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# Biopolymer Gels: Nanostructure and Macroscopic Properties

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**Abstract** Small-angle neutron scattering (SANS) has been used to investigate the effect of salts (NaCl and $CaCl_2$) on the structure of DNA and polyacrylic acid (PAA) gels. In the absence of salt a distinct correlation peak is observed in the SANS spectra of both systems indicating that electrostatic interactions play an important role in the organization of the polymer chains. When the salt concentration is increased, the peak position shifts to smaller values of the scattering vector $q$, and progressively vanishes. Osmotic swelling pressure measurements show that Ca ions reduce the swelling pressure and lead to the collapse of these gels. The Ca/Na ion exchange process does not affect the shear modulus of PAA gels. However, the shear modulus of DNA gels decreases with increasing Ca ion concentration at high swelling degrees, and increases at low swelling degrees. The results indicate that changing the ionic composition provides a simple way to control the nanoscale structures and properties in polyelectrolyte gels.

**Keywords** DNA · Gel · Polyelectrolyte · Shear modulus · Small-angle neutron scattering

## Introduction

Colloids, polymers and biomaterials are increasingly important, from both fundamental and applied viewpoints. The demand for materials with controlled structure and morphology at all dimensions from nanoscale to macroscale is growing rapidly. Nanostructures may confer beneficial properties on biomaterials with advanced functionality. Living organisms tailor biological materials into highly complex functional structures exerting control on composition, interactions and architecture. Biological systems operate at the cellular and subcellular levels; therefore, material properties including structure, osmotic and mechanical properties must be determined to dimensions below 100 nm.

An emerging area of practical significance relates to biomimetic polymer networks and gels. Recently designed synthetic polymers mimic the hierarchical structure and function of biological macromolecules, such as DNA, proteins, as well as biological membranes and cells. Understanding the underlying physical characteristics of these systems enables molecular and nanometer scale manipulation with the aim of engineering useful and novel properties. Examples of applications include responsive biomaterials in tissue repair, e.g., "smart hydrogel scaffolds" for tissue engineering, medical implants for diagnosis and therapy, and in-vivo drug-delivery.

The main focus of our research is on the roles that nanoscale structures and interactions play in determining the macroscopic properties of polyelectrolyte gels. In hydrogels different kind of interactions (electrostatic, van der Waals, hydrophobic interactions, hydrogen bonding, etc.) play a role in driving the formation of complex hierarchical structures. These interactions are governed by a combination of structural properties at the micro- and nanoscale as well as by macroscopic physical parameters such as ionic strength and solvent quality. It is well known that many natural and synthetic polyelectrolytes

(e.g., DNA, polyacrylic acid) exhibit a strong sensitivity to ionic strength and, in particular, to counterion valence. Changes in the ionic environment impact the structure and dynamic properties of these polymers and, at high ionic strength lead to their precipitation. The complexity of the behavior of charged macromolecular systems necessitates an investigation of the structure and physical properties on all length scales from the atomic to the macroscopic level. Small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) are well-suited methods for such studies since enhanced spatial resolution is crucial. These techniques allow us to investigate biopolymer molecules and assemblies in their natural environment and to correlate the changes in environmental conditions (e.g., ionic composition, solvent quality) with physical properties.

We developed a multiscale approach to examine the structural hierarchy, phase behavior and equilibrium properties of polymer gels. In the present work we report SANS measurements that probe the structure over a wide range of length scales (1–500 nm) and provide insight into the hierarchical organization of polymer gels. A comparison is made between the main structural features of a synthetic (polyacrylic acid sodium salt) (PAA) and a biopolymer (DNA) gel. Osmotic swelling pressure measurements and shear modulus measurements are used to determine the macroscopic properties of the same gels.

# Theory

The total free energy change, $\Delta F$, associated with the swelling of a covalently cross-linked polymer network can be given as a sum of three terms [1]

$$\Delta F = \Delta F_{\text{mix}} + \Delta F_{\text{el}} + \Delta F_{\text{ion}} , \tag{1}$$

where $\Delta F_{\text{mix}}$ is the mixing, $\Delta F_{\text{el}}$ is the elastic, and $\Delta F_{\text{ion}}$ is the ionic contribution of the free energy.

In weakly cross-linked gels the elastic contribution can be approximated by the Gaussian theory of rubber elasticity [1–3]. In polyelectrolyte gels, in the presence of large amount of added salt, the electrostatic interactions are screened, and the ionic term is not expected to play a significant role. However, ionic interactions may modify the mixing free energy contribution. For neutral polymer gels the mixing pressure can be given by the Flory–Huggins theory [1], based on the lattice model of polymer solutions

$$
\begin{aligned}
\Pi_{\text{mix}} &= -\frac{1}{v_1} \frac{\partial \Delta F_{\text{mix}}}{\partial n_1} \\
&= -\frac{RT}{v_1} \left[ \ln(1-\varphi) + \varphi + \chi_0 \varphi^2 + \chi_1 \varphi^3 \right] ,
\end{aligned} \tag{2}
$$

where $\varphi$ is the volume fraction of the polymer, $v_1$ is the molar volume of the solvent, $n_1$ is the number of the moles of the solvent, $R$ is the gas constant, $T$ is the absolute temperature, and $\chi_0$ and $\chi_1$ are constants that depend on the polymer–solvent interactions.

The neutron scattering intensity of a neutral semi-dilute polymer solution can be described by a Lorentzian function [4]

$$I(q) = \frac{A}{\left(1 + q^2 \xi^2\right)} , \tag{3}$$

where $A$ is a constant, $\xi$ is the polymer–polymer correlation length, and $q$ is the scattering vector.

The scattering intensity from gels contains another contribution due to structural features frozen in by the cross-links [4–6]. Thus, the gel signal is given by

$$I(q) = \frac{A}{\left(1 + q^2 \xi^2\right)} + B(q) , \tag{4}$$

where the functional form of the second term is defined by the details of the gel structure.

# Experimental

Gel Preparation

Polyacrylic acid (PAA) gels were prepared by free-radical polymerization in aqueous solution from partially neutralized acrylic acid monomers at 30% (w/w) monomer concentration in the presence of 0.3% $N,N'$-methylenebis(acrylamide) cross-linker as described previously [7]. After gelation the remaining acrylic acid units were neutralized by 0.1 M NaOH solution.

DNA gels were made from deoxyribonucleic acid sodium salt (Sigma). The molecular weight determined by ultracentrifugation was $1.3 \times 10^6$ Da. DNA gels were prepared [8] from a 3% (w/w) solution by cross-linking with ethyleneglycol diglycidyl ether at pH = 9.0 using TEMED to adjust the pH.

Both PAA and DNA gels were swollen in NaCl solution, and then the concentration of the $CaCl_2$ in the surrounding NaCl solution was gradually increased.

Small-angle Neutron Scattering

SANS measurements were made on gels using the NG3 instrument [9] at the National Institute of Standards and Technology (NIST, Gaithersburg MD). Gel samples were placed into standard NIST sample cells. The sample cell consisted of 1 mm thick quartz windows separated by a 2 mm thick spacer. The $q$ range explored was $0.003 \, \text{Å}^{-1} \leq q \leq 0.2 \, \text{Å}^{-1}$, and counting times from twenty minutes to two hours were used. $D_2O$ was the solvent. After radial averaging, detector response and cell window scattering were applied. The neutron scattering intensities were calibrated using absolute intensity standards. All experiments were carried out at $25 \pm 0.1 \,^{\circ}\text{C}$.

Swelling Pressure and Elastic Modulus Measurements

Swelling pressure measurements were made by equilibrating the gels with aqueous solutions of poly(vinyl pyrrolidone) ($M_n = 29$ kDa) of known osmotic pressure [10, 11]. 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). Swollen networks were uniaxially compressed (at constant volume) between two parallel flat plates. The stress-strain isotherms were determined in the range of the deformation ratio $0.7 < \Lambda < 1$.

The data were analyzed using the relation [2]

$$\sigma = G \left( \Lambda - \Lambda^{-2} \right) , \tag{5}$$

where $G$ is the shear modulus and $\sigma$ is the nominal stress (related to the undeformed cross-section of the gel cylinder). The absence of volume change and barrel distortion was checked by measuring the dimensions of the deformed and undeformed gel cylinders.

## Results and Discussion

Small-Angle Neutron Scattering Measurements

Figure 1 shows the SANS spectra of DNA and PAA gels (inset) measured in $D_2O$ at different NaCl concentrations. All the spectra exhibit two common features: low-$q$ clustering and high-$q$ solvation. The upturn in $I(q)$ at approximately $q < 0.01$ Å$^{-1}$ indicates domain formation generally observed in polyelectrolyte solutions [12–14]. The size of the clusters exceeds the resolution of the SANS experiment. Solvation is governed by the thermodynamic interactions between the polymer and the solvent molecules [15]. In the salt-free solutions the scattering curves for both gels exhibit a distinct correlation peak at a finite value of $q$, a behavior typical of weak polyelectrolyte systems. In the DNA gel the peak occurs at $q_0 \approx 0.07$ Å$^{-1}$ corresponding to an average distance of $d_0 = 2\pi/q_0 \approx 90$ Å between the charged domains. In the PAA gel the polyelectrolyte peak is not well resolved from the low-$q$ clustering feature. In salt solutions ions screen the charges, and the polyelectrolyte peak position is shifted towards lower values of $q$. In the DNA gel the correlation peak moves from $q_0 \approx 0.07$ Å$^{-1}$ (without salt) to $q_o \approx 0.04$ Å$^{-1}$ (in 10 mM NaCl solution) indicating that the size of the charged domains increases by roughly 80%. In 40 mM NaCl solution the polyelectrolyte peak has completely disappeared and the curve only exhibits a shoulder at $q \approx 0.04$.

The SANS data can be analyzed using a simple equation that reproduces the main characteristic features of the
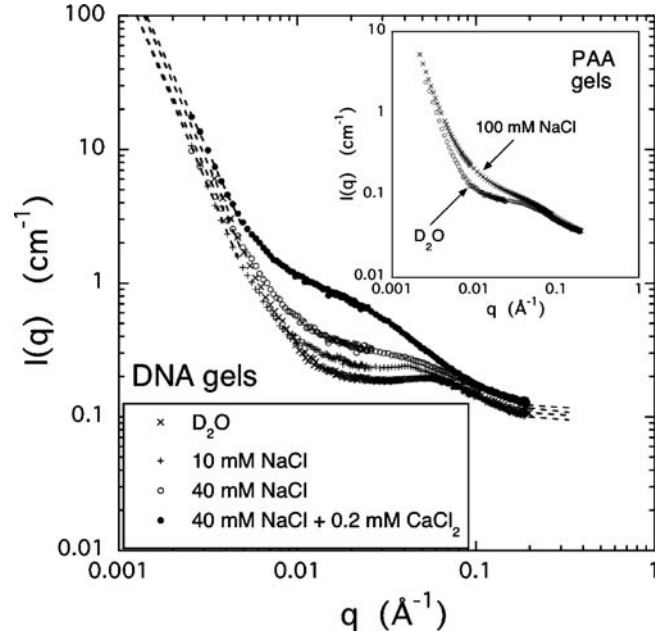


**Fig. 1** SANS intensity from DNA gels in equilibrium with $D_2O$ solutions containing NaCl (0, 10 and 40 mM) and NaCl + CaCl$_2$ (40 mM NaCl + 0.2 mM CaCl$_2$). The *inset* shows the SANS spectra of PAA gels in pure $D_2O$ (*lower curve*) and in 100 mM NaCl (in $D_2O$) (*upper curve*)

scattering curves [14, 15]

$$I(q) = \frac{A}{1 + |q - q_0|^2 \, \xi^2} + \frac{B}{q^m} + C . \tag{6}$$

In this equation $q_0$ is the peak position. $A$, $B$, $C$ and $m$ are constants. $C$ is mostly due to incoherent "background" scattering, which is independent of $q$. The dashed lines show the fits of Eq. 6 to the SANS spectra. For small values of $q$ ($< 0.01$ Å$^{-1}$) both DNA and PAA gels exhibit a power law behavior with a slope $-3.4 < m < -4$, that can be attributed to scattering from interfaces. Rough colloids give slopes between $-3$ and $-4$, whereas smooth colloids give slope of $-4$ (Porod scattering) [16, 17]. In the intermediate $q$-range ($0.01$ Å$^{-1} < q < 0.08$ Å$^{-1}$) the first term of Eq. 6 satisfactorily describes the experimental data. In the high $q$-region ($q > 0.08$ Å$^{-1}$) the scattering intensity is governed by the local geometry of the polymer molecules. We note that small ions are not visible in the SANS experiment; only their influence on the polymer conformation and the thermodynamic properties of the system is detectable.

The upper curve in Fig. 1 shows the SANS spectrum of a DNA gel measured in 40 mM NaCl containing 0.2 mM CaCl$_2$. Ca/Na ion exchange modifies the electrostatic interactions between the DNA strands and affects their organization. In the low-$q$ region Ca ions only slightly influence the slope of the scattering curve. In gels covalent

cross-links lead to a local decrease in chain mobility, and prevent significant structural reorganization. At intermediate length scales the scattering intensity from the Ca-containing gel significantly exceeds that from the other three samples. The increase of intensity is consistent with a system approaching phase separation. The present DNA gel undergoes phase separation at approximately 0.3 mM $CaCl_2$ concentration (in the surrounding 40 mM NaCl solution). In the high-$q$ region calcium ions do not influence the SANS signal, indicating that the chain geometry (cross-section of the DNA molecule) remains unchanged.

## Osmotic Pressure and Shear Modulus Measurements

In this section we focus on the macroscopic elastic and osmotic properties of PAA and DNA gels, and relate the macroscopic behavior to structural features identified by SANS.

The dependence of the swelling degree $(1/\varphi)$ on the $CaCl_2$ concentration for DNA and PAA gels swollen in 10 mM NaCl solution is plotted in Fig. 2. With increasing $CaCl_2$ concentration both systems display an abrupt volume change. The sharp variation of the swelling degree indicates that this transition is a highly cooperative process.
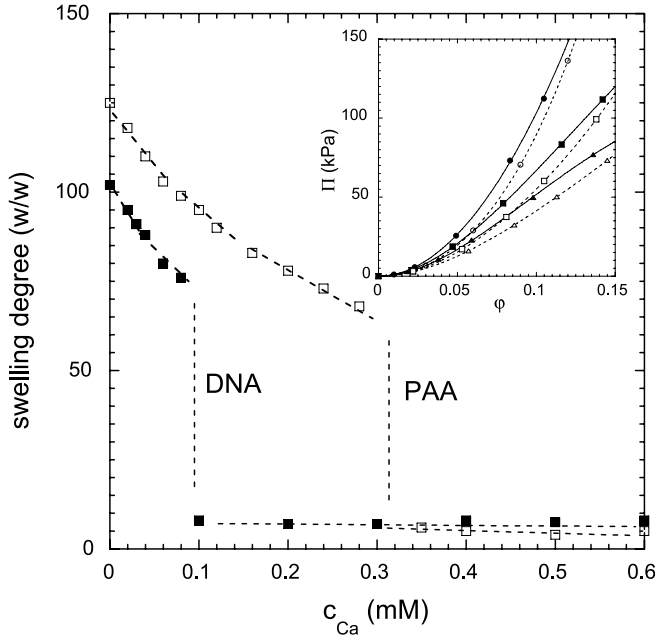
Equation 1 predicts that the swelling pressure of the gel $\Pi_{sw}$ is the sum of elastic ($\Pi_{el}$), mixing ($\Pi_{mix}$) and ionic ($\Pi_{ion}$) pressure contributions [1]

$$\Pi_{sw} = \Pi_{el} + \Pi_{mix} + \Pi_{ion} . \tag{7}$$

In what follows we investigate the effect of ions on the individual terms of Eq. 7.

The elastic contribution can be estimated from the shear modulus $G$ of the gel [2]

$$\Pi_{el} = -G = -KRT\nu\varphi^n , \tag{8}$$

where $\nu$ is the concentration of the elastic chains in the swollen network, and $K$ is a constant that depends on the functionality of the cross-links. According to the classical theory of rubber elasticity the value of the exponent $n$ is 1/3 [1, 2].

The inset in Fig. 2 illustrates the dependence of the osmotic pressure $\Pi_{mix}$ on the polymer volume fraction $\varphi$ for DNA (continuous curves) and PAA (dashed curves) gels. Each data set was measured at constant $CaCl_2$ concentration. The osmotic pressure gradually decreases as Ca ions replace Na ions, which implies that the osmotic compression modulus $K_{os}(= \varphi\partial\Pi_{sw}/\partial\varphi)$ also decreases with increasing Ca concentration. The decrease in $K_{os}$ is reflected by an increase in the SANS intensity (see Fig. 1). We note that at the phase transition both scattering intensity and correlation length ($\xi$) diverge.

Figures 3 and 4 show the variation of the shear modulus as a function of the polymer volume fraction for PAA



**Fig. 2** Dependence of the swelling degree of DNA and PAA gels on the $CaCl_2$ concentration of the surrounding 10 mM NaCl solution. *Inset* shows the variation of the osmotic pressure with the polymer volume fraction for the same gels at three different calcium concentrations (DNA gels: ● 0 mM $CaCl_2$, ■ 0.03 mM $CaCl_2$, ▲ 0.06 mM $CaCl_2$; PAA gels: ○ 0 mM $CaCl_2$, □ 0.1 mM $CaCl_2$, △ 0.2 mM $CaCl_2$)
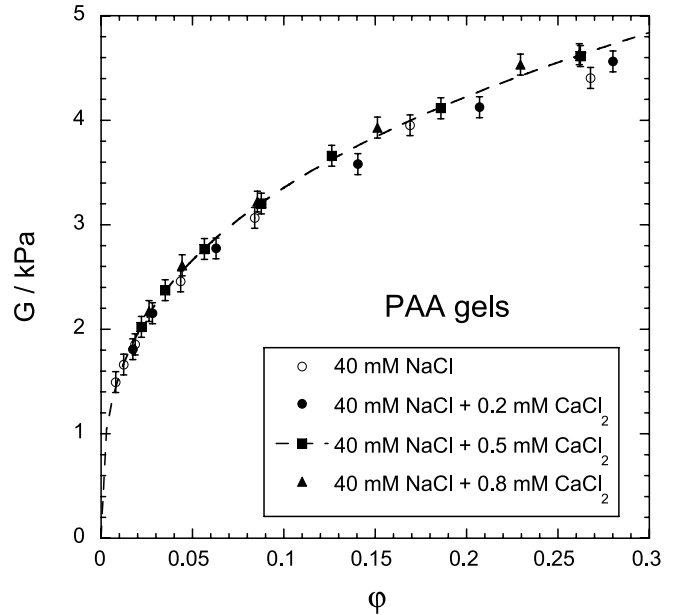


**Fig. 3** Variation of the shear modulus of PAA gels with the polymer volume fraction in salt solutions containing 40 mM NaCl and different amounts of $CaCl_2$. *Dashed curve* is a power law fit to Eq. 8 ($n = 0.34$)
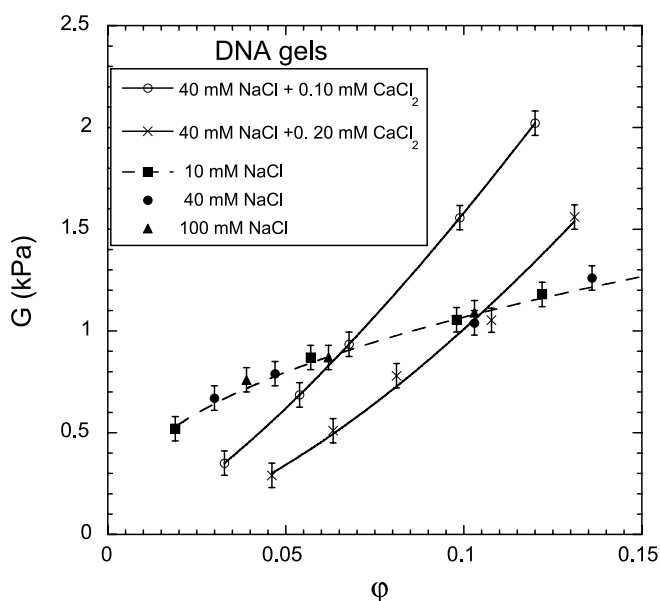
**Fig. 4** Variation of the shear modulus in DNA gels with the DNA volume fraction in salt solutions containing different amounts of NaCl and CaCl$_2$. The *dashed line* through the 10 mM NaCl data is a power law fit to Eq. 8 ($n = 0.42$)

and DNA gels swollen in NaCl solutions containing different amounts of CaCl$_2$. In PAA gels $G$ is practically independent of the ion concentration and ion valence, implying that Ca ions do not form additional "cross-links" between the negatively charged PAA chains. The dashed curve through the experimental points is the fit of Eq. 8 to the data. The value 0.34 obtained for the exponent is close to that predicted by the theory of rubber elasticity. In DNA gels $G$ is hardly affected by the NaCl concentration. However, addition of Ca ions modifies $G$. It is well known that dissolved DNA spontaneously forms liquid crystalline regions (mesophases). SANS measurements show that Ca ions only slightly affect the gel structure in the low-$q$ region (see Fig. 1). Replacing Na with Ca ions reduces the electrostatic repulsion between the charged domains producing an increase in the elastic modulus. This is observed at high DNA concentration where the elastic moduli of the

Ca-containing gels exceed that of the Ca-free gels. However, at low DNA concentration the elastic modulus decreases with increasing Ca content. In highly swollen gels the DNA-rich zones become separated by regions of lower DNA concentration. The elastic modulus of such systems is governed by the properties of the "soft" regions as indicated by the decrease of $G$.

## Conclusions

SANS and osmotic pressure measurements reveal similarities between the structure and macroscopic properties of PAA and DNA gels. In the absence of added salt the SANS spectra of both systems exhibit a correlation peak which progressively disappears as the salt (NaCl) concentration increases at constant polymer concentration. The data also show that on addition of salt the position of the correlation peak shifts to the lower $q$-region.

Ca ions reduce the osmotic swelling pressure and induce volume transition in both gel systems. Addition of Ca ions enhances the scattering intensity as expected upon approaching phase transition.

Shear modulus measurements reveal important differences between the elastic properties of PAA and DNA gels. In PAA gels the shear modulus is practically independent of the CaCl$_2$ concentration of the surrounding solution indicating that Ca ions do not form cross-links. 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 gels.

The results illustrate that changing the ionic environment in polyelectrolyte gels allows us to modify the organization of the polymer segments at the nanoscale level without significantly influencing the network structure at larger length scales.

## References

1. Flory PJ (1953) Principles of Polymer Chemistry. Cornell University, Ithaca
2. Treloar LRG (1976) The Physics of Rubber Elasticity. Clarendon, Oxford
3. Horkay F, McKenna GB (2007) Mark JE (ed) Gels, Physical Properties of Polymers Handbook. Springer, New York
4. Bastide J, Candau SJ (1996) Cohen Addad JP (ed) Physical Properties of Polymeric Gels. Wiley, Chichester
5. Horkay F, Hecht AM, Mallam S, Geissler E, Rennie AR (1991) Macromolecules 24:2896
6. Horkay F, Grillo I, Basser PJ, Hecht AM, Geissler E (2002) J Chem Phys 117:9103
7. Horkay F, Tasaki I, Basser PJ (2000) Biomacromolecules 1:84
8. Horkay F, Basser PJ (2004) Biomacromolecules 5:232
9. NIST Cold Neutron Research Facility (1999) NG3 and NG7 30-meter SANS Instruments Data Acquisition Manual, January 1999
10. Vink H (1971) Eur Polym J 7:1411

11. Horkay F, Zrínyi M (1982) Macromolecules 15:1306
12. Moan M (1978) J Appl Cryst 11:519
13. Prabhu VM, Muthukumar M, Wignall GW, Melnichenko YB (2003) J Chem Phys 119:4085–4098
14. Hammouda B, Horkay F, Becker M (2005) Macromolecules 38:2019
15. Hammouda B, Ho D, Kline S (2004) Macromolecules 37:6932
16. Bale HD, Schmidt PW (1984) Phys Rev Lett 53:596
17. Glatter O, Kratky O (1982) Small Angle X-ray Scattering. Academic Press, New York