

# Osmotic Behavior of DNA Gels Swollen in Physiological Salt Solutions

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## INTRODUCTION

Gaining insight into the physical and chemical details of the macromolecular interactions that determine cell physiology requires thorough study of both the structure of macromolecular complexes and the forces that govern their assembly. During the past decade extensive investigations have been made to understand the effect of cations on the physical properties and structure of DNA in solution.<sup>1,2</sup> An important constraint in studying ion-polymer interactions in solution is that multivalent ions lead to phase separation, even at relatively low ion concentration. The use of cross-linked networks extends the region in which the system remains stable, since in gels macroscopic phase separation does not occur. Here we focus on the effect of calcium ions on the osmotic properties of DNA gels under near-physiological conditions using complementary osmotic and scattering techniques.

## THEORY

The free energy of a gel can be expressed as a sum of three terms<sup>3</sup>, corresponding to the mixing, the elastic and the ionic contributions, respectively

$$\Delta F = \Delta F_{mix} + \Delta F_{el} + \Delta F_{ion} \quad (1)$$

The contribution of the elastic term in lightly cross-linked networks can be described by the Gaussian theory of rubber elasticity.<sup>3</sup> In fully neutralized polyelectrolytes, in the presence of added salt, the ionic term is not expected to play an explicit role. Ionic interactions, however, may modify the mixing free energy contribution. In neutral polymer solutions the Flory-Huggins theory<sup>3</sup>, based on the lattice model of solutions, expresses the mixing pressure as

$$\Pi_{mix} = \partial \Delta F_{mix} / \partial n_1 = - (RT/v_1) [\ln(1-\phi) + \phi \chi_1 \phi^2 + \chi_2 \phi^3] \quad (2)$$

where  $\phi$  is the volume fraction of the polymer,  $v_1$  is the molar volume of the solvent,  $R$  is the gas constant,  $T$  is the absolute temperature and  $\chi_1$  and  $\chi_2$  are constants that depend on the strength of the interactions.

The neutron scattering intensity from a neutralized polyelectrolyte gel can be given by a sum of thermodynamic and static components<sup>4</sup>

$$I(q) = I_{dyn}(q) + I_{stat}(q) \quad (3)$$

$$\frac{1}{I(q)} = \frac{1}{I_{stat}(q)} + \frac{1}{I_{dyn}(q)} \left[ \frac{kT}{M_{os}} \frac{1}{(1-\phi)L} \frac{1}{q^2} \frac{1}{\phi^2} \frac{1}{\phi^2} \right] \quad (3)$$

where  $\xi^2$  is a contrast factor,  $k$  is the Boltzmann constant,  $\phi$  and  $L$  are correlation lengths,  $q$  is the scattering vector,  $A$  and  $n$  are constants. The first term in eq 3 describes the thermodynamic concentration fluctuations the amplitude of which is governed by the longitudinal osmotic modulus  $M_{os}$  of the gel, while the second term arises from concentration fluctuations frozen-in by the cross-links.

## EXPERIMENTAL

DNA gels were made from DNA sodium salt (Sigma,  $M=1.3 \times 10^6$ ) by cross-linking with ethyleneglycol diglycidyl ether at  $\text{pH} = 9.0$ .

Osmotic measurements were made in aqueous solutions containing 100 mM NaCl and increasing amounts of  $\text{CaCl}_2$ . These DNA gels exhibit a reversible volume transition at approximately 0.3 mM  $\text{CaCl}_2$  concentration.

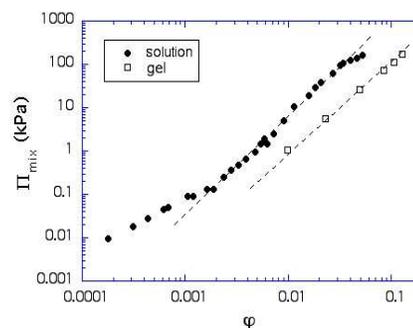
The shear modulus was obtained from uniaxial compression measurements performed on cylindrical gel specimens using a TA.XT2I HR Texture Analyser (Stable Micro Systems, UK).

Small-angle neutron scattering SANS measurements were made at the National Institute of Standards and Technology (NIST, Gaithersburg, MD) on the NG3 instrument at two sample-detector distances, 2.5 m and 13.1 m, with incident wavelength 8 Å. Corrections for incoherent background, detector response and cell window scattering were applied.

All measurements were carried out at  $25 \pm 0.1$  °C.

## RESULTS AND DISCUSSION

In Figure 1 the dependence of the osmotic mixing pressure on the volume fraction of the DNA is plotted for a gel (open squares) and for the uncross-linked DNA solution (filled circles).



**Figure 1.** Dependence of the osmotic pressure on the polymer volume fraction for DNA solutions<sup>5</sup> (in 10 mM tris-EDTA buffer) and DNA gels (in 10 mM NaCl).

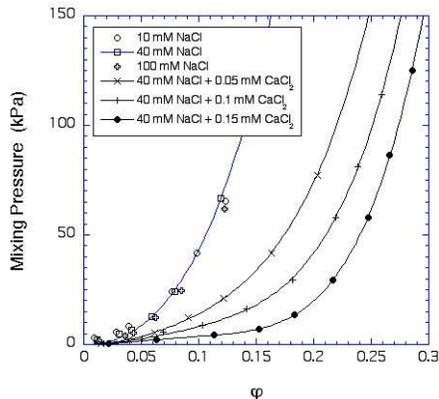
There are two important differences between the gel and the solution data:

- (i) the osmotic mixing pressure of the cross-linked DNA is lower than that of the corresponding solution, and
- (ii) in the gel the linear region ( $\Pi_{mix} \sim \phi$ ) at low concentration is absent.

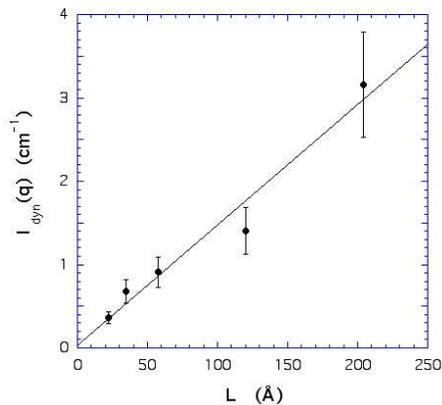
The reduction of the mixing pressure, observed in many other systems<sup>6</sup>, is usually attributed to permanent elastic constraints generated by the cross-links, which reduce the degree of freedom of the polymer chains. At low polymer concentration no linear region is present because the cross-linked system does not exist in the dilute concentration regime (i.e., below the overlap concentration). In the semi-dilute concentration region ( $\phi_{DNA} > 0.001$ ) both data sets can be fairly well described by a simple power law. The slopes of the straight lines are  $2.36 \pm 0.06$  (gel) and  $2.52 \pm 0.05$  (solution), which exceed that predicted by the scaling theory<sup>7</sup> for a neutral semi-dilute polymer solution in a good solvent ( $\Pi_{mix} \sim \phi^{2.31}$ ).

In Figure 2 the osmotic mixing pressure is plotted for DNA gels equilibrated with solutions containing 40 mM NaCl and

different amounts of  $\text{CaCl}_2$ . The curves through the data points show the least squares fits to eq. 2. The results indicate that calcium ions primarily affect  $\chi_1$ , while  $\chi_b$  only weakly depends on the  $\text{CaCl}_2$  concentration (see Table 1).



**Figure 2.** Osmotic mixing pressure of DNA gels as a function of DNA volume fraction in 40 mM NaCl solutions containing different amounts of  $\text{CaCl}_2$ .



**Figure 3.** Neutron scattering intensity from thermodynamic concentration fluctuations  $I_{dyn}(q)$  as a function of the correlation length  $L$ .

**Table 1. The Flory-Huggins Interaction Parameter for DNA Gels Swollen in 40 mM NaCl Solutions Containing Different Amounts of  $\text{CaCl}_2$**

Sample	$\chi_b$	$\chi_1$
40 mM NaCl	0.480	0.25
40 mM NaCl + 0.05 mM $\text{CaCl}_2$	0.487	0.39
40 mM NaCl + 0.10 mM $\text{CaCl}_2$	0.489	0.41
40 mM NaCl + 0.15 mM $\text{CaCl}_2$	0.495	0.42

The values obtained for  $\chi_b$  ( $\approx 0.49$ ) indicate that these DNA gels are close to the theta condition ( $\chi_b = 0.5$ ), where repulsive interactions between neighboring polymer strands compensate the attractive forces. Scaling theory predicts that at the theta condition the intensity scattered by thermodynamic concentration fluctuations (first term of eq 3), is proportional to the thermodynamic correlation length. Figure 3 shows the variation of  $I_{dyn}(q)$  calculated from macroscopic osmotic pressure measurements as a function of  $L$  obtained from the analysis of the SANS spectra. The linear relationship indicates that the osmotic behavior of DNA resembles neutral polymer systems in the theta condition.

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