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Thermoresponsive *N,N*-dialkylacrylamide copolymer blends as DNA sieving matrices with a thermally tunable mesh size

In an earlier study we showed that a blend of thermoresponsive and nonthermoresponsive hydroxyalkylcelluloses could be used to create a thermally tunable polymer network for double-stranded (ds) DNA separation. Here, we show the generality of this approach using a family of polymers suited to a wider range of DNA separations: a blended mixture of *N*,*N*-dialkylacrylamide copolymers with different thermoresponsive behaviors. A mixture of 47% w/w *N*,*N*-diethylacrylamide (DEA)/53% w/w *N*,*N*-dimethylacrylamide (DMA) (DEA47; thermoresponsive, transition temperature = 55°C in water) and 30% w/w DEA/70% w/w DMA (DEA30; nonthermoresponsive, transition temperature $>$ 85°C in water) copolymers in the ratio of 1:5 w/w DEA47:DEA30 was used to separate a dsDNA restriction digest (ΦX174-*Hae*III). We investigated the effects of changing mesh size on dsDNA separation, as controlled by temperature. We observed good DNA separation performance with the copolymer blend at temperatures ranging from 25°C to 48°C. The separation selectivity was evaluated quantitatively for certain DNA fragment pairs as a function of temperature. The results were compared with those obtained with a control matrix consisting only of the nonthermoresponsive DEA30. Different DNA fragment pairs of various sizes show distinct temperature-dependent selectivities. Over the same temperature range, no significant temperature dependence of selectivity is observed for these DNA fragment pairs in the nonthermoresponsive control matrix. Overall, the results show similar trends in the temperature dependency of separation selectivity to what was previously observed in hydroxyalkylcellulose blends, for the same DNA fragment pairs. Finally, we showed that a ramped temperature scheme enables improved separation in the blended copolymer matrix for both small and large DNA fragments, simultaneously in a single capillary electrophoresis (CE) run.

Keywords: DNA separation / DNA sieving matrix / Thermoresponsive polymer
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1 Introduction

In a recent report, we demonstrated the use of a blend of thermoresponsive and nonthermoresponsive polymers, based on hydroxyalkylcellulose derivatives, to create a DNA sieving matrix with a "thermally tunable mesh size", *i.e.*, temperature-dependent selectivity [1]. In that work, hydroxyethylcellulose (HEC, nonthermoresponsive) was used as a "scaffold" for DNA separation, with "gaps" filled by thermoresponsive hydroxypropylcellulose (HPC) at low temperature, and left more open as the thermoresponsive constituent "shrinks" with increasing temperature due to a gradual volume-phase transition. This blended HEC/HPC network was used to separate a dsDNA digest (ΦX174-*Hae*III) at a series of different temperatures (25°C–38°C). The selectivities of separate for several pairs of DNA fragments were evaluated, and we found that small fragments (118–194 bp), large fragments (1078–1353 bp) and fragments with similar size (271–281 bp) show different temperature-dependent selectivities, upon changing the physical properties of the sieving matrix with temperature. This was the first report that blends of thermoresponsive and nonthermoresponsive polymers can enable a novel strategy to address the

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Abbreviations: DEA, *N,N*-diethylacrylamide; **DMA**, *N,N*-dimethylacrylamide; **HEC**, hydroxyethylcellulose; **HPC**, hydroxypropylcellulose; **LCST**, lower critical solution temperature; **PDI**, polydispersity index; R_g, radius of gyration of the polymer coils; **TBE**, Tris-borate-EDTA

different sieving matrix requirements for high-resolution electrophoretic separation of differently sized DNA fragments. We further showed that if the temperature was ramped over an appropriate range during electrophoresis, the HEC/HPC blend with thermally tunable mesh size can be used to achieve optimal separation for DNA fragments of different sizes simultaneously, in a single CE analysis.

In the present work, we extend our experimental study to another type of thermoresponsive and nonthermoresponsive polymer blend, comprised of copolymers of *N,N*-dialkylacrylamides. Acrylamide derivatives of this class have been shown to be useful for a wider range of DNA separations than hydroxyalkylcelluloses, including long-read DNA sequencing [2–11]. This is partly because acrylamide-based polymers can be synthesized with targeted average molecular weights, facilitating matrix optimization. Moreover, we reasoned that our ability to tailor the thermoresponsive behavior of *N,N*-dialkylacrylamide copolymers *via* control of monomer composition [12] should in principle allow application of this strategy over different temperature ranges, with the use of different copolymer blends. For example, it has been shown that the optimal DNA sequencing temperatures for certain *N,N*-dialkylacrylamide copolymers lies in the range of 50°C – 70°C, depending on composition [6]. With a careful choice of the polymer blend component and the temperature during analysis, it may be possible to obtain improved performance with these materials for DNA sequencing applications.

Thermoresponsive sieving networks based on copolymers of *N,N*-dialkylacrylamides have previously been developed as DNA sequencing matrices in our laboratory [5, 13]. The development of matrices exhibiting a "thermally controlled viscosity switch" [14] is motivated by the problem of loading highly viscous, entangled polymer solutions, which are necessary for high-resolution DNA sequencing separations [15–18], into capillary or chip microchannels. "Thermothinning" polymer networks undergo a thermodynamically driven volume-phase transition at a given lower critical solution temperature (LCST), accompanied by a dramatic decrease in viscosity, in response to a change in temperature over a narrow range [19]. The thermoresponsive behavior of the copolymer network effectively decouples matrix loading and DNA sieving performance: with the use of this kind of "thermothinning" polymer solutions, the polymer matrices can be loaded rapidly into microchannels with low pressure (50 psi) at temperatures above the LCST, while upon cooling below the LCST, the entangled polymer networks are restored for effective DNA sieving [14]. With a DNA sequencing matrix based on a 42% w/w *N*,*N*-diethylacrylamide (DEA)/58% w/w *N*,*N*-dimethylacrylamide (DMA) (DEA42) at 7.0% w/v in 1 × Tris-*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid-EDTA (TTE) buffer, we were able to obtain a read length of 575 bases in 93 min, with 98.5% accuracy of base-calling [5, 14].

In this study, as with our previous studies of an HEC/HPC blend [1], we harness the thermoresponsive behavior of LCST-exhibiting polymers to control the physical state of entanglement of the polymer network and to improve the selectivity of the matrix for dsDNA separation. In particular, we employed a mixture of random copolymers: 47% DEA/53% DMA (w/w; DEA47) (thermoresponsive, transition temperature = 55°C in 1 × Tris-borate-EDTA (TBE) buffer) and 30% DEA/70% DMA (w/w; DEA30) (nonthermoresponsive, transition temperature $>$ 85°C in 1 × TBE) in the ratio of 1:5 (w/w) DEA47:DEA30 to formulate a DNA separation matrix with a total of 3% w/w polymer in 1 × TBE (pH 8.3). We hypothesized that, as with the HEC/HPC system, the DNA sieving performance of this polymer network could be modulated by the shrinking/swelling cycle of the thermoresponsive copolymer constituent (DEA47) when temperature is changed within a certain range just below the LCST, while the entangled network properties of the nonthermoresponsive copolymer "scaffold" should remain essentially unchanged. The study was planned to provide information about the temperature dependency of the separation selectivity of the matrix for differently sized DNA fragments, and to allow us to design an appropriate temperature-control strategy for high-resolution DNA separations over a wider DNA size range. By studying this related but novel system, we hoped to learn whether the phenomena observed in previous work with the HEC/HPC system could be generally applied with different classes of LCST-exhibiting polymers. The results obtained in this study with dsDNA could have valuable implications for designing more sophisticated strategies for other genomic separations. In particular, high-throughput DNA sequencing involves the size-based separation of single-stranded DNA fragments ranging from a few bases to a few thousand bases. Hence, uniformly high resolution of ssDNA separation, over this large size range, is critical for long-read DNA sequencing analyses presently carried out in genome centers by capillary array electrophoresis. Our goal is to implement thermoresponsive polymer networks for DNA separation on temperature-controlled microfluidic devices [20–23], which should offer both cost and throughput advantages relative to capillary array electrophoresis. However, with their shorter separation channel lengths, electrophoresis chips place even more stringent demands on the DNA separation matrix than capillary-based systems.

2 Materials and methods

2.1 Polymer synthesis and characterization

Materials and methods for the chemical synthesis and physical characterization of DMA/DEA copolymers were described in detail in a previous report [4]. Briefly, ultrapure (> 99.5%) DMA and DEA (Monomer-Polymer Dajac Labs, Feasterville, PA, USA) were randomly copolymerized by aqueous-phase free-radical polymerization. An aqueous solution with 7% w/v total monomer concentration was thermostatted at 47°C, and degassed with nitrogen prior to initiation. Initiator V-50 (2,2'-azobis(2-amidinopropane) dihydrochloride; Wako Chemical USA, Richmond, VA, USA) was dissolved in water and injected into the reaction flask. After 16 h, the resulting mixture was allowed to come to room temperature, poured into 100 kDa molecular weight (MW) cutoff cellulose ester membranes (Spectrum Laboratories, Rancho Dominguez, CA, USA), and dialyzed against deionized, distilled water for 10 days with frequent water changes to remove unreacted monomer and low-MW polymer. The polymer solution was then frozen and lyophilized (Labconco, Kansas City, MO, USA), resulting in a white, stiff, foam-like polymer material that was then redissolved in aqueous electrophoresis buffer by slow rotation overnight at room temperature (Roto-Torque; Cole-Parmer Instrument, Vernon Hills, IL, USA).

2.2 Molar mass characterization

The molar mass distributions of the DMA/DEA copolymers were determined by first fractionating the polymer samples by gel permeation chromatography (GPC) prior to analysis by on-line multiangle laser light scattering (MALLS) and refractive index detection, using a Waters 2690 Alliance Separations Module (Milford, MA, USA) with Shodex (New York, NY, USA) OHpak columns SB-806 HQ, SB-804 HQ, and SB-802.5 HQ connected in series. In this tandem GPC-MALLS mode, the effluent from the GPC systems flows into the Dawn DSP laser photometer and Optilab interferometric refractometer (both, Wyatt Technology, Santa Barbara, CA, USA). Sample aliquots of 100 μL (sample concentration, 0.5 mg/mL) were injected into the system (mobile phase, 0.1 M NaCl, 50 mM NaH_2PO_4, and 200 ppm NaN_3 (pH 4.6); flow rate, 0.30 mL/min). The tandem GPC-MALLS data were processed with ASTRA® for Windows® software from Wyatt Technology. A detailed discussion of using GPC-MALLS for accurate polymer characterization is given in another article [24].

2.3 dsDNA sample and separation matrices

A solution of ΦXC174-*Hae*III restriction fragments (New England BioLabs, Beverly, MA, USA) was diluted to 50 mg/mL in distilled, deionized water. An aqueous buffer consisting of 50 mM Tris, 50 mM boric acid, and 2 mM EDTA (Amresco, Solon, OH, USA and Sigma, St. Louis, MO, USA) with pH 8.3 was used to dissolve the polymer in predetermined amounts to make up a blended matrix of DEA/DMA copolymers containing 2.5% w/w DEA30 and 0.5% w/w DEA47. A control (nonthermoresponsive) matrix was prepared by dissolving 3.0% w/w DEA30 in 1 × TBE buffer. The same separation matrix solutions were used as the running buffer in CE experiments, for the respective matrices.

2.4 CE

For CE experiments we employed a single fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), 30 cm in length (25 cm to the detector), with 75 μm ID and 360 μm OD, internally coated with a covalently attached layer of linear polyacrylamide (LPA) according to the method of Hjertén [25]. CE was carried out in a BioFocus Capillary Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA) in reversed-polarity mode. DNA samples were injected electrokinetically at the cathodic end of the capillary, with a field strength of 500 V/cm for 3 s. A field strength of 265 V/cm was applied for electrophoretic DNA separations. Electrophoresis runs were performed at seven different temperatures, and DNA detection was by UV absorbance at 260 nm.

2.5 Visible spectrophotometry

The turbidity of the polymer matrix as a function of temperature (to characterize the "cloud-point" phase transition behavior associated with the LCST) was characterized with a Cary 500 UV-visible-NIR spectrophotometer (Varian, Walnut Creek, CA, USA) with a circulating water bath to control the temperature. The polymer solution was heated and cooled at a rate of 5°C/min, and absorbance data were collected at 500 nm. The data we present were reproducible upon multiple cycles of repeated heating and cooling at this rate.

3 Results and discussion

3.1 Polymer matrix characterization

Based on our experience with the HEC/HPC blend, we expected to observe a subtle, but distinct effect on DNA separation selectivity with the change of the sieving matrix mesh size, upon the change of CE run temperature. Obtaining similar results with an alkylacrylamide system, however, is important for proving that the phenom-

enon is general, and applicable to different classes of thermoresponsive and nonthermoresponsive polymer blends. Also, the temperature-dependent DNA separation properties observed for a DMA/DEA copolymer system can be used as a guide to design advanced temperature control schemes for more interesting applications, such as DNA sequencing with extended read length in microfluidic devices with advanced spatial and temporal temperature control [23].

The physical properties of the two DMA/DEA copolymers used in this study are summarized in Table 1. The weight-average molar mass obtained with our high-yielding solution polymerization protocol (> 85% yield of polymer, based in initial mass of monomer and mass of polymer obtained) was typically in the range of 1.5–4.5 MDa. In

Table 1. Physical properties of DMA and DEA copolymers

Polymer	Weight-average molar mass (MDa)[a)]	PDI[a)]	Measured R_g (nm)[a)]
30% DEA/70% DMA (w/w; DEA30)	4.34	1.53	142
47% DEA/53% DMA (w/w; DEA47)	2.62	1.36	110

a) Data represent the average of the results from three analyses (standard deviation, ~ 5%).

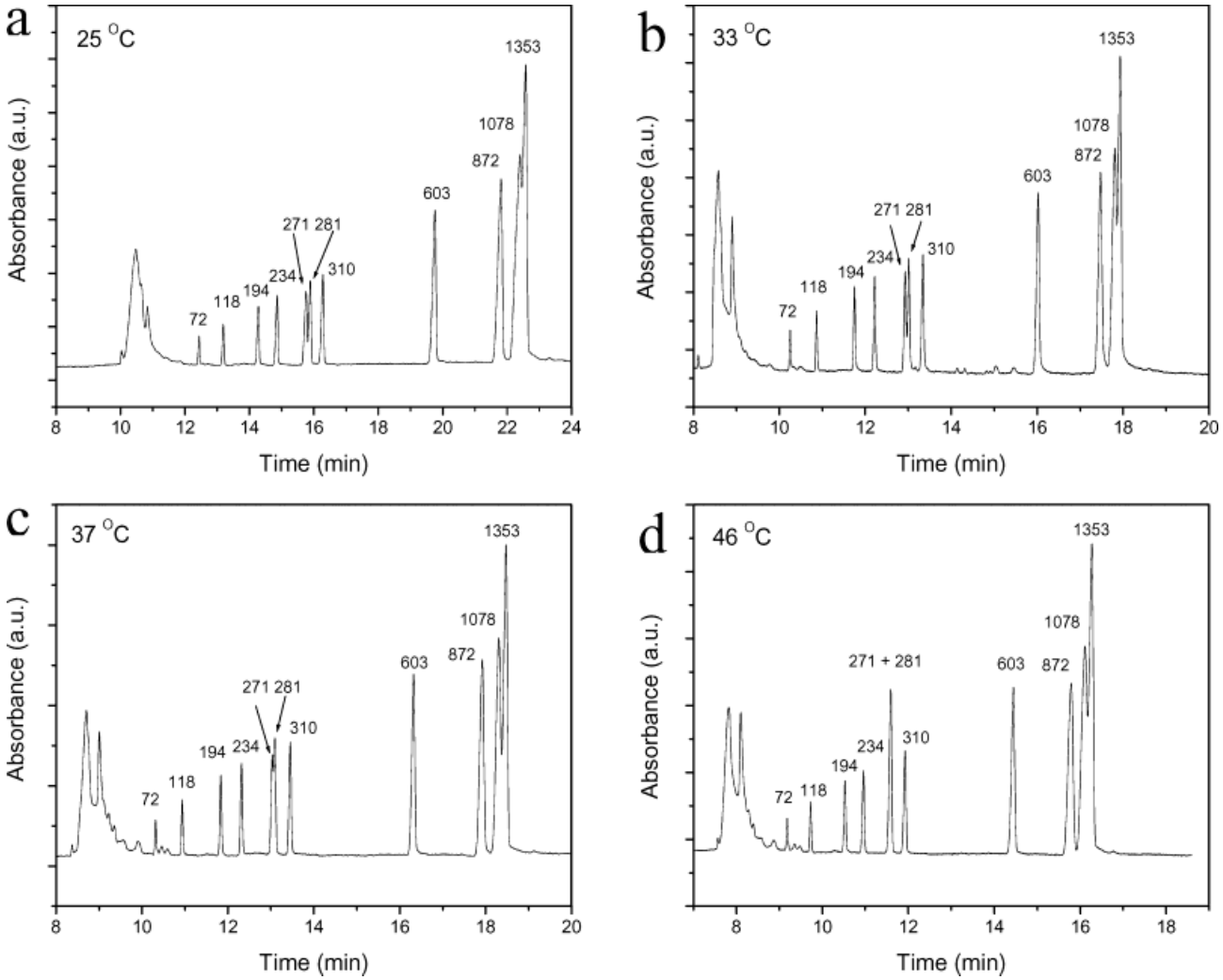

Figure 1. Representative electropherograms of the CE separation of a ΦX174-*Hae*III dsDNA restriction digest at (a) 25°C; (b) 33°C; (c) 37°C; (d) 46°C. CE conditions: reversed polarity; electrokinetic injection at 500 V/cm for 3 s; run voltage, 265 V/cm; current, 25°C: 7.2 μA; 33°C: 7.6 μA; 37°C: 7.8 μA; 46°C: 8.2 μA.

aqueous solution, these high-molar mass copolymers form a highly entangled network, as is essential for high-resolution DNA separation. Previous ^{1}H NMR studies [4] have confirmed that they are truly random copolymers, with comonomer composition essentially the same as the monomer ratio in the reaction vessel. Our previous studies have also shown that polymer hydrophobicity can have deleterious effects on DNA separation [4]. Co-polymerizing DEA (the thermoresponsive monomer) with DMA is a good strategy for reducing the overall polymer hydrophobicity, while also giving a measure of control over the specific phase transition behavior of the copolymer [12]. Even with significant incorporation of DEA, the copolymers are readily soluble in aqueous 1 × TBE buffer and remain soluble over the temperature range used, resulting in a homogenous solution suitable for electrophoretic DNA separation. The copolymers used in this study were chosen to have similar chemical structures but distinct thermoresponsive properties. In particular, the close similarity of the two copolymers in terms of monomer composition ensures that the two copolymers are completely miscible in aqueous solution. This is confirmed by the sensitive UV detection allowable in the blended matrix over a wide temperature range, as seen in Figs. 1a–d.

Temperature-ramped visible spectrophotometry experiments show that while DEA30 is nonthermoresponsive up to 85°C (*i.e.*, no LCST-induced volume phase transition is observed for a 3% w/v solution in 1 × TBE, data not shown), a thermally induced coil-to-globular transition of DEA47 occurs at around 55°C, as indicated by the sharp increase in matrix turbidity seen at that temperature in Fig. 2a. The hysteresis observed between the heating and cooling curves is the result of the finite time required for collapsed, partially aggregated polymer coils to re-dissolve upon cooling below the phase transition. For a polymer blend consisting of 2.5% w/w DEA30 and 0.5% w/w DEA47 in 1 × TBE (Fig. 2b) (note that this is the same total polymer concentration) an LCST-type transition is observed at about the same temperature (56°C), showing that the overall volume phase transition behavior of the copolymer blend is dictated by the thermoresponsive constituent of the matrix. However, a comparison of the two absorbance *vs.* temperature curves (Figs. 2a and b) reveals that in the case of the polymer blend containing a large fraction of nonthermoresponsive DEA30 (80% by weight), the transition is significantly less sharp. As the thermoresponsive DEA47 polymer is the minor constituent, a more gradual response will be expected when the collapsing DEA47 particles are embedded in a nonthermoresponsive DEA30 matrix. This broadened transition behavior is to our advantage, since we hope to employ a gradual temperature ramp to modulate the physical properties of the entangled polymer network, while avoiding the onset of turbidity, which disallows UV detection.

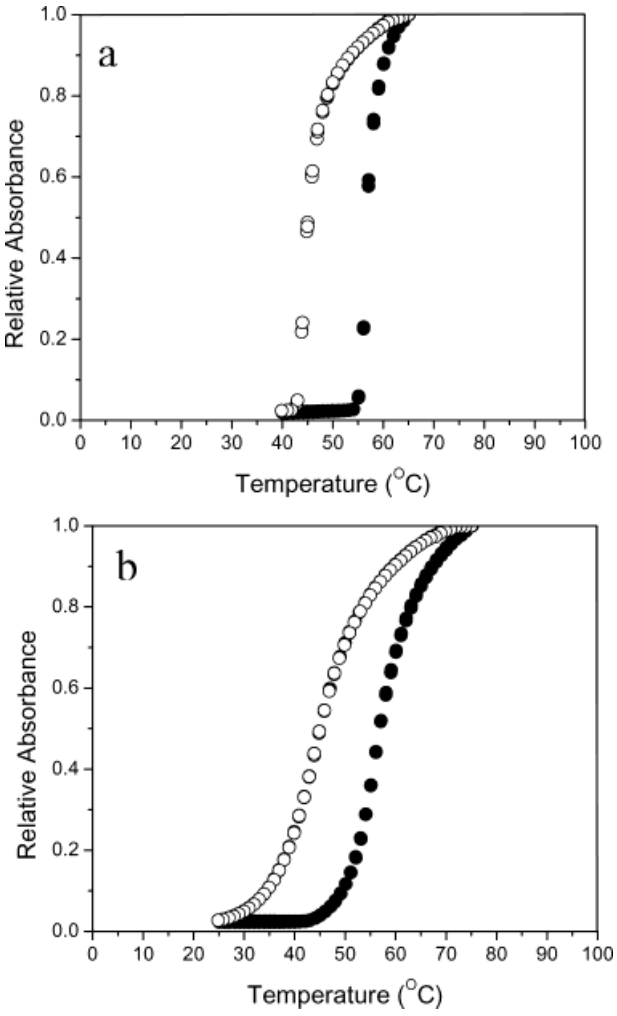

Figure 2. Temperature-dependent absorbance curves for (a) 3% w/w DEA47 blend in 1 × TBE; (b) 2.5% w/w DEA30 and 0.5% w/w DEA47 blend in 1 × TBE. Closed circles depict heating data, and open circles depict cooling data (5°C/min). The plots represent data obtained in three consecutive cycles of heating and cooling (curves were reversible and reproducible).

3.2 DNA separation performance

The DNA sieving performance of the blended matrix at 25°C, 33°C, 37°C, and 46°C are illustrated in the electropherograms shown in Figs. 1a–d. (Note that all of these temperatures are well below the mid-point of the LCST transition, and hence the solution is still optically clear.) Control experiments were performed with a nonthermoresponsive, 3% w/w DEA30 matrix over the same temper-

ature range (electrophoresis data not shown). We find that both the blended matrix and the control matrix provide good dsDNA separation performance over the temperature range investigated. At lower temperatures, better resolution is obtained for small DNA (compare, in particular, the resolution of the peaks for the 271 bp/281 bp fragments), whereas somewhat improved resolution of large DNA (1078 bp/1353 bp fragments) can be observed at high temperatures. While it appears that all dsDNA fragments are reasonably well resolved over this temperature range without a dramatic improvement in peak spacing, the subtle effect of temperature on matrix performance can be more clearly determined by quantitative data analysis. We calculated the selectivity of DNA separation as a function of temperature for pairs of small DNA fragments ($<$ 200 bp), large fragments ($>$ 1 kbp), and DNA fragments of similar size (Δbp = 10 bp), and compared the results we obtained with our previous observations of the HEC/HPC system. Selectivity, *S*, is defined as

$$S = (\Delta\mu/\mu_{avg})/\Delta bp \quad (1)$$

where $\Delta\mu$ is the difference in the electrophoretic mobility of the two DNA fragments, μ_{avg} is the average of the two mobilities, and Δbp is the difference in DNA size in base pairs (bp). As in our previous study, we chose to compare selectivities, rather than resolution, to emphasize peak separation only, and neglect any differences in peak width that might occur from run to run, as peak widths can be affected by numerous (nonmatrix-related) factors such as the state of the wall coating or the injection conditions.

Figures 3a–c show the temperature-dependent selectivity of DNA separation for different pairs of fragments including 118–194 bp, 271–281 bp, and 1078–1353 bp, respectively. On each plot, we show the selectivity of separation for a DNA pair for both the blended matrix (closed circles) and the nonthermoresponsive control (open circles). A distinct maximum in the selectivity is observed for the 118–194 bp dsDNA fragments at around 35°C. On the other hand, the selectivity for the 271–281 bp fragments decreases monotonically with increasing temperature, with a dramatic (sigmoidal) decrease between 30°C and 40°C, and levels out at temperatures above 40°C. However, a significant improvement in the peak separation of the larger 1078–1353 bp fragments is observed between 30°C and 40°C, with the rate of increase in separation selectivity diminishing above 40°C. The essentially sigmoidal shape of the curves in Figs. 2b and c is intriguing, and we are not presently sure of its origin. When comparison is made with the control matrix, which is done by using 3% w/w nonthermoresponsive DEA30, it can be seen that the blended matrix gives a much stronger temperature dependency. This verifies that the thermoresponsive constituent plays a major role in the altered

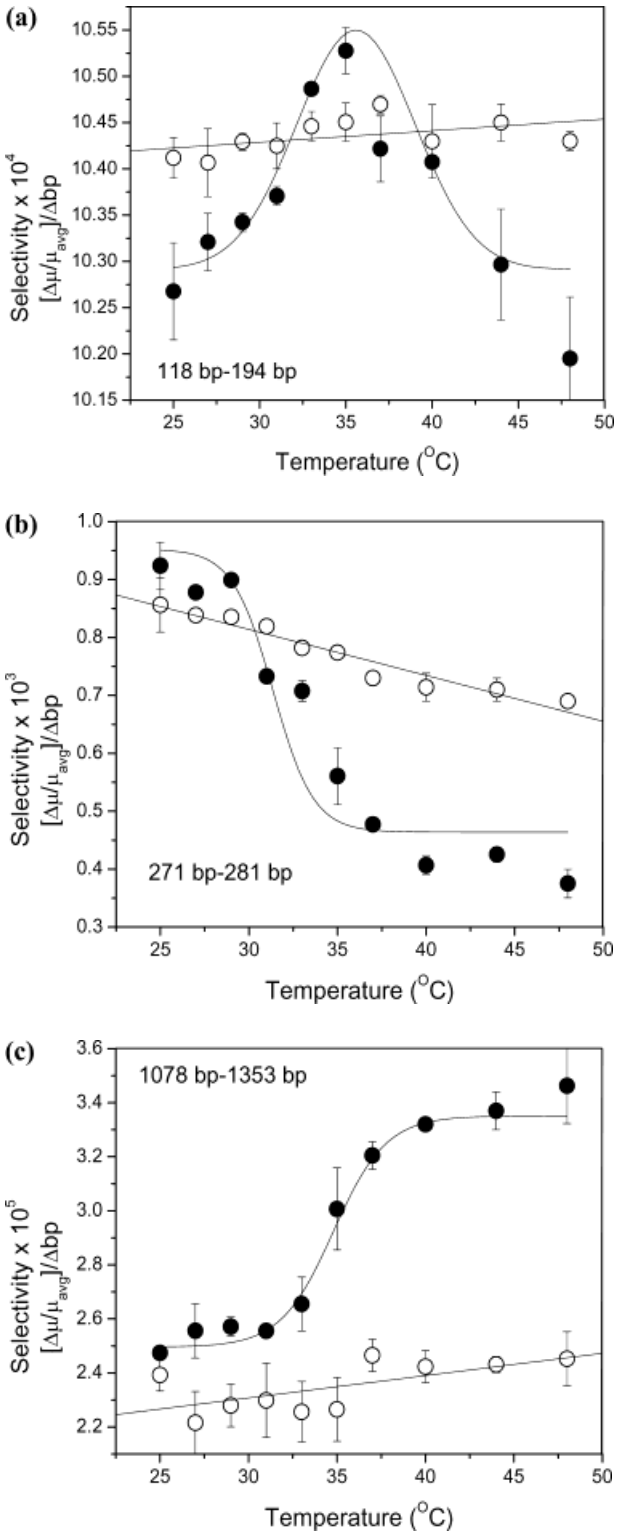

Figure 3. Selectivity as a function of temperature for (a) 118–194 bp; (b) 271–281 bp; (c) 1078–1353 bp. Closed circles represent the data obtained in 2.5% w/w DEA30 and 0.5% w/w DEA47 blend in 1$\times$ TBE. Open circles represent the data obtained in 3% w/w DEA30 blend in 1$\times$ TBE (control).

selectivity, much greater than one would expect for the sole effect of increased electrophoretic mobility with temperature [26].

Interestingly, the temperature-dependent selectivity for different pairs of DNA fragments in this DMA/DEA copolymer system follows trends that are quite similar to those we observed for the HEC/HPC system [1]. The only major differences are the temperatures at which the changes in selectivity occur; these appear to be dependent on the specific phase transition behaviors (LCSTs) of the respective thermoresponsive polymer networks (42°C for HEC/HPC, 56°C for DEA/DMA). While these polymers have very different chemical structures and polydispersities, these findings support the hypothesis that we can use thermoresponsive polymer networks to manipulate the sieving network properties, and hence effect a degree of control over analyte transport in microchannel electrophoresis. These results also illustrate the feasibility of applying this concept over different temperature ranges for various applications, depending upon the analytes, by simply using different blends of thermoresponsive and nonthermoresponsive polymers.

The physical picture that leads to the observed temperature-dependent selectivities was discussed qualitatively in our previous report [1]. We believe that the change of polymer mesh properties with temperature in this type of thermoresponsive polymer mixture can be considered to be essentially analogous to differences arising in network properties as polymer concentration is changed, which of course also leads to differences in DNA separation performance. In addition, it is possible that DNA conformation is also temperature-dependent, which could lead to different separation selectivities, as it is known that the relative dimensions of the DNA analytes and the mesh of the sieving network are important [27]. That is, just as there is an "optimal" polymer concentration to resolve DNA fragments in a certain size range (which is specific to the particular polymer and depends also on polymer molecular weight [28]), there is an optimum temperature for separating DNA of different sizes in blended thermoresponsive networks [28, 29]. At low temperature, when the thermoresponsive constituent is in a solvent-swollen state, the overall matrix forms a more entangled and constrictive network, which is more favorable for small-DNA separation [28, 30]. The unusual temperature-dependent selectivity behavior observed for the 118–194 bp fragments (*i.e.*, the maximum in selectivity) is perhaps due to the distinct migration behavior of these short, relatively rigid dsDNA molecules. Since the most effective DNA sieving is expected when the mesh size of the polymer matrix is comparable to the radius of gyration of the polymer coils (R_g) of the DNA molecules [27], the maximum in selectivity which is observed most likely correlates with a temperature that creates the optimal entangled network properties ("mesh size") for that particular DNA size. On the other hand, the monotonic decrease of temperature-dependent selectivity that we observe for the 271–281 bp fragments suggests that small DNA fragments having a very small difference in chain length are always best separated in a more constrictive matrix. This is perhaps analogous to the separation of small ssDNA sequencing fragments, which always require a highly entangled polymer network for the separation of molecules differing by only one DNA base in length [16]. Finally, the increase of selectivity for the larger, 1078–1353 bp DNA fragments with increased temperature corresponds to the general observation that large dsDNA molecules are best separated in a less concentrated polymer network with a more open mesh [28, 31, 32]. The Karger group [15] has shown that the selectivity of ssDNA separation, for larger sequencing fragments ($>$ 600 bases), improves in lower-concentration solutions of high-molecular-weight LPAs.

3.3 Temperature ramping experiments

With the knowledge of the different temperature-dependent separation selectivities for different pairs of DNA fragments, we designed a more interesting temperature ramping scheme, that we could implement in a single CE run. The time-dependent temperature profile we executed during the 20 min electrophoresis run is recorded in Fig. 4, and includes a steady increase between 25°C

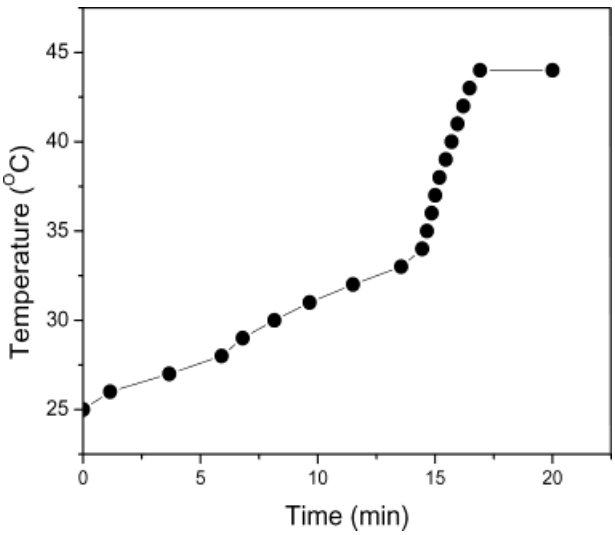

Figure 4. The temporal temperature profile of the temperature-ramping experiment. The profile is obtained by setting up a "timed event" in the Bio-Rad CE instrument software. Temperature was observed as a function of time and plotted.

and 33°C, followed by a rapid increase to 44°C. With this temperature-ramping scheme, we hoped to achieve improved (close to optimal) separation of both small and large DNA fragments simultaneously, which is not achievable in one CE run with a single temperature. When we carried out a CE separation with this temperature profile, high-resolution dsDNA separation was achieved. The calculated selectivities for the various DNA fragment pairs are given in Table 2, along with the corresponding temperature in a constant-temperature CE run at which selectivity at that level is achieved. It is observed that the selectivities in the temperature-ramped run are almost at their optimum values, for each DNA pair. The temperature-ramping scheme has the advantage of resolving small DNA fragments at low temperature (with almost optimal selectivity) at the beginning of the CE run, while with the gradual temperature ramping, large DNA fragments were well resolved as the run goes on. These data show therefore that while optimal separations for differently sized dsDNA fragment pairs cannot be achieved simultaneously at a single temperature, this strategy provides a solution to address one of the intrinsic limitations of matrix-based DNA electrophoretic separation. Although the advantages of this approach for dsDNA separations are modest, the benefit may be more significant for more challenging tasks such as DNA sequencing and genotyping separations. We predict that even subtle effects on the sieving matrix mesh size will have a significant effect on the separation of single-base ladder of ssDNA fragments with chain length, spanning three orders of magnitude. Even slight improvements in DNA sequencing read length (say 50–80 bases), representing a 10% longer read, would allow easier sequence assembly and a net cost reduction for ongoing genome projects.

Table 2. Separation selectivity achieved in the temperature-ramping CE run

DNA fragments	Selectivity in temperature-ramped CE	Comparable to selectivity as achieved at (°C)
118– 194 bp	10.36×10^{-4}	30
271– 281 bp	8.21×10^{-3}	32
1078–1353 bp	3.58×10^{-5}	46

4 Concluding remarks

We have applied thermoresponsive polymer networks based on blends of *N*,*N*-dialkylacrylamide copolymers as DNA sieving matrices to verify and generalize the concept of "dynamic porosity". Blends of DMA/DEA copolymers having distinct thermoresponsive properties were used to create a DNA sieving matrix with thermally tunable mesh size. We find that the polymer blend provided high-resolution separation of dsDNA at various temperatures ranging from 25°C to 48°C. The selectivities of separation for several pairs of DNA fragments were evaluated and we find that small fragments (118–194 bp), large fragments (1078–1353 bp) and fragments with similar size (271–281 bp) show different temperature-dependent selectivities, as a result of changing polymer network properties with temperature. A maximum selectivity of separation is observed for the smaller, 118–194 bp DNA fragments at around 35°C. On the other hand, the selectivity for similarly sized 271–281 bp fragments in this blended matrix decreases monotonically with temperature, with a dramatic decrease between 30°C and 40°C. Significant improvement in the separation of the larger, 1078–1353 bp DNA fragments is observed between 30°C and 40°C. Moreover, the separation selectivities for different pairs of DNA fragments show a temperature dependency similar to what was observed in HEC/HPC blends. These results verify and generalize the idea of using blended thermoresponsive and nonthermoresponsive polymer networks as a way to control DNA transport properties in microchannel electrophoresis. The distinct thermoresponsive behaviors of the two systems suggest the applicability of this concept at different temperature ranges for various applications, depending upon the analytes of interest, by the use of tailored copolymer blends. Finally, a temperature-ramping strategy was applied to enhance the separation selectivity for various DNA fragments simultaneously in a single CE run. The results presented here address the problem of the different matrix requirements of electrophoretic DNA separation for fragments in different size ranges, by implementing a sophisticated strategy and materials with controlled properties. In the future work we will apply this strategy to more challenging tasks, such as DNA sequencing on temperature-controlled microfluidic devices.

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