COMPARISON BETWEEN THE EFFECT OF CALCIUM IONS ON THE OSMOTIC PROPERTIES OF SYNTHETIC AND BIOPOLYMER GELS

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Introduction

Gels are particularly suitable to study ion-polymer interactions. Monovalent-divalent cation-exchange plays central role in various physiological processes such as nerve excitation and muscle contraction It is natural to use synthetic polyelectrolyte gels as model systems to demonstrate principles, develop and verify new experimental approaches.[1,2] They are relatively easy to make, their structure can be well-controlled, and they are sensitive to different external stimuli such as temperature, solvent composition, and pH. However, previous studies on synthetic polyelectrolyte gels were not performed in conditions that are biologically relevant. In this work we compare experimental findings obtained by macroscopic osmotic and mechanical measurements, and small angle neutron scattering for weakly cross-linked sodium polyacrylate gels (SPA) and DNA gels. These gels swell in physiological salt solution and exhibit a reversible volume transition when calcium ions are added.[3,4]

Experimental

SPA gels were made by free-radical polymerization at 30% (w/w) acrylic acid monomer concentration in the presence of 0.3% N,N'-methylenebis(acrylamide) cross-linker as described previously.[3] After gelation the remaining acrylic acid units were neutralized by NaOH.

DNA gels were made from deoxyribonucleic acid sodium salt (Sigma). The molecular weight determined by ultracentrifugation was 1.3×10^6 Da. DNA gels were prepared[4] from a 3% (w/w) solution by cross-linking with ethyleneglycol diglycidyl ether at pH = 9.0 using TEMED to adjust the pH.

Both SPA and DNA gels were swollen in 40 mM NaCl solution, and then the concentration of $CaCl_2$ in the surrounding NaCl solution was gradually increased up to 2.0 mM.

Swelling pressure measurements were made by equilibrating the gels with aqueous solutions of poly(vinyl pyrrolidone) (M_n = 29 kDa) of known osmotic pressure.[5,6] The penetration of the polymer into the swollen network was prevented by a semipermeable membrane.

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[7] 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

Figure 1 shows the dependence of the swelling degree ($1/\varphi$) on the $CaCl_2$ concentration for SPA and DNA gels swollen in 40 mM NaCl solution. With increasing $CaCl_2$ concentration both systems display a sudden volume change. The sharp variation of the swelling degree indicates that the transition is a cooperative process.

In the case of a polyelectrolyte gel the swelling pressure Π_{sw} is given as the sum of elastic Π_{el}, mixing Π_{mix} and ionic Π_{ion} contributions[8]

$$\Pi_{sw} = \Pi_{el} + \Pi_{mix} + \Pi_{ion} \qquad (1)$$

In what follows we investigate the effect of calcium ions on the terms of eq. (1).

The elastic contribution can be expressed by the shear modulus *G* of the gel[9]

$$\Pi_{el} = -G = -\,ART\nu\,\varphi^{1/3} \qquad (2)$$

where ν is the concentration of the elastic chains in the network, φ is the volume fraction of the polymer, R is the gas constant, T is the absolute temperature, and A is a constant that depends on the functionality of the cross-links.

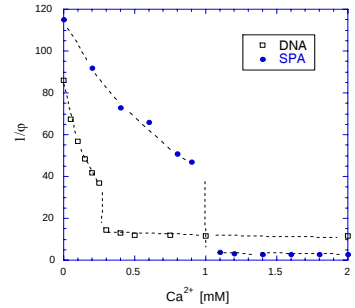

Figure 1. Variation of the swelling degree of SPA and DNA gels as a function of the $CaCl_2$ concentration. The gels were swollen in 40 mM NaCl solution.

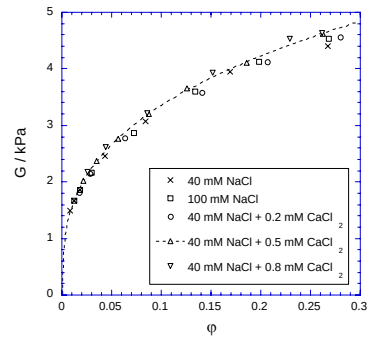

Figure 2a. Dependence of the shear modulus of SPA gels on the polymer volume fraction in 40 mM NaCl solution containing different amounts of $CaCl_2$.

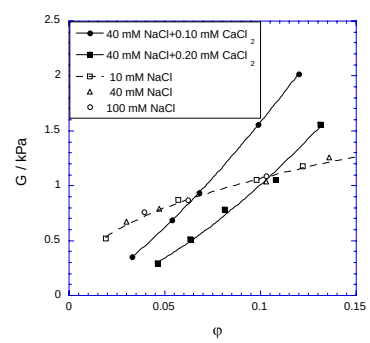

Figure 2b. Variation of the shear modulus of DNA gels with the polymer volume fraction in 40 mM NaCl solution containing different amounts of $CaCl_2$.

In **Figure 2** the effect of Ca^{2+} ions on the shear moduli of SPA and DNA gels is compared. In SPA gels *G* is practically independent of the $CaCl_2$ concentration (Figure 2a). This result implies that Ca^{2+} ions make no significant contribution to the cross-link density. In contrast, the elastic

properties of DNA gels are substantially modified by Ca^{2+} ions (Figure 2b). It is likely that Ca^{2+} ions replace condensed sodium ions, and reduce the repulsion between DNA strands. Molecular association creates DNA-rich domains separated by regions of diminished DNA concentration. The elastic modulus of a gel containing concentrated zones embedded into a soft elastic matrix is primarily governed by the properties of the matrix. This picture is consistent with the observed decrease of *G* at high swelling degrees in the presence of Ca^{2+} ions. At higher DNA concentration, however, the elastic modulus of the Ca-containing gel exceeds that of the corresponding Ca-free gel.[4]

The mixing contribution Π_{mix} can be given by a Flory-type equation

$$\Pi_{mix} = -(RT/v_1)[\ln(1-\varphi) + \varphi + \chi_0\varphi^2 + \chi_1\varphi^3] \qquad (3)$$

where v_1 is the molar volume of the solvent, χ_0 and χ_1 are constants.

In **Figure 3** is shown the variation of χ_0 and χ_1 with the $CaCl_2$ concentration for SPA and DNA gels. In both systems the second order interaction parameter χ_0 varies weakly with the polymer concentration, while the third order interaction parameter χ_1 exhibits a sharp, jump-like increase at low $CaCl_2$ concentration. The variation of χ_1 may be related to the effect of Ca^{2+} ions on the flexibility of the polyeletrolyte chains.

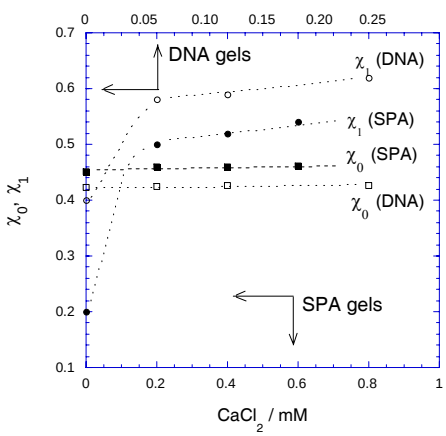

Figure 3. Variation of the Flory-Huggins interaction parameter as a function of the $CaCl_2$ concentration in DNA gels swollen in 40 mM NaCl solution.

Figure 4 shows the neutron scattering intensity *I(q)* as a function of the scattering vector *q* for SPA and DNA gels. The main features of the scattering spectra of these gels are similar. At low *q* a power law behavior is observed caused by scattering from surfaces. At intermediate *q* the scattering intensity varies as q^{-1}. This behavior is typical of scattering from rod-like structures. At high *q* the scattering response can be described by the Ornstein-Zernike expression. The continuous curve through the SPA data points is the least squares fit of eq. 4 to the data[10]

$$I(q) = \left[\frac{\Delta\rho^2 kT\varphi^2}{M_{os}}\frac{1}{(1+qL)}\frac{1}{\left(1+q^2\xi^2\right)} + C\varphi^n\right] \qquad (4)$$

where $\Delta\rho^2$ is a contrast factor, *k* is the Boltzmann constant, ξ and *L* are correlation lengths, *q* is the scattering vector, and *C* and *n* are constants. The first term in eq 4 describes the thermal concentration fluctuations the amplitude of which is governed by the longitudinal osmotic modulus $M_{os}(=\varphi\partial\omega/\partial\varphi+4G/3)$ of the gel, while the second term arises from concentration fluctuations frozen-in by the cross-links.

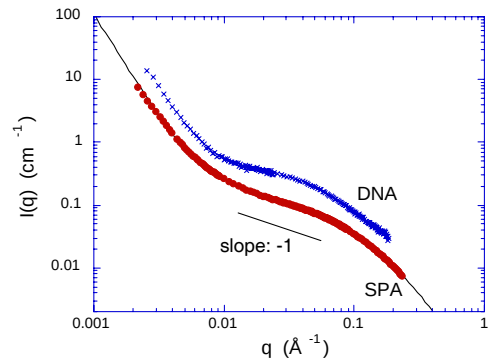

Figure 4. SANS spectra of SPA and DNA gels in 40 mM NaCl solution containing 0.1 mM $CaCl_2$.

Conclusions

Osmotic and scattering measurements reveal close similarities between the structure and macroscopic osmotic properties of weakly cross-linked SPA and DNA gels swollen in nearly physiological salt solutions. Addition of $CaCl_2$ induces a reversible volume transition in both systems. Below the transition calcium ions produce a sudden increase in the third order Flory-Huggins interaction parameter, while the second order interaction parameter only weakly increases. SANS measurements indicate that the main features of the scattering spectra are identical. Shear modulus measurements reveal differences between the elastic properties of SPA and DNA gels. The shear modulus of SPA gels is practically independent of the $CaCl_2$ concentration of the surrounding salt solution. The shear modulus of Ca-containing DNA gels is smaller at low DNA concentration, and greater at high DNA concentration than that of the corresponding Ca-free DNA gel.

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