JOURNAL OF Applied Polymer SCIENCE

Swelling Behavior of Physical and Chemical DNA Hydrogels

Pinar Karacan, Husniye Cakmak, Oguz Okay
Department of Chemistry, Istanbul Technical University, 34469 Istanbul, Turkey
Correspondence to: O. Okay (E-mail: okayo@itu.edu.tr)

ABSTRACT: DNA hydrogels were prepared from aqueous solutions of double-stranded DNA (about 2000 base pairs long) by physical and chemical means. Physical gels were obtained via denaturation–renaturation cycle of 5% aqueous DNA solutions between 25 and 90°C. Although physical DNA gels exhibit a high modulus of elasticity, the crosslinks holding the DNA network together are destroyed during the expansion of gels in water or in dilute salt solutions. It was shown that these gels can be used for the controlled release of DNA in aqueous media. Chemical DNA gels formed using ethylene glycol diglycidyl ether crosslinker are stable in water with a wide range of swelling ratios that could be adjusted by the amount of DNA at the gel preparation. Swelling behavior of chemical DNA gels in acetone/water mixtures as well as in aqueous salt solutions is very similar to that of synthetic polyelectrolyte hydrogels. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 3330–3337, 2013

KEYWORDS: gels; biomaterials; crosslinking; nucleic acids; swelling

Received 30 July 2012; accepted 4 September 2012; published online 21 September 2012
DOI: 10.1002/app.38550

INTRODUCTION

All living cells contain deoxyribonucleic acid (DNA) molecules carrying genetic information in their base sequences. DNA is a biopolymer composed of building blocks called nucleotides consisting of deoxyribose sugar, a phosphate group and four bases, adenine, thymine, guanine, and cytosine.[1] DNA has a double-helical conformation in its native state, which is stabilized by hydrogen bonds between the bases attached to the two strands. When a DNA solution is subjected to high temperatures, the hydrogen bonds holding the two strands together break and the double helix dissociates into two single strands having a random coil conformation.[2] This transition from double-stranded (ds) to single-stranded (ss) DNA is known as denaturation or melting and can be reversed by slow cooling of dilute DNA solutions. DNA denaturation was extensively investigated in the past few decades as it gives useful information on DNA regarding its structure and stability. Typically, melting curves of DNA are obtained by monitoring the UV absorption at 260 nm.[2–4] The disruption of base stacking decreases the electronic interaction between the bases so that it becomes easier for an electron to absorb a photon. Thus, denaturation leads to the hyperchromic effect, i.e., the increased absorption of light.

DNA hydrogel is a network of crosslinked DNA strands swollen in aqueous solutions.[5,6] Such soft materials are a good candidate to make use of the characteristics of DNA such as coil-globule transition, biocompatibility, selective binding, and molecular recognition.[7,8] DNA hydrogels were prepared starting from branched DNA molecules via ligase-mediated reactions.[9] These hydrogels can also be prepared by crosslinking of DNA in aqueous solutions using diepoxides as chemical crosslinking agents.[10–19] Epoxide groups can react with the amino groups on the nucleotide bases to form interstrand crosslinks leading to the formation of a three-dimensional DNA network. We have recently focused on developing responsive DNA hydrogels with a wide range of tunable properties such as the conformation of the network strands,[17] viscoelasticity,[16] nonlinear elasticity,[18] and porosity.[19] It was shown that DNA can also be physically crosslinked by subjecting DNA solutions to heating-cooling cycles.[16,18] Heating of semidilute solutions of ds-DNA above its melting temperature results in the dissociation of the double helix into flexible single strand fragments. On cooling back to the room temperature at a slow rate, the dissociated strands cannot reorganize to form the initial double stranded conformation. Hence, the hydrogen bonds formed between strands belonging to different ds-DNA molecules act as physical junction zones leading to the formation of gels with a modulus of elasticity between 10^1 and 10^4 Pa. The effects such as the concentration of DNA solution, pH, the presence of a crosslinker, and the duration of the heating period on the viscoelastic properties of the physical gels were investigated before.[16–18]

In the present study, we investigate the swelling behavior of both physically and chemically crosslinked DNA hydrogels in water and in aqueous salt solutions. Our aim is to compare the swelling behavior of DNA hydrogels with that of synthetic polyelectrolyte hydrogels. Physical gels were prepared by subjecting

aqueous solutions of 5% DNA to heating–cooling cycles between below and above the melting temperature of DNA. Such physical gels were prepared before, on a small scale, between the parallel plates of rheometer,[18] but their swelling behavior and stability in aqueous media have not been investigated. As will be seen below, although the physical crosslinks holding the DNA network together are destroyed during the expansion of the physical gels in water, they can be used for the controlled release of ds-DNA in aqueous environment. Chemical DNA hydrogels were prepared by the solution crosslinking of DNA at three different concentrations using ethylene glycol diglycidyl ether (EGDE) crosslinker. Chemical gels are stable in water and exhibit swelling behavior very similar to synthetic polyelectrolyte hydrogels.

EXPERIMENTAL

Materials

DNA gels were made from DNA sodium salt from salmon testes (DNA, Sigma). According to the manufacturer, the % G—C content of the DNA used 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 1.3×10^6 g/mol, which corresponds to $\sim$ 2000 bp. The crosslinker EGDE (Fluka), the catalyst *N,N,N′,N′*-tetramethylethylenediamine (TEMED, Merck), acridine orange (Aldrich), and sodium bromide (NaBr, Merck) were used as received.

Preparation of DNA Gels

Physical DNA gels were prepared starting from 5 (wt/vol %) aqueous DNA solutions. DNA was first dissolved in 4.0 m*M* NaBr at 35°C for one day. The solution was then transferred into several plastic syringes of 4.5 mm internal diameters. The syringes were placed in a temperature controlled oven and heated from 25 to 90°C with a heating rate of 5°C/min, kept at 90°C for 30 min, subsequently cooled down to 25°C within 7 h. The heating-cooling cycle was carried out several times to check the reproducibility of the results.

For the chemical crosslinking reactions, DNA dissolved in 4.0 m*M* NaBr, as described above, was mixed with EGDE crosslinker and then with TEMED catalyst (0.44 vol/vol %). DNA concentration at crosslinking was 6, 8, and 9.3 wt/vol %. The crosslinker (EGDE) content of the reaction solution was expressed as EGDE %, which represents the mass of pure EGDE per 100 g of DNA. DNA solution containing EGDE and TEMED was then transferred into several plastic syringes of 4.5 mm internal diameters and they were placed in an oven at 50°C for 4 h to complete the gelation reactions.

Swelling Measurements

DNA gels taken from the syringes were cut into samples of about 5 mm in length. Then, each sample was placed in an excess of water or aqueous NaBr solutions at 24 ± 1°C. To reach swelling equilibrium, the sample was immersed in solution for at least two weeks replacing the solution many times. The swelling equilibrium was tested by monitoring the diameter of the gels using an image analyzing system consisting of a microscope (XSZ single Zoom microscope), a CDD digital camera (TK 1381 EG) and a PC with the data analyzing system Image-Pro Plus. The swelling equilibrium was also tested by weighing the gel samples. The equilibrium volume and the equilibrium weight swelling ratios, $V_{\mathrm{rel,\ eq}}$ and $m_{\mathrm{rel,\ eq,}}$ respectively, were calculated as:

$$V_{\mathrm{rel,\,eq}} = (D/D_0)^3 \quad (1)$$

$$m_{\mathrm{rel,\,eq}} = m/m_0 \quad (2)$$

where D and D_0 are the diameters of the gels after equilibrium swelling and after preparation, respectively, m and m_0 are the corresponding masses of the gel samples. Swelling tests of the gels in aqueous acetone solutions of various compositions were also carried out as described above. The swelling measurements were conducted on at least six gel samples prepared under the same experimental condition and the results were averaged. The extent of solubilization of gels during their swelling was monitored by UV spectroscopic measurements.

UV Spectrophotometry

The amount of DNA released from DNA gels during their swelling was determined by UV spectroscopic measurements. The concentration of DNA in the external solution was monitored on a T80 UV–vis spectrometer using the molar extinction coefficient ε_{260} determined as 6900 M^{-1} cm^{-1} at 260 nm.

Rheological Experiments

Physical DNA gels were also prepared between the parallel plates of the rheometer (Gemini 150 Rheometer system, Bohlin Instruments) equipped with a Peltier device for temperature control. The upper plate (diameter 40 mm) was set at a distance of 500 μm before the onset of the reactions. During all rheological measurements, a solvent trap was used to minimize the evaporation. Further, the outside of the upper plate was covered with a thin layer of low-viscosity silicone oil to prevent evaporation of solvent. A frequency of $\omega = 6.28$ rad/s and a deformation amplitude $\gamma_0 = 0.01$ were selected to ensure that the oscillatory deformation is within the linear regime. The heating-cooling cycle between 25 and 90°C was carried out, as described earlier,[17] with a heating rate of 3.25°C/min, keeping at 90°C for 10 min, subsequently cooling down to 25°C with a rate of 1.08°C/min, and finally keeping at 25°C for 40 min. Thereafter, frequency-sweep tests at $\gamma_0 = 0.01$ were carried out.

Mechanical Tests

Uniaxial compression measurements were performed at 24°C on DNA gel samples by using an apparatus previously described.[20] Briefly, a cylindrical gel sample of about 5 mm in length was placed on a digital balance (Sartorius BP221S, readability and reproducibility: 0.1 mg). A load was transmitted vertically to the gel through a rod fitted with a PTFE end-plate. The compressional force acting on the gel was calculated from the reading of the balance. The resulting deformation was measured after 3 s of relaxation by using a digital comparator (IDC type Digimatic Indicator 543–262, Mitutoyo), which was sensitive to displacements of 10^{-3} mm. The elastic modulus G was determined from the initial slope of linear dependence:

$$\sigma_{\mathrm{nom}} = G\left(\lambda - \lambda^{-2}\right) \quad (3)$$

where σ_{nom} is the nominal stress, i.e., the force acting per unit crosssectional area of the undeformed gel specimen, and λ is the deformation ratio (deformed length/initial length).

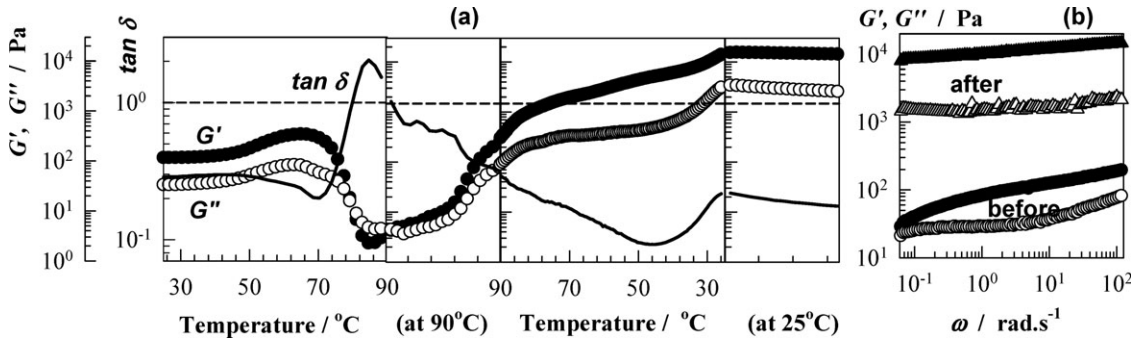

Figure 1. (a) Elastic modulus G' (●), viscous modulus G'' (○) and the loss factor tan δ (line) of 5% DNA solution during the heating-cooling cycle at $\omega = 6.28$ rad/s, (b) G' (●) and G'' (○) of the physical gels at 25°C before (circles) and after the cycle (triangles) as a function of the frequency ω. $\gamma_o = 0.01$. The dashed horizontal line in (a) represents the condition tan $\delta = 1$.

ATR-FTIR Measurements

Physical gels dissolved in water were frozen at −86°C for 1 day before being freeze-dried at −40°C/0.12 mbar for 1 day and −60°C/0.01 mbar for an additional 1 day. Spectra of the freeze-dried samples were collected using a single bounce diamond attenuated total refractance (ATR) module on a Fourier-transform infrared (FTIR) spectrometer (Nicolet Nexus 6700) equipped with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. The resolution of each spectrum was 4 cm^{-1}, and 64 interferograms were coadded in the range of 500–4000 cm^{-1}.

Acridine Orange Staining of Gels

Swollen chemical gel samples were immersed in acridine orange solutions (68 μg/mL) for 1–2 min, then washed with water, and the stained gel samples were immediately examined under UV light at 366 nm.

RESULTS AND DISCUSSION

Physical Gels

Physical gels were prepared starting from aqueous 5% ds-DNA solutions by a heating-cooling cycle between 25 and 90°C. The viscoelastic behavior of the DNA solution during the course of the heating and cooling periods was first investigated within the rheometer at a frequency of 6.28 rad/s and at strain amplitude γ_o of 1%. Figure 1(a) shows the variations of the elastic modulus G', viscous modulus G'', and the loss factor tan δ of the solution during the heating-cooling cycle. During the heating period and particularly above 70°C, the dynamic moduli of the solution rapidly decrease while the loss factor tan δ increases, indicating the occurrence of a conformational transition in ds-DNA molecules. Moreover, at 90°C as well as during the cooling period back to 25°C, the dynamic moduli again increase and finally attain very larger values. For example, G' becomes 15 kPa after the cycle, as compared to its initial value of 0.1 kPa. The results were found to be reproducible in several separate runs.

As mentioned in the Introduction section, the dramatic changes in the dynamic moduli of the DNA solution during the heating and cooling periods are related with the denaturation and renaturation transitions of DNA molecules. The hydrogen bonds in DNA base pairs holding the two strands together break in the course of the heating process, so that the double-helix dissociates into ss-DNA strands. Since, ss-DNA is a flexible polymer compared to semiflexible ds-DNA, the formation of flexible ss-DNA strand portions in ds-DNA molecules initially increases the number of entanglements so that both G' and G'' first slightly increase between 45°C and 70°C [Figure 1(a)]. However, as the temperature further increases, the extent of denaturation increases and finally, the double-helix completely dissociates into two single strands having a random coil conformation. The radius of gyration of ds-DNA of 2000 bp, as used in the present experiments, is 106 nm in water, as compared to 20 nm for the corresponding ss-DNA.[16] This indicates that each DNA molecule will occupy about 150-fold smaller volume after denaturation, which is reflected in Figure 1(a) as a gel-to-sol transition at 80°C, i.e., as the increase of tan δ from below to above unity. Moreover, the increase of the moduli during cooling back to 25°C is due to the renaturation of DNA above its critical entanglement concentration. The hydrogen bonds reformed during cooling between strands belonging to different ds-DNA molecules act as physical crosslinks so that the elastic modulus increases. Very large elastic and viscous moduli of the final gel also reveal formation of a poorly connected three-dimensional DNA structure with the presence of mobile aggregates of DNA. Figure 1(b) shows the mechanical spectra of 5% DNA solution at 25°C before and after the heating-cooling cycle. The measurements were conducted at $\gamma_0 = 0.01$ and over the frequency range 0.063–130 rad/s. The initial DNA solution exhibits so-called weak gel behavior with frequency dependent moduli, with G' approaching to G'' at low frequencies. After the cycle, both G' and G'' are essentially independent of frequency over the range of investigated, indicating the dominant viscoelastic relaxations of the physical DNA network are at lower frequencies; that is, the relaxation time of the DNA network is long. Such rheological behavior matches of the characteristics of a strong gel.

To obtain cylindrical gel samples suitable for the swelling tests, the heating-cooling cycles were conducted using DNA solutions in sealed plastic syringes of about 4.5 mm in diameter. It was observed that, although the initial DNA solution flowed under gravity, the physical gels formed after the cycle preserved their shapes and they exhibited an elastic modulus of 2.1 ± 0.3 kPa after a relaxation time of 3 s. The conformation of DNA strands

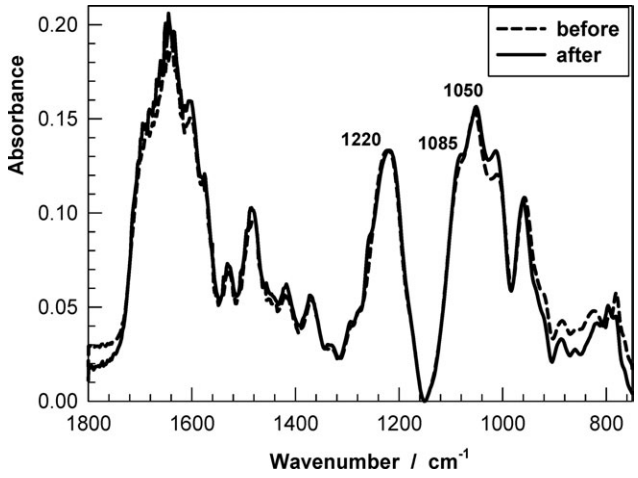

Figure 2. ATR-FTIR spectra of dried DNA before (dashed curve) and after gelation (solid curve).

in the physical gels was investigated by ATR-FTIR measurements. The spectra of dried DNA before and after the heating cooling cycle are shown in Figure 2. The peak at 1220 cm^{-1} assigned to PO^{2-} antisymmetric stretching vibration is known to be invariant of DNA conformation,[21] and therefore, it was used for the normalization of the spectra. The peaks in the range of 1550–1750 cm^{-1} characterizing base pairing and base stacking remained unchanged after gelation indicating that DNA conformation remains unchanged after the cycle. The peaks at 1085 and 1050 cm^{-1} corresponding to PO^{2-} symmetric stretching vibration and C—O stretching vibration of phosphodiester, respectively, characterize backbone conformation. It was reported that the intensity of these peaks decreases during denaturation due to the conformational changes in deoxyribose-phosphate backbone upon disruption of the DNA helical structure.[21–23] The intensity of these peaks also remained unchanged after gelation supporting that the DNA strands in the physical gels are in double stranded conformation. The conformation of DNA before and after heating-cooling cycle was also tested by determining the molar extinction coefficient ε_{260} of DNA, isolated from the gels, at 260 nm. Absorbance of DNA solutions at 260 nm is known to increase as the structure of DNA transforms from double to single strand; in case of complete denaturation, it increases 40% because of the change of the molar absorption coefficient of the aromatic rings.[24] DNA solutions were prepared by dissolving the physical gels in water and, they were diluted to 10–65 μg DNA/mL with water for the determination of ε_{260}. The results were compared with those of starting DNA solutions. ε_{260} was found to be constant at 6900 ± 100 M^{-1} cm^{-1} before and after gelation indicating that the physical gels consist of ds-DNA strands.

Physical gel samples were subjected to swelling tests in aqueous NaBr solutions of various concentrations (C_{salt}) between 0 and 10^{-1} M. Figure 3(a) shows the relative weight swelling ratio m_{rel} of gels at time t plotted against the swelling time t. The semilogarithmic plot was chosen for clearer representation of the changes in m_{rel} at short times. In salt solutions below 10^{-2} M NaBr, m_{rel} first increases with increasing swelling time, and after attaining a maximum value, it again decreases. By UV–vis measurements conducted on external salt solutions, it was found that the decrease in m_{rel} is related with the disintegration of DNA chains from the gel network. This is illustrated in Figure 3(b), where the released amount of DNA, with respect to its total amount in the gel, is plotted against the swelling time in NaBr solutions. While about 80% of ds-DNA in the physical gels released within one day in 0–10^{-4} M NaBr, a much slower release over a time scale of many days was achieved at higher salt concentrations.

Thus, the hydrogen bonds acting as physical crosslink zones between ds-DNA strands are destroyed during the expansion of the gels in dilute salt solutions. However, at higher salt concentrations, the gels immersed in solutions remain stable over many days [Figure 3(a)]. For example, in 10^{-2} M NaBr, the gels dissolved after about 16 days while in 10^{-1} M NaBr they remained stable over the whole swelling time studied (42 days). The results thus show that both the release rate and the released amount of DNA could be adjusted by the salt concentration in

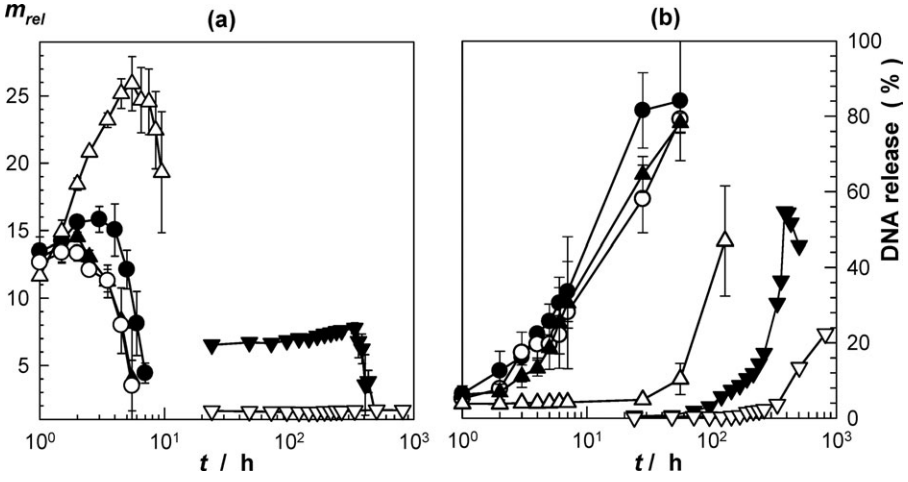

Figure 3. The relative weight swelling ratio m_{rel} (a) and the released amount of DNA from the gels (b) shown as a function of the swelling time t in NaBr solutions. C_{salt} = 0 (●), 10^{-5} (○), 10^{-4} (▲), 10^{-3} (△), 10^{-2} (▼), and 10^{-1} M (▽).

Table I. Swelling Ratios $m_{rel,\ eq}$, Elastic Moduli G, and Conformation of the Network Chains of DNA Gels

			$m_{rel,eq}$			
Code	DNA (%)	EGDE (%)	Water	1*M* NaBr	G/Pa	Type
1 a	6	10	-	-	-	-
1 b	6	20	100 (20)[a]	2.9 (0.2)[a]	190 (20)[a]	ss
2	8	10	55 (4)	1.6 (0.1)[a]	460 (60)[a]	ss
3	9.3	10	26 (3)	1.8 (0.2)[a]	1450 (90)[a]	ds

[a]Standard deviations 4.

the solution. This also suggests that the physical DNA gels prepared without a crosslinker can be used for the controlled release of ds-DNA in aqueous solutions.

Chemical Gels

Chemical DNA gels were prepared by crosslinking of ds-DNA at 50°C using 10% EGDE crosslinker in the presence of 0.44% TEMED. The gelation time was set to 4 h. Preparation conditions of gels and their swelling and elastic properties are collected in Table I. Gels denoted by 1 a formed at 6% DNA were too weak to withstand the swelling pressure of water, and they dissolved within hours. Increasing the amount of EGDE to 20% provided formation of gels (Gel-1 b) that are stable in water for 3 weeks while dissolving at longer swelling times. The other gels formed at 8 and 9.3% DNA, denoted by Gel-2 and Gel-3, respectively, were stable in water. UV–vis measurements conducted during their swelling indeed showed that the released amount of DNA from the gel network is less than 5%. To demonstrate the conformation of DNA chains, the gel samples were stained with acridine orange and the stained samples were examined under UV light at 366 nm. Gels 1 b and 2 fluoresce red indicating predominantly ss-DNA conformation of the network chains, while Gel-3 formed at 9.3% DNA fluoresces green due to the monomeric acridine orange bound to ds-DNA chains (Table I). This finding is in accord with our previous work showing that the crosslinking reactions conducted below 9% DNA are accompanied by the denaturation of DNA fragments.[16,17]

Table I also shows that both the swelling ratio and the elastic modulus of the hydrogels could be varied over a wide range by changing the initial DNA concentration at the gel preparation. Gel 1 b exhibits an equilibrium weight swelling ratio $m_{rel,\ eq}$ of about 100 corresponding to a DNA concentration of 0.06 (wt/vol %) in swollen gel. The response of DNA strands in such a highly swollen gel to an external stimulus was investigated by immersing the gel samples, at the state of preparation, into acetone/water mixtures of various compositions. Filled symbols in Figure 4 (a, b) represent the equilibrium weight $m_{rel,\ eq}$ and volume swelling ratios $V_{rel,\ eq}$ of gels plotted against the acetone content. At low acetone concentrations the gels are swollen, at high concentrations they are collapsed. The transition from swollen to collapsed state occurs continuously between 60 and 70% acetone, during which both the mass and the volume of gel changes about 130 times. We observed that the gel does not shrink homogeneously in the phase transition region; part of

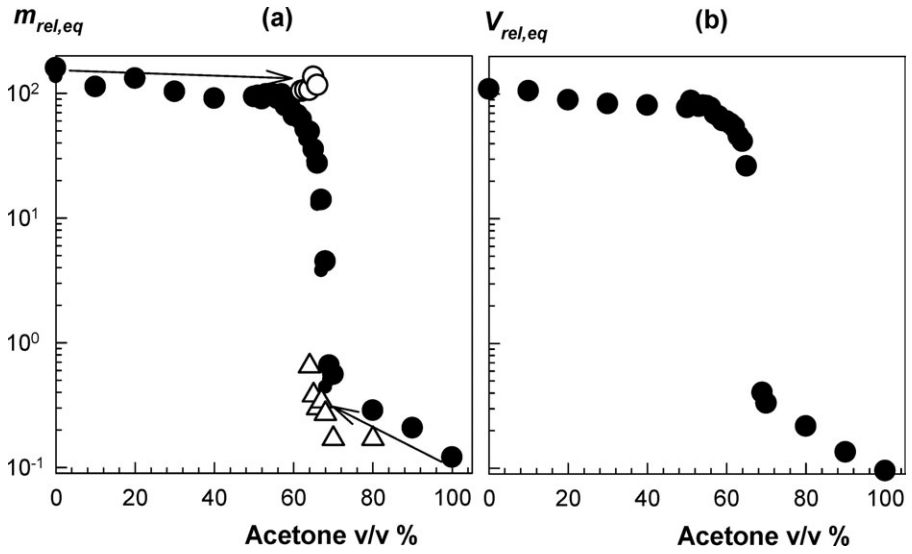

Figure 4. Variations of the equilibrium weight $m_{rel,\ eq}$ (a) and volume swelling ratios $V_{rel,\ eq}$ (b) of Gel-1 b with the acetone content of the external acetone/water mixture. Open symbols in (a) were obtained by immersing swollen (circles) and collapsed gel samples (triangles) into acetone/water mixtures, as indicated by the arrows.

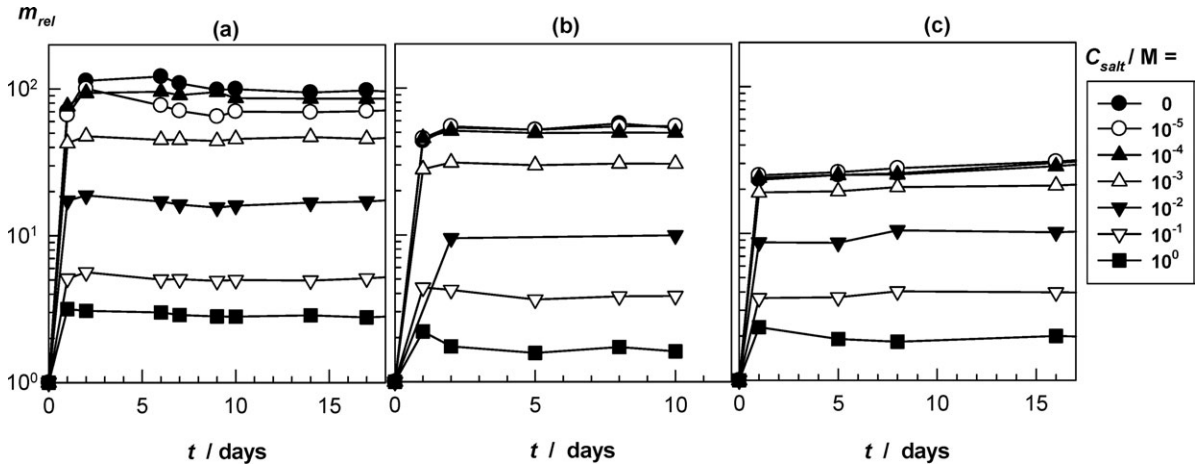

Figure 5. Swelling kinetics of Gels-1 b (a), -2 (b), and -3 (c) in aqueous NaBr solutions as the dependence of m_{rel} on the swelling time t.

the gel sample underwent collapse whereas the other part remained in the swollen state indicating hindered diffusion of solvent molecules between the inside and outside of the gel phase and the existence of kinetically frozen structures. Because of the distortion of the shape of cylindrical gel samples in this region, no volume data were available between 65 and 69% acetone.

To gain more insight about this nonequilibrium behavior, gels equilibrium swollen in water as well as collapsed in acetone were immersed into acetone/water mixtures. Open circles and open triangles in Figure 4(a) represent the swelling data obtained from the initially swollen and collapsed gels, respectively. Interestingly, swollen and collapsed gels do not change their states between 64 and 66% acetone. For example, in 65% acetone, the gels remained stable after 3 months both in swollen and in collapsed states depending on their history. Such a hysteresis behavior, most likely caused by the kinetically frozen structures in DNA gels, was reported before in synthetic polyelectrolyte hydrogels.[25–28] Thus, DNA-rich collapsed regions block the solvent diffusion outside the gel sample preventing the equilibration of the gel volume with surroundings. We have to note that, in case of DNA cryogels, a discrete phase transition was observed at 51% acetone, during which the gel mass changes about 8 times.[19] The shift of the critical acetone concentration toward higher acetone content in the present gels indicates that the phase transition requires poorer solvent conditions than the macroporous DNA gels. This may be related to the high water content of nonporous gels, which produces different solvent composition between the inside and outside of the gel phase.

The gel samples were subjected to swelling tests in aqueous NaBr solutions of various concentrations C_{salt} between 0 and 1*M*. Figure 5 shows the relative weight swelling ratio m_{rel} of gels plotted against the swelling time. The gel samples swell to equilibrium state within 1–2 days while at longer times, the gel mass remains constant. This is in contrast to the physical gels described in the previous section (Figure 3), and indicates the stability of the chemically crosslinked DNA network structure. Moreover, the swelling tests conducted both under light and in dark environment showed no difference between the swelling results revealing that the network chains are not disrupted under the action of light.

In Figure 6, the equilibrium weight swelling ratio $m_{rel,\ eq}$ of DNA hydrogels is plotted against C_{salt}. Filled and open symbols represent data obtained under light and no light, respectively. It is seen that the equilibrium data are also not affected from the presence of light. This is in contrast with a previous report showing disruption of DNA gels crosslinked with EGDE by sunlight exposure.[29] Below 10^{-4} M NaBr, the swelling ratio of gels remains almost unchanged while further increase in C_{salt} leads to a rapid deswelling. The decreased swelling ratio of DNA hydrogels with increasing salt concentration is due to decrease in the concentration difference of counterions inside and outside the gel. The fixed phosphate residues of DNA strands confined to the gel phase require equal number of counterions (Na^+) to stay within the gel to achieve electroneutrality. There are two DNA counterions per base pair inside DNA gels, but not all of these counterions are free and osmotically effective in gel swelling. According to Manning,[30] the charge density parameter ξ determines whether the counterion condensation can occur. The condition for condensation is $\xi \geq 1\ z^{-1}$ where z is the valence of the counterion. For ds-DNA in aqueous solutions, $\xi = 4.17$ so that counterion condensation is predicted.[30,31] The fraction of free, uncondensed counter ions is then given by $z^{-1}\xi^{-1} = 0.24$ for monovalent ions. Thus, 24% of the counterions on DNA strands are free and expected to participate in the osmotic pressure of the system. These counter ions are responsible for the swelling of DNA gels in water or dilute salt solutions. In 1.0*M* NaBr solution, all DNA gels exhibit low $m_{rel,\ eq}$ values (between 1.6 and 2.9) because of screening of charge interactions within the hydrogel.

It would be interesting to compare the experimental swelling results with those of synthetic polyelectrolyte hydrogels. Figure 7(a) compares the swelling ratios of DNA hydrogels (filled symbols) with those of ionic polyacrylamide (PAAm) hydrogels (open symbols), taken from the literature.[32] Ionic PAAm hydrogels contain 18, 60, and 80 mol % strong electrolyte 2-acrylamido-2-methylpropane sulfonic acid sodium salt (AMPS) units.

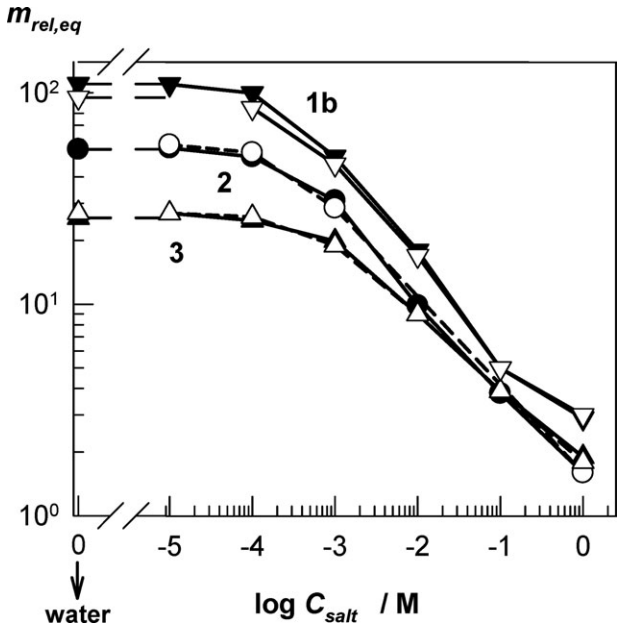

Figure 6. Variation of the swelling ratio $m_{rel,\ eq}$ of DNA hydrogels with the NaBr concentration in the external solution. Experimental data obtained under light and no light are shown by the filled and open symbols, respectively. Gel codes are indicated.

The volume swelling ratios q_v of PAAm hydrogels with respect to dry state reported in Ref. 32 were converted to $m_{rel,\ eq}$ using the equation

$$m_{rel,eq} = C_0\left[1+\frac{(q_v-1)}{d_2}\right] \quad (4)$$

where C_0 is the initial monomer concentration at the gel preparation (0.05 g/mL) and d_2 is the PAAm density (1.35 g/mL). In Figure 7(b), the swelling data of both DNA and ionic PAAm hydrogels are replotted as the normalized gel mass m_{norm} with respect to the swollen gel mass in water. The results clearly show that the behavior of both ss- and ds-DNA hydrogels is very similar to that of synthetic polyelectrolyte gels. Thus, although the details of DNA network strands determines the properties such as selective binding and molecular recognition, such details do not affect the swelling properties of DNA hydrogels.

Assuming that the osmotic pressure of a DNA gel is the sum of three contributions, namely osmotic pressures due to DNA–solvent mixing (π_{mix}), because of deformation of DNA network chains to a more elongated state (π_{el}), and due to the nonuniform distribution of mobile counterions between the gel and the solution (π_{ion}), swelling behavior of DNA gels can be predicted using the Flory-Rehner theory of swelling equilibrium.[33] Detailed equations describing the equilibrium between gel and salt solutions were given before for ionic hydrogels.[32] Using the DNA–water interaction parameter χ given in literature,[11] and assuming that 24% of the counterions on DNA strands are effective in gel swelling, solution of these equations for the present DNA gels leads to swelling ratios, which are much larger than the experimental values shown in Figure 6. Such a discrepancy between theory and experiment was also observed before in synthetic polyelectrolyte hydrogels due to the fact that the number of osmotically effective counterions in gels is much smaller than the total number of free counterions.[20,32] Moreover, nonGaussian elasticity of the network chains is another reason for this discrepancy. We measured the elastic modulus of DNA gels (Gel-3) in equilibrium with NaBr solutions of various concentrations. Although the swelling ratio decreases from 27 to 1.5 with increasing salt concentration from 0 to 1*M* (Figure 6), the elastic modulus of gels was found to be constant at 1.47 ± 0.23 kPa. According to the theory of rubber elasticity of Gaussian chains, the elastic modulus G scales with the swelling ratio as $G \sim m_{rel,eq}^{-1/3}$,[34] so that the modulus should decrease with an exponent of −1/3 with increasing degree of swelling. This indicates non-Gaussian behavior of swollen DNA gels due to the

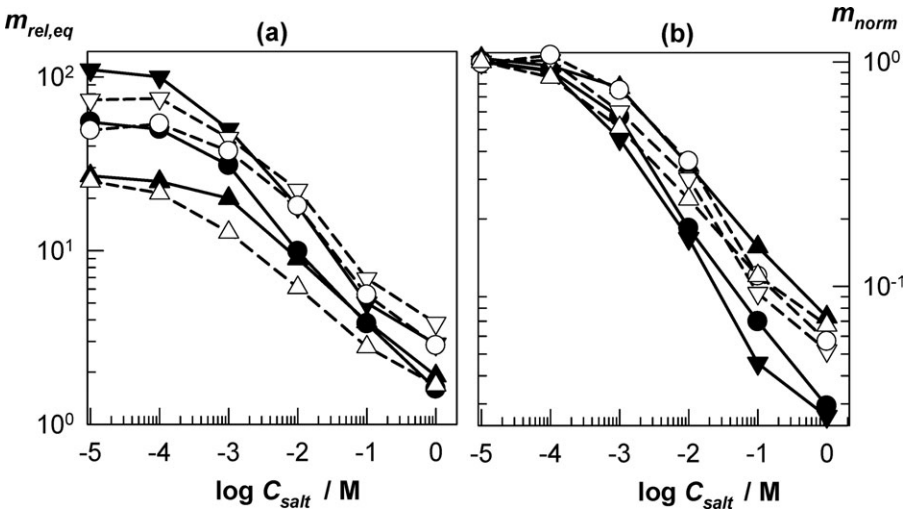

Figure 7. $m_{rel,\ eq}$ (a) and m_{norm} (b) shown as a function of salt concentration for DNA (filled symbols) and PAAm hydrogels containing AMPS units (open symbols, taken from Ref. 32). DNA Gel-1 b (▼), -2 (●), and -3 (▼). AMPS mole fraction = 0.18 (△), 0.60 (○), and 0.80 (△). NaBr and NaCl are salts used for DNA and PAAm hydrogels, respectively.

limited extensibility of the network chains, as observed before in synthetic hydrogels.[20,35]

CONCLUSIONS

Physical and chemical DNA hydrogels were prepared from aqueous ds-DNA solutions. Physical gels generated via heating-cooling cycle of 5% aqueous DNA solutions are not stable in water, indicating that the physical crosslinks are destroyed during the swelling process. It was found that both the release rate and the released amount of DNA from the physical gels can be adjusted by changing the salt concentration in the external solutions. In this way, the release of ds-DNA molecules from the gels can be varied between a few hours and 10s of days. This suggests that the physical gels can be used for the controlled release of DNA in aqueous media. In contrast, chemically crosslinked DNA gels formed using EGDE crosslinker are stable in water and their swelling ratios could be adjusted by the amount of DNA at the gel preparations. Swelling behavior of chemical DNA gels in acetone/water mixtures as well as in aqueous salt solutions is very similar to that of ionic synthetic polyelectrolyte hydrogels.

ACKNOWLEDGMENTS

The authors greatly acknowledge the financial supported by Turkish Academy of Sciences (TUBA) and Scientific and Technical Research Council of Turkey (TUBITAK); contract grant number TBAG 211T044.

REFERENCES

1. Xu, J.; LaBean, T. H.; Craig, S. L. In Supramolecular Polymers; Ciferri, A., Ed.; CRC Press: Boca Raton, FL, **2004**; Chapter 12, p 445.
2. Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. Nucleic Acids: Structures, Properties, and Functions. University Science Books: Sausalito, CA, **2000**.
3. Freifelder, D.; Davison, P. F. *Biophys. J.* **1963**, *3*, 49.
4. Ivanov, V.; Zeng, Y.; Zocchi, G. *Phys. Rev.* **2004**, *E70*, 051907.
5. Alemdaroglu, F. E.; Hermann, A. *Org. Biomol. Chem.* **2007**, *5*, 1311.
6. Okay, O. *J. Polym. Sci. Part B. Polym. Phys.* **2011**, *49*, 551.
7. Murakami, Y.; Maeda, M. *Biomacromolecules* **2005**, *6*, 2927.
8. Ishizuka, N.; Hashimoto, Y.; Matsuo, Y.; Ijiro, K. *Colloids Surf. A* **2006**, 284–285, 440.
9. Um, S. H.; Lee, J. B.; Park, N.; Kwon, S. Y.; Umbach, C. C. *Nat. Mater.* **2006**, *5*, 797.
10. Amiya, T.; Tanaka, T. *Macromolecules* **1987**, *20*, 1162.
11. Horkay, F.; Basser, P. *J. Biomacromolecules* **2004**, *5*, 232.
12. Costa, D.; Miguel, M. G.; Lindman, B. *J. Phys. Chem. B* **2007**, *111*, 8444.
13. Costa, D.; Miguel, M. G.; Lindman, B. *J. Phys. Chem. B* **2007**, *111*, 10886.
14. Costa, D.; Miguel, M. G.; Lindman, B. *Adv. Coll. Int. Sci.* **2010**, *158*, 21.
15. Costa, D.; Hansson, P.; Schneider, S.; Miguel, M. G.; Lindman, B. *Biomacromolecules* **2006**, *7*, 1090.
16. Topuz, F.; Okay, O. *Macromolecules* **2008**, *41*, 8847.
17. Topuz, F.; Okay, O. *Biomacromolecules* **2009**, *10*, 2652.
18. Orakdogen, N.; Erman, B.; Okay, O. *Macromolecules* **2010**, *43*, 1530.
19. Orakdogen, N.; Karacan, P.; Okay, O. *Reac. Funct. Polym.* **2011**, *71*, 782.
20. Gundogan, N.; Melekaslan, D.; Okay, O. *Macromolecules* **2002**, *35*, 5616.
21. Lee, S. L.; Debenedetti, P. G.; Errington, J. R.; Pethica, B. A.; Moore, D. J. *J. Phys. Chem. B* **2004**, *108*, 3098.
22. Matsuno, H.; Nakahara, J.-I.; Tanaka, K. *Biomacromolecules* **2011**, *12*, 173.
23. Whelan, D. R.; Bambery, K. R.; Heraud, P.; Tobin, M. J.; Diem, M.; McNaughton, D.; Wood, B. R. *Nucleic Acids Res.* **2011**, *39*, 5439.
24. Planken, K. L.; Koenderink, G. H.; Roozendaal, R.; Philipse, A. P. *J. Colloid Interface Sci.* **2005**, *291*, 120.
25. Okay, O.; Akkan, U. *Polym. Bull.* **1998**, *41*, 363.
26. Philippova, O. E.; Pieper, T. G.; Sitnikova, N. L.; Starodoubtsev, S. G.; Khokhlov, A. R.; Kilian, H. G. *Macromolecules* **1995**, *28*, 3925.
27. Siegel, R. A. *Adv. Polym. Sci.* **1993**, *109*, 233.
28. Kayaman, N.; Okay, O.; Baysal, B. M. *Polymer Gels Networks* **1997**, *5*, 339.
29. Costa, D.; Valente, A. J. M.; Pais, A. C. C. C.; Miguel, M. G.; Lindman, B. *Colloids Surf A: Physicochem. Eng. Aspects* **2010**, *354*, 28.
30. Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.
31. Sun, M.; Pejanovic, S.; Mijovic, J. *Macromolecules* **2005**, *38*, 9854.
32. Okay, O.; Sariisik, S. B.; Zor, S. D. *J. Appl. Polym. Sci.* **1998**, *70*, 567.
33. Flory, P. J. Principles of Polymer Chemistry; Cornell University Press: Ithaca, New York, **1953**.
34. Treloar, L. R. G. The Physics of Rubber Elasticity; Oxford University Press: Oxford, **1975**.
35. Gundogan, N.; Melekaslan, D.; Okay, O. *Eur. Polym. J.* **2003**, *39*, 2209.