participate in the osmotic fluctuations. At higher $q$-values a shoulder is observed analogous to that in a neutral gel.

SANS measurements reveal similarities between the structure of neutralized synthetic and biopolymer systems.

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