# Osmotic Observations on Chemically Cross-Linked DNA Gels in Physiological Salt Solutions

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Neutralized DNA gels exhibit a reversible volume transition when $CaCl_2$ is added to the surrounding aqueous NaCl solution. In this paper, a systematic study of the osmotic and mechanical properties of Na−DNA gels is presented to determine, qualitatively and quantitatively, the effect of Ca−Na exchange on the volume transition. It is found that in the absence of $CaCl_2$ the DNA gels exhibit osmotic behavior similar to that of DNA solutions with reduced DNA concentration. At low $CaCl_2$ concentration, the gel volume gradually decreases as the $CaCl_2$ concentration increases. Below the volume transition, the concentration dependence of the osmotic pressure can be satisfactorily described by a Flory−Huggins-type equation. The $Ca^{2+}$ ions primarily affect the third-order interaction term, which strongly increases upon the introduction of $Ca^{2+}$ ions. The second-order interaction term only slightly depends on the $CaCl_2$ concentration. It is demonstrated that DNA gels cross-linked in solutions containing $CaCl_2$ exhibit reduced osmotic mixing pressure. The concentration dependence of the shear modulus of DNA gels can be described by a single power law. The scaling exponent is practically independent of the NaCl concentration and increases with increasing $CaCl_2$ content.

## Introduction

In typical mammalian cells, DNA is concentrated in the nuclei,[1,2] where its volume fraction ($\varphi$) is approximately 0.01. Within bacterial viruses, however, DNA concentration may approach $\varphi \approx 0.5$.

It is known that addition of certain cations (e.g., spermidine, $Co(NH_3)_6^{3+}$) to a DNA solution leads to the formation of compact ordered structures such as rods or toroids.[3−6] It was also observed that the effect of alkaline earth metal ions on DNA conformation is less pronounced than that of transition metal ions.[7] The former ions bind primarily to the phosphate groups, while the latter ions interact with both the phosphate groups and the heterocyclic atoms of the DNA bases. Transition metal ions disrupt base pairing and link different DNA sites.[5,7] DNA compaction plays a central role in many biological processes, for example, in gene therapy because large extended chains cannot be delivered into the cells.

DNA is a charged semiflexible polymer. In general, the behavior of a polyelectrolyte solution strongly depends on the salt concentration and on electrostatic and hydrophobic interactions.[8,9] Ion condensation leads to a decrease in the electrostatic interactions between the charged groups. The counterions in the immediate vicinity of the charged macromolecules are electrostatically associated rather than immobilized at specific sites. High valence ions screen the electrostatic interactions and change the activity of the water. Ion binding governs the interactions in metal ion−polyanion systems, but the linkage between electrolyte activity and DNA compaction is not fully understood.[7,10] Elucidation of the effect of ions on the structure of DNA requires the knowledge of the parameters that control its thermodynamic properties.

The aim of the present paper is to investigate the osmotic properties of DNA gels in aqueous solutions containing NaCl and $CaCl_2$. $Ca^{2+}$ ions do not cause DNA condensation (generally, in water, condensation requires cations of charge +3 or greater), but they produce a reversible volume change. We are particularly interested in the volume transition that occurs when $Ca^{2+}$ ions are introduced into DNA gels swollen in NaCl solutions under nearly physiological conditions. Conventionally, metal ion−polyelectrolyte interaction is investigated in dilute solutions. In DNA solutions, above a critical $CaCl_2$ concentration, phase separation occurs. Gels allow higher ion and polymer concentrations without causing macroscopic phase separation. Studies on gels may also shed light on the packing of DNA molecules in confined geometries such as cell nuclei.

In this work, the DNA concentration, the total salt concentration, and the ratio of monovalent ($Na^+$) to divalent ($Ca^{2+}$) cations are varied in a wide range around the physiological concentration. The variation of the osmotic swelling pressure is determined as a function of the swelling degree of the gel, and the ionic composition of the surrounding solution. We intend to answer the following specific questions: What is the effect of added salts (NaCl and $CaCl_2$) on the osmotic properties of neutralized DNA gels? Does the osmotic pressure of the cross-linked DNA differ from that of the corresponding DNA solution? How does the equilibrium swelling degree of a DNA gel vary when $Ca^{2+}$ ions are introduced? How does the Ca−Na cation exchange modify the thermodynamic interaction in DNA gels?

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## Theory

A thermodynamic framework describing swelling equilibrium of charged polymer gels was first proposed by Katchalsky and Flory.[11,12] At equilibrium, there is a balance between the network pressure and the osmotic pressure of the ions. In gels swollen in pure water (i.e., in the absence of added salt), the osmotic pressure is due to counterions that are confined inside the volume of the gel immersed in water. In the presence of added salt, however, the osmotic pressure is associated with the establishment of a Donnan equilibrium.

The swelling pressure of a neutral polymer gel is given as the sum of an elastic pressure term, $\Pi_{el}$, due to stretching of the chains and a mixing term, $\Pi_{mix}$, acting to swell the network.[12]

$$\omega = \Pi_{el} + \Pi_{mix} \tag{1}$$

In the simplest case, when the network chains do not interact with each other (phantom network theory[12,13]), $\Pi_{el}$ is given as

$$\Pi_{el} = -A\nu RT\varphi^{1/3} = -G \tag{2}$$

where $\varphi$ is the volume fraction of the polymer, $A$ is a constant of the order unity, $\nu$ is the concentration of the elastic chains, $R$ is the gas constant, and $T$ is the absolute temperature. The prefactor $A$ depends on the topology of the network (e.g., functionality of cross-links). $G$ is the shear modulus of the gel. We note that physical cross-links, such as entanglements, act similarly to chemical cross-links and increase the shear modulus. Equation 2 is derived for a network of Gaussian chains. For very short chains, the main chain extension even in the unstrained state exceeds that for which the Gaussian approximation is valid. A non-Gaussian treatment is essential for the accurate description of the elastic response of such systems.[13]

The form of the mixing term is similar to that of a semidilute polymer solution[14]

$$\Pi_{mix} = BRT\varphi^{m} \tag{3}$$

where $B$ is a constant and the exponent $m$ depends on the thermodynamic quality of the solvent. According to the scaling theory,[14] $m = 2.31$. It should be noted that the simple scaling relationship given by eq 3 is not applicable for concentrated polymer solutions. In the latter case, higher-order terms must be introduced

$$\Pi_{mix} = a\varphi^2 + b\varphi^3 + ... \tag{4}$$

where $a$ and $b$ are constants. The coefficient $a$ vanishes at the $\theta$ point where the second virial coefficient also vanishes.

It is generally found that the osmotic pressure of polymer solutions can be satisfactorily described over a wide range of concentration using the Flory−Huggins-type expression[12]

$$\Pi_{mix} = -(RT/V_1)[\ln(1-\varphi) + \varphi + \chi_0\varphi^2 + \chi_1\varphi^3] \tag{5}$$

where $V_1$ is the molar volume of the solvent and $\chi_0$ and $\chi_1$ are constants.

In the case of a polyelectrolyte gel, there is an additional contribution to the swelling pressure, $\Pi_{ion}$, due to the presence of charged groups.[12,15] Thus, the total swelling pressure is given as

$$\omega = \Pi_{el} + \Pi_{mix} + \Pi_{ion} \tag{6}$$

At equilibrium with a pure solvent, $\omega = 0$. Although the network structure allows ions to pass through it, Donnan equilibrium requires charge neutrality inside and outside the gel. We have to distinguish between two cases:[16,17]

For gels swollen in a pure solvent (or in very dilute salt solutions, when the concentration of the counterions $c_c$ is much higher than that of the salt ions $c_s$), the ionic contribution is given as

$$\Pi_{ion} = RTfc_c \qquad c_c \gg c_s \tag{7}$$

where $f$ is the osmotic activity coefficient.

For gels swollen in the presence of large amount of added salt, we have

$$\Pi_{ion} = RTf^2c_c^{\,2}/(4c_s) \qquad c_c \ll c_s \tag{8}$$

At high salt concentration, the ions screen the Coulomb repulsions between the charged groups of the DNA.

Combining eqs 2−8 yields the swelling pressure of the polyelectrolyte gel

$$\omega = -A\nu RT\varphi^{1/3} - (RT/V_1)[\ln(1-\varphi) + \varphi + \chi_0\varphi^2 + \chi_1\varphi^3] + RTf^2c_c^{\,2}/(4c_s + fc_c) \tag{9}$$

It should be noted that for DNA gels the counterion concentration is equal to the concentration of the phosphate groups, that is, $c_c = c_{DNA}(\text{g L}^{-1})/330$.

## Experimental Part

**Preparation of DNA Gels.** DNA gels were made from deoxyribonucleic acid sodium salt (Na−DNA from salmon testes, Sigma). According to the manufacturer the % G−C content of this DNA is 41.2%, and the melting temperature is reported to be 87.5 °C in 0.15 M sodium chloride plus 0.015 M sodium citrate. The molecular weight determined by ultracentrifugation is $1.3 \times 10^6$, which corresponds to approximately 2000 base pairs.

First, DNA was dissolved in a HEPES buffer (pH = 7.0); then the solutions were dialyzed against distilled water. DNA gels were made by cross-linking[18] with ethylene glycol diglycidyl ether (2%) at pH = 9.0 using TEMED to adjust the pH. The DNA concentration at cross-linking was 3% (w/w). The gels were equilibrated in NaCl solutions containing different amounts of $CaCl_2$ (0−0.2 mM).

**Osmotic and Mechanical Measurements.** Deswelling was achieved by enclosing the DNA gels in a semipermeable membrane surrounded by poly(vinyl pyrrolidone) solutions (PVP, molecular weight = 29 kDa) of known osmotic pressure.[19,20] The membrane prevented the diffusion of the PVP molecules into the swollen network. After equilibrium (approximately 4−5 days) was attained, the concentrations in both phases were measured. At equilibrium, the swelling

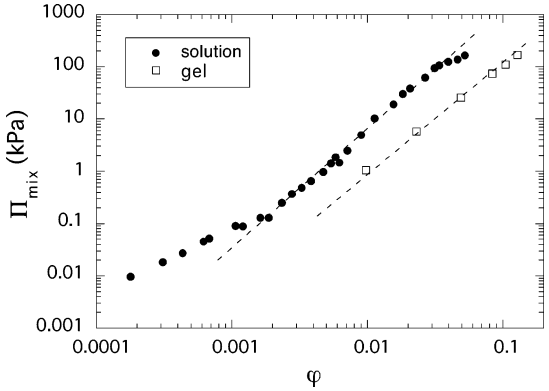


**Figure 1.** Double logarithmic plot of the osmotic pressure, $\Pi_{mix}$, as a function of the DNA volume fraction: (□) DNA gel in 10 mM NaCl solution; (●) DNA solution in 10 mM Tris−EDTA buffer. The solution data were taken from ref 21.

pressure of the gel, $\omega$, is equal to the osmotic pressure of the surrounding polymer solution.

The shear modulus of the DNA gels was determined using a TA.XT2I HR texture analyzer (Stable Micro Systems, U.K.). The measurements were performed under uniaxial compression on cylindrical specimens in equilibrium with salt solutions. The shear modulus, $G$, was calculated from the nominal stress, $\sigma$ (force per unit undeformed cross-section), using the relation[13]

$$\sigma = G(\Lambda - \Lambda^{-2}) \tag{10}$$

where $\Lambda$ is the deformation ratio. Measurements were performed in the range $0.7 < \Lambda < 1$. The absence of volume change and barrel distortion during these measurements was checked.

Both osmotic and mechanical measurements were carried out at 25 ± 0.1 °C.

## Results and Discussion

In polyelectrolyte solutions and gels, ions and polymeric species contribute to the osmotic pressure. In DNA, the counterions prevent the separation of strands in the double helix structure. However, the details of the interactions between polyanion, counterions, and added salt are not fully understood.

Our discussion of obtained experimental results falls into three sections. First the osmotic properties of DNA gels swollen in nearly physiological NaCl solutions are discussed. The osmotic pressure of the gel is obtained by subtracting the elastic pressure from the total swelling pressure. A comparison is made between the osmotic response of a chemically cross-linked DNA gel and that of the corresponding un-cross-linked solution. In the second section, we focus on the effect of $Ca^{2+}$ ions on the swelling transition and on the osmotic mixing pressure of DNA gels. The interaction between the $Ca^{2+}$ ions and DNA molecules is analyzed in the framework of the Flory−Huggins theory. Finally, we discuss the results of shear modulus measurements performed on gels swollen in NaCl solutions containing different amounts of $CaCl_2$.

**Effect of Monovalent Ions on the Osmotic Properties.** In Figure 1, the dependence of the osmotic pressure is plotted on the volume fraction of the DNA for a gel (open squares) and for the un-cross-linked DNA solution (filled circles). (The latter curve was constructed using osmotic pressure data reported in ref 21.) In the solution at low concentration ($\varphi_{DNA} < 0.001$), the osmotic pressure varies linearly with the DNA concentration. This linear behavior is characteristic of dilute polymer systems. (We note that in ref 21 at high DNA concentration ($\varphi_{DNA} > 0.025$) a second linear region is identified suggesting that the osmotic pressure is governed by the counterions. The osmotic activity coefficient estimated from this linear region is $f = 0.245$. Because of the limited amount of data at high DNA concentration, we do not discuss the osmotic behavior in this region.)

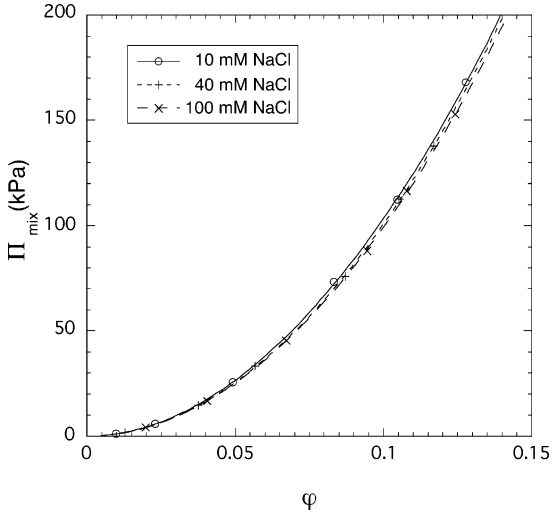


**Figure 2.** Variation of the osmotic mixing pressure as a function of the DNA volume fraction in gels swollen in NaCl solutions: (○) 10 mM NaCl; (+) 40 mM NaCl; (×) 100 mM NaCl.

There are two important differences between the gel and the solution data: (i) the osmotic pressure of the cross-linked DNA is significantly lower than that of the corresponding solution, and (ii) in the gel, the linear region ($\Pi \propto \varphi$) at low concentration is absent. The reduction of the osmotic mixing pressure, observed in many other polymer systems,[22] is usually attributed to permanent elastic constraints generated by the cross-links, which reduce the degree of freedom of the polymer chains. Consequently, a certain fraction of the polymer cannot participate fully in the concentration fluctuations that control the osmotic properties. 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 semidilute concentration region ($\varphi_{DNA} > 0.001$), both data sets can be fairly well described by a simple power law. The slopes of the straight lines are 2.36 ± 0.06 (gel) and 2.52 ± 0.05 (solution), which slightly exceed that predicted for neutral semidilute polymer solutions ($\Pi \propto \varphi^{2.31}$). To obtain agreement between the solution and the gel results, the concentration of the latter must be multiplied by 1.75. The present finding suggests that the gel behaves like a DNA solution with a lower effective concentration.

Figure 2 shows the mixing pressure of a DNA gel measured in NaCl solutions ($c_{NaCl} = 10$, 40, and 100 mM). The weak variation of the mixing pressure with the NaCl concentration indicates that the osmotic properties of these

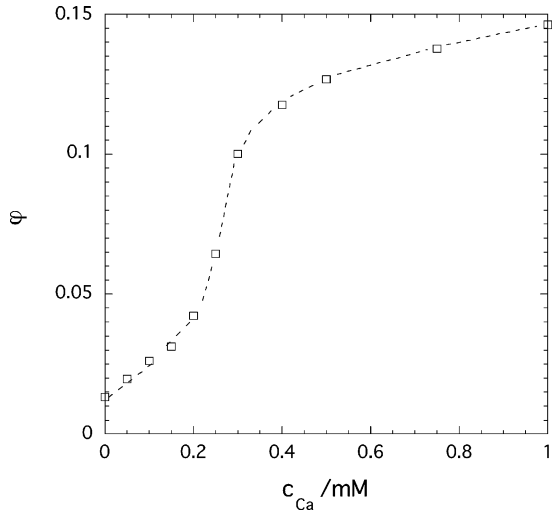


**Figure 3.** Variation of DNA volume fraction in a gel swollen in 40 mM NaCl solution as a function of the $CaCl_2$ concentration.

DNA gels are primarily governed by the polymeric contribution (second term in eq 9); the ionic contribution to the mixing pressure is negligible. The lines through the data points show the least-squares fits to eq 5. The fits yield $0.41 < \chi_0 < 0.43$ and $0.39 < \chi_1 < 0.41$.

The existence of a concentration range in DNA solutions in which the osmotic pressure is virtually independent of the concentration of the monovalent salt was observed by Raspaud et al.[21] The present results suggest that in gels this behavior exists in a wider DNA and NaCl concentration range than in solution.

**Effect of $Ca^{2+}$ Ions on the Osmotic Properties of DNA Gels.** The effect of $Ca^{2+}$ ions on the equilibrium swelling behavior of a DNA gel is illustrated in Figure 3, which shows the variation of the DNA volume fraction $\varphi$ as a function of the $CaCl_2$ concentration in the surrounding 40 mM NaCl solution. At low $CaCl_2$ concentration, $\varphi$ gradually increases with the addition of $CaCl_2$. At a critical concentration ($c_{Ca} \approx 0.25$ mM), however, a sudden volume change occurs over a relatively narrow concentration range of $CaCl_2$. Above $c_{Ca} \approx 0.25$ mM, the swelling degree only slightly depends on the $CaCl_2$ concentration of the surrounding liquid. We note that the swelling−shrinking process induced by $Ca^{2+}$ ions is reversible.

In Figure 4, the osmotic mixing pressure is plotted for DNA gels equilibrated with solutions containing 40 mM NaCl and different amounts of $CaCl_2$. The curves through the data points show the least-squares fits to eq 5. The dependences of the parameters $\chi_0$ and $\chi_1$ obtained from the fits are displayed in Figure 5 (×, +). It is apparent that $\chi_0$ is practically independent of the calcium concentration, while $\chi_1$, after an initial jump, exhibits a weak, nearly linear increase with increasing $CaCl_2$ concentration. Qualitatively similar behavior has been reported for synthetic polyelectrolyte systems when multivalent ions were introduced into networks swollen in monovalent salt solutions.[23,24]

To gain further insight into the effect of the $Ca^{2+}$ ions on the osmotic properties, we prepared gels by cross-linking the DNA in solutions containing 40 mM NaCl and 10 mM $CaCl_2$. In this experiment, both salts were added to the DNA solution prior to the cross-linker.

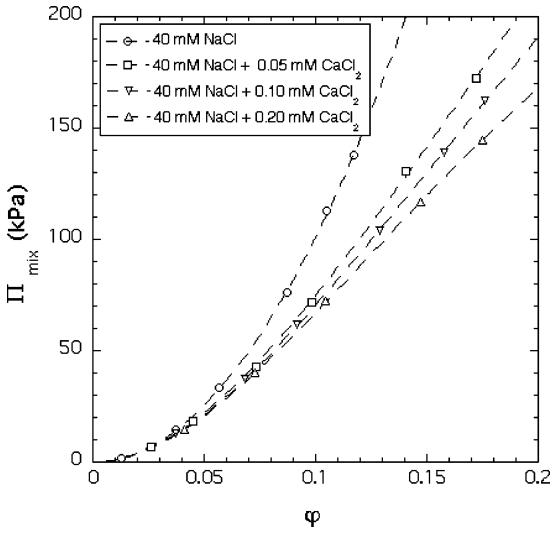


**Figure 4.** Osmotic mixing pressure of DNA gels as a function of DNA volume fraction measured in 40 mM NaCl solutions containing different amounts of $CaCl_2$: (○) 0; (□) 0.05; (▽) 0.10; (△) 0.20 mM.

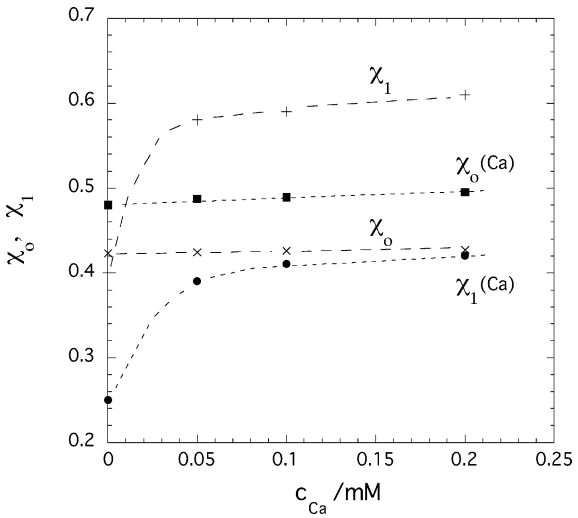


**Figure 5.** Variation of the interaction parameters $\chi_0$ and $\chi_1$ as a function of the $CaCl_2$ concentration of the surrounding 40 mM NaCl solution: (×,+) DNA gels cross-linked in Ca-free solutions; (●,■) DNA gels cross-linked in the presence of $CaCl_2$.

As can be seen in Figure 6, the mixing pressure of these DNA gels is significantly diminished, indicating that $Ca^{2+}$ ions alter the solvent quality and presumably the network structure. In addition, the replacement of $Na^+$ ions by $Ca^{2+}$ reduces the number of osmotically effective counterions. The parameters calculated from the fits to eq 5 are shown in Figure 5 (●,■). Although the numerical values of $\chi_0$ and $\chi_1$ significantly differ from those obtained for DNA gels cross-linked in the absence of $CaCl_2$, both systems exhibit qualitatively similar behavior: increasing the $Ca^{2+}$ concentration increases the value of $\chi_1$, while $\chi_0$ varies little.

It is known that in polymer solutions the third-order thermodynamic interaction parameter (which is proportional to $\chi_1 - {}^1/_3$) is governed primarily by the chain stiffness.[14] The data shown in Figure 5 suggest that $Ca^{2+}$ ions may modify the stiffness of the DNA chains. This result is in qualitative agreement with the mechanism proposed by Rouzina and Bloomfield[25] to describe the effect of nonspecifically bound cations on DNA strands. According to their model multivalent cations bind at the entrance to the B-DNA

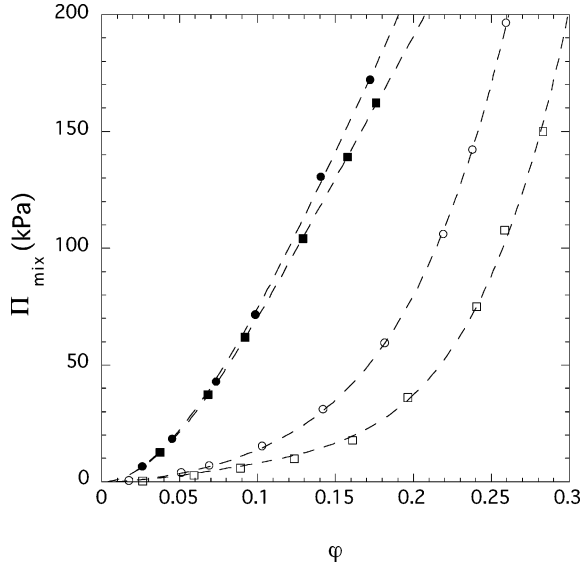


**Figure 6.** Comparison between the osmotic mixing pressures of DNA gels cross-linked in the presence (open symbols) and in the absence (filled symbols) of $CaCl_2$. Osmotic measurements were made in 40 mM NaCl solutions containing 0.05 mM $CaCl_2$ (circles) and 0.10 mM $CaCl_2$ (squares).

major groove, between two phosphate strands, electrostatically repelling sodium counterions from the neighboring phosphates and causing the chains to kink in response to the high charge of the counterion. The attractive interaction between localized ions and the unscreened phosphates leads to groove closure, accompanied by bending toward the cation. In this way, $Ca^{2+}$ ions can induce DNA bending, that is, reduce the apparent stiffness of the chain. However, Zhang et al.[26] have argued that the increased chain flexibility due to high valence cations arises from the smaller spatial extent of the counterion cloud, which dresses the polyelectrolyte chain backbone.

**Effect of $Ca^{2+}$ Ions on the Elastic Modulus of DNA Gels.** Measurement of the elastic modulus of DNA gels provides an independent estimate of the interaction between the $Ca^{2+}$ ions and DNA molecules. Multivalent cations are expected to increase the elastic modulus because they form intermolecular bridges that act as cross-links between polymer chains. In Figure 7, the variation of the shear modulus $G$ on the DNA volume fraction is shown. In these lightly cross-linked gels, the value of $G$ is small and is practically independent of the NaCl concentration over the whole concentration range explored. The concentration dependence of the shear modulus can be described by the equation $G \propto \varphi^{0.42\pm0.04}$ (dashed curve in Figure 7). The exponent is higher than that predicted by the classical theory of rubber elasticity[13] for gels consisting of flexible chains ($G \propto \varphi^{1/3}$). Indeed, DNA is a relatively rigid polymer as a consequence of its double helical nature. It is possible that, with increasing DNA concentration, structural changes occur in the gels (e.g., liquid crystal formation, hydrogen bonding). DNA mesophases are expected to increase the elastic modulus of the gel. The relatively small deviation from the behavior of networks built from flexible chains, however, suggests that the elasticity of these highly swollen DNA gels is mainly dominated by entropic rather than energetic effects,

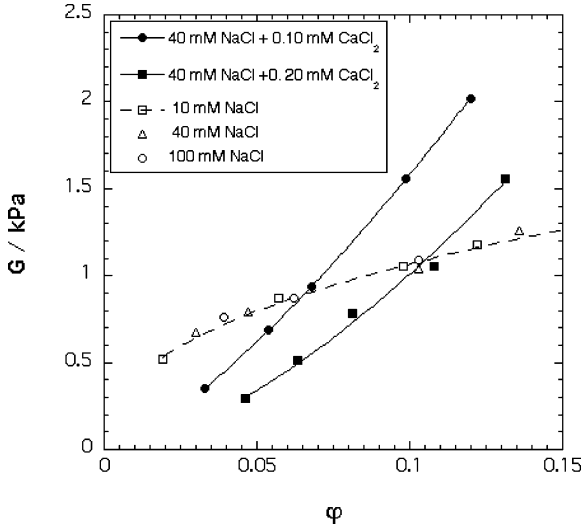


**Figure 7.** Dependence of the shear modulus $G$ on the DNA volume fraction in NaCl solutions (□, 10 mM NaCl; △, 40 mM NaCl; ○, 100 mM NaCl) and in solutions containing 40 mM NaCl and different amounts of $CaCl_2$ (●, 0.10 mM $CaCl_2$; ■, 0.20 mM $CaCl_2$).

that is, the elements can rotate more or less freely around the network junctions if the topological constraints are not too strong.

Introduction of $Ca^{2+}$ ions substantially modifies the elastic properties of DNA gels. The slope of the $G$ vs $\varphi$ graph in the presence of $CaCl_2$ is different from that in the absence. It seems plausible to assume that $Ca^{2+}$ ions form bridges between the charged phosphate groups. This explanation, however, fails to describe the decrease of the shear modulus at low DNA concentration in the presence of $CaCl_2$. Because divalent counterions compensate more efficiently for the charge of the polyanion, it is more likely that the $Ca^{2+}$ ions replace the condensed sodium counterions and reduce chain repulsion. Molecular association creates DNA-rich domains separated by regions of diminished DNA concentration. In general, the elastic modulus of a gel containing concentrated zones embedded into a soft elastic matrix is primarily governed by the properties of the matrix. The observed decrease of $G$ at a high swelling degree in the presence of $Ca^{2+}$ ions is in qualitative agreement with this picture.

Given the absence of a network model that incorporates all of these effects in a consistent manner, it is very difficult to quantitatively interpret the experimental results. For gels made of rigid rodlike chains,[27] it is predicted that the shear modulus scales with the polymer volume fraction as $G \propto \varphi^{3/2}$. The experimentally observed exponents in the present DNA gels are in this range (1.35 for the gel in 0.1 mM $CaCl_2$ solution and 1.56 for the gel in 0.2 mM $CaCl_2$ solution).

## Conclusions

It is demonstrated that the osmotic pressure of DNA gels is smaller than that of the corresponding un-cross-linked DNA solutions. The DNA gel behaves like a semidilute solution in which the DNA concentration is decreased, indicating that the reduction of the osmotic pressure is primarily caused by the presence of cross-links. Its osmotic properties are governed by the polymer concentration over an extended concentration range. The apparent reduction of

the counterion contribution may be caused by the elastic pressure that counteracts the osmotic pressure exerted by the counterions.

DNA gels exhibit a reversible volume change upon the addition of $CaCl_2$. $Ca^{2+}$ ions cause a pronounced reduction in the osmotic mixing pressure. The concentration dependence of the osmotic pressure of both Ca-free and Ca-containing gels can be satisfactorily described by a Flory−Huggins-type equation. $Ca^{2+}$ ions primarily affect the third-order interaction term, while the second-order term only slightly depends on the $CaCl_2$ concentration. It is likely that $Ca^{2+}$ ions replace the $Na^+$ counterions and more effectively compensate for the net charge of the polyanion. These results suggest that the volume transition is largely governed by association of the DNA chains caused by charge neutralization rather than by binding of the calcium ions.

The dependence of the shear modulus of lightly cross-linked gels on the DNA concentration can be described by a power law. The exponent in NaCl solutions is $0.42 \pm 0.04$, and its value increases with increasing $CaCl_2$ content. The shear modulus of the Ca-containing gels at high degrees of swelling is, however, diminished. Reduction of the shear modulus is inconsistent with the assumption that $Ca^{2+}$ acts as a cross-linker. It is more likely that the gel contains polymer-rich domains dispersed in a continuous matrix of lower polymer concentration.

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