Review

Brett A. Buchholz
Wei Shi
Annelise E. Barron

Department of Chemical Engineering,
Northwestern University,
Evanston, IL, USA

Microchannel DNA sequencing matrices with switchable viscosities

We review the variety of thermo-responsive and shear-responsive polymer solutions with "switchable" viscosities that have been proposed for application as DNA sequencing matrices for capillary and microfluidic chip electrophoresis. Generally, highly entangled polymer solutions of high-molar mass polymers are necessary for the attainment of long DNA sequencing read lengths ($>$ 500 bases) with short analysis times ($<$ 3 h). However, these entangled polymer matrices create practical difficulties for microchannel electrophoresis with their extremely high viscosities, necessitating high-pressure loading into capillaries or chips. Shear-responsive (shear-thinning) polymer matrices exhibit a rapid drop in viscosity as the applied shear force is increased, but still require a high initial pressure to initiate flow of the solution into a microchannel. Polymer matrices designed to have thermo-responsive properties display either a lowered (thermo-thinning) or raised (thermo-thickening) viscosity as the temperature of the solution is elevated. These properties are generally designed into the polymers by the incorporation of moderately hydrophobic groups in some part of the polymer structure, which either phase-separate or hydrophobically aggregate at higher temperatures. In their low-viscosity states, these matrices that allow rapid loading of capillary or chip microchannels under low applied pressure. The primary goal of work in this area is to design polymer matrices that exhibit this responsive behavior and hence easy microchannel loading, without a reduction in DNA separation performance compared to conventional matrices. While good progress has been made, thermo-responsive matrices have yet to offer sequencing performance as good as nonthermo-responsive networks. The challenge remains to accomplish this goal through the innovative design of novel polymer structures.

Keywords: Capillary electrophoresis / DNA separation matrices / DNA sequencing / Review / Shear-responsive / Thermo-responsive EL 4933

Contents

Correspondence: Professor Annelise E. Barron, Northwestern University, Department of Chemical Engineering, 2145 Sheridan Road, Rm. E136, Evanston, IL 60208, USA
E-mail: a-barron@northwestern.edu
Fax: + 847-491-3728

Abbreviations: CAE, capillary array electrophoresis; **DEA**, *N,N*-diethylacrylamide; **DMA**, *N,N*-dimethylacrylamide; **HEC**, hydroxyethyl cellulose; **HPC**, hydroxypropyl cellulose; **LCST**, lower critical solution temperature; **LPA**, linear polyacrylamide; $\mathbf{M_w}$, weight-average molar mass; **pDEA**, linear poly-*N,N*-diethylacrylamide; **pDMA**, linear poly-*N,N*-dimethylacrylamide; **PEO**, poly(ethylene oxide); **pNIPA**, poly-*N*-isopropylacrylamide; **PPO**, poly(propylene oxide); **TBE**, Tris-borate-EDTA buffer

1 Introduction

The existing method of choice of the Human Genome Project for high-throughput DNA sequencing is automated capillary array electrophoresis (CAE), in which DNA is separated by its migration through matrices of entangled polymer solutions [1, 2]. The goal of the evolving genome projects is to achieve continual increases in sequencing efficiency and throughput, as well as continual decreases in the cost-per-base. Fused-silica capillary arrays, used in CAE, are costly, and high-throughput

 0173-0835/02/1005–1398 $17.50+.50/0

sequencing is still more expensive than genome centers would like. The translation of DNA sequencing technology onto less expensive microfluidic chips is one way in which sequencing cost could be reduced. However, there must be significant technical advances in both device and separation matrix engineering for chips to compete with present CAE instruments, which are well optimized [3].

Currently, DNA sequencing by CAE using entangled polymer solutions is becoming increasingly popular, because of its high degree of automation and reproducible performance. Moreover, the applicability of these instruments for different types of genetic analyses could be much broader, since entangled polymer matrices can provide high-resolution separations of both single-stranded (ss) and double-stranded (ds) DNA fragments, depending on their formulation. Typically, capillary arrays can be used for hundreds of experiments in series, merely by using pressure to replace the viscous polymer matrix after each run. Numerous types of polymers have been utilized as DNA separation matrices, including most notably linear polyacrylamide (LPA) [4], polydimethylacrylamide (pDMA) [5], and poly(ethylene oxide) (PEO) [6]. In commercial sequencing instruments, DNA separation matrices based on entangled networks of LPA or pDMA are most widely used, and typically generate read lengths of 550–650 bases per capillary within 2–4 h of electrophoresis [3].

In order to attain the longest read lengths, the polymers used in DNA sequencing matrices must be of high molar mass ($>$ 2–4 MDa), so that the entangled networks they form are robust to DNA migration. However, in a 7 M urea buffer at the polymer concentration necessary for sequencing, high-molar-mass polymer solutions normally exhibit extremely high zero-shear viscosities, as has been discussed in a recent review article [7]. Polymer chemistry can also have a dramatic effect on matrix viscometric properties. For instance, a 2.5% w/v solution of pDMA with a weight-average molar mass (M_w) of 5.2 MDa was shown to have comparatively lower viscosity than a solution of LPA having a similar M_w, due to a lower level of polymer entanglement resulting from greater pDMA hydrophobicity which results in smaller coil size [8]. Nevertheless, the zero-shear viscosity of the pDMA network was still extremely high, nearly 30 000 cP [5]. This general viscometric property of entangled polymer networks (strong resistance to flow) makes filling of the capillaries or chip microchannels with polymer solutions difficult. The problem can be overcome by the application of a high capillary loading pressure (*e.g.*, 1000 psi or greater), which can be generated by some of the commercial capillary array instruments [7].

On the other hand, the separation of dsDNA fragments, also valuable for some types of genetic analyses such as restriction mapping and allelic genotyping, does not necessarily require a highly entangled matrix of high-molar-mass polymers [9]. Lower-concentration, less viscous solutions can be employed for the separation of DNA fragments in the double-stranded form, as single-base resolution is not typically a necessity [7]. The use of less concentrated polymer solutions allows the replacement of dsDNA separation matrices from capillaries at relatively low applied pressure. Most types of polymer matrices function well for dsDNA separation; sequencing applications are much more challenging.

Microfluidic chip systems are a promising future step in genetic analysis technology development, offering faster parallel DNA sequencing separations ($\sim$ 550 bases in under 30 min [10–14]) and potentially a lower per-lane cost than capillary arrays [15]. However, the problem of high separation matrix viscosity is magnified on microfluidic chips, which are less easy to engineer for high-pressure matrix replacement than CAE systems. Furthermore, bonded microfluidic devices may or may not be able to withstand high matrix loading pressures, depending on the materials from which the chip is fabricated and the method of bonding the base plate to the cover plate [16]. Some plastic devices can only withstand 50 psi applied pressure [16], while 200 psi is a typical cut-off for many glass chips. Glass microchips are reasonably expensive to manufacture and at present do not allow rapid on-line loading and reloading of separation matrices [17]. One group has shown that separation matrices can be loaded into glass microchips with a special high-pressure device, but this step must be done by taking the chip off-line, and hence would interrupt the sequencing workflow [18]. Unless chips can be engineered for rapid, on-line separation matrix replacement, allowing hundreds of sequencing runs in series, they may not offer a significant cost advantage over CAE, given that fused-silica capillary arrays can be reused for 150–200 sequencing runs in series while being kept in place in the instrument. On the other hand, if DNA sequencing matrices could be replaced rapidly from chips under a low applied pressure, then microfluidic chips would clearly offer higher sequencing throughput at much lower cost than CAE.

Although less entangled, semidilute hydroxyethylcellulose (HEC) solutions [19] or low-molar mass hydroxypropylmethylcellulose (HPMC) solutions [20] have relatively low viscosities and can be used very efficiently for dsDNA fragment separations, they generally cannot meet the more demanding resolution requirements of DNA sequencing applications [21]. Low-molar-mass pDMA solutions were also shown to have relatively low viscosities,

and a read length of 600 bases in 2 h has been claimed for these matrices, however, in general the read lengths they generate are substantially shorter than those typically provided by LPA matrices [22].

Given the difficulties inherent in engineering chip devices for high-pressure replacement of viscous polymer solutions necessary for high-performance DNA sequencing, this review focuses on the novel strategies in chemical and physical design of polymer matrices that have been devised to address these problems. Specifically, this review concentrates on the polymeric matrices utilized for microchannel DNA separations that exhibit some type of "viscosity switch", which results from stimulus-responsive viscometric properties.

2 Shear-responsive polymer solutions

DNA sequencing matrices composed of high-molar-mass, entangled, water-soluble polymers such as LPA typically have extremely high zero-shear viscosities. However, these semidilute polymer solutions behave as non-Newtonian fluids under flow, where their viscosities are strongly dependent on the magnitude of the shear rate applied to the solutions. As the shear rate applied to the fluid is increased above a critical value, the viscosity decreases exponentially, as a result of the progressive disentanglement of polymer chains under flow [23, 24]. This effect is known as "shear thinning" [25]. As the force applied to the matrix at the capillary entrance increases, the polymer chains begin to align in the extensional flow field. This chain alignment lowers the overall viscosity of the solution, as there are fewer entanglements between polymer chains to resist flow. To initiate extensional flow of these high zero-shear viscosity solutions into microchannels, a hand-held syringe (which can generate $>$ 10 000 psi if a narrow-bore syringe is used) generally needs to be utilized to load the solution [4].

Replaceable matrices comprised of LPA, which is the best example of a shear thinning matrix, have provided excellent DNA sequencing results. Initial capillary electrophoresis results obtained with matrices containing a single, high-M_w LPA polymer at a concentration of 2% w/v led to a read length of 1000 bases in 80 min at 96.8% base-calling accuracy, which was a major breakthrough in sequencing read-length extension [26, 27]. Further optimization of this matrix was accomplished through reformulation based on polymer molar mass distributions [28]. It was determined that a low concentration of high-molar-mass LPA (typically $\sim$ 2%) gives excellent resolution for large DNA sequencing fragments, but poorer resolution of very small DNA fragments ($<$ 150 bases) [28]. The resolution of smaller DNA fragments in these less concentrated polymer matrices can be improved by increasing the total polymer concentration, irrespective of the average molar mass of the polymers added. Hence, read length was increased by adding a small amount of a low-molar-mass LPA to a DNA sequencing matrix comprised predominantly of high-molar-mass LPA. With full optimization, a solution of 2% w/w high-molar-mass LPA (M_w 17.1 MDa) and 0.5% w/w low-molar mass-LPA (M_w 268 kDa) [29] delivered a read length of 1300 bases in a little over 2 h, with a 98.5% base-calling accuracy (overall average of 1250 bases at 98.5% base-calling accuracy), an achievement that remains unequaled by any other sequencing technology [4].

To alleviate the difficulties for microchannel electrophoresis imposed by the high zero-shear viscosities of conventional polymer matrices such as LPA, especially for DNA sequencing on microfluidic chips, various groups have worked to manipulate the chemical and physical structure of the polymers to alter the viscometric properties of their solutions. Establishing a basis for this work, a study was undertaken of the effect of applied shear on the viscosity of four different entangled polymer matrices based on linear poly-*N,N*-dialkylacrylamide derivatives having varying degrees of hydrophobicity as a result of their different chemical structures [8]. Each polymer solution exhibited shear-thinning behavior, with the shear thinning much more pronounced in the most hydrophilic polymer studied, which was LPA. The other, less hydrophilic polymer solutions could be shown to have a lower initial entanglement densities because of their greater coil densities in water, and therefore to offer less resistance than LPA to microchannel flow at low shear. However, as the polymers' chemical structure increased in hydrophobicity, it was also observed that the shear-thinning behavior of the matrices became less dramatic [8].

3 Thermo-responsive polymer solutions

In addition to altering the shear-thinning behavior of the polymer solutions, manipulations of the chemical structure of the polymers can also be used to endow the polymer solutions with thermo-responsive properties. These temperature-sensitive polymer solutions have been designed as both thermo-thinning and thermo-thickening DNA separation matrices [3, 30, 31]. These thermo-responsive matrices are designed to allow a decoupling of the loading and sieving properties of the DNA separation matrices, and hence to allow rapid capillary or chip loading under a low applied pressure (*e.g.*, 50–100 psi). The challenge has been to produce polymer matrices with a useful "viscosity switch" that also give excellent DNA sequencing performance, based on the properties of the entangled networks that they form in their fully extended, most viscous state.

3.1 Thermo-thinning polymer solutions

Several different types of polymer solutions with thermo-thinning properties have been used for DNA sequencing separations, as shown in Table 1 and schematically presented in Fig. 1, including most importantly poly-*N,N*-dialkyacrylamides such as pDMA/*N*,*N*-diethylacrylamide (DEA) copolymers (Fig. 1a). At room temperature, these moderately hydrophobic copolymers can form an entangled network in aqueous solution much like LPA; however, the viscosity of their solutions decreases rapidly upon heating to a certain critical elevated temperature, due to a thermodynamic solubility-to-insolubility phase transition. More specifically, upon heating to a particular phase transition temperature, the polymer chains collapse in coil volume, due to increased intrapolymer hydrophobic interactions that result from the weakening of hydrogen-bonding interactions between polymer chain and solvent. This volume phase transition is shown schematically in Fig. 2 [3]. (For more detailed discussion of this phenomenon, see [3].) The temperature at which this phase transition occurs is known as the lower critical solution temperature (LCST) or cloud point temperature, and is dictated by the chemical structure of the polymer as well as by the solvent conditions [32–35]. In some cases, detailed below, a tremendous reduction in solution

Table 1. Summary of the properties and performance of thermo-responsive polymer solutions for DNA separations by microchannel electrophoresis

Polymers	M_W (kDa)	Transition temperature	Operating polymer concentration and temperature	Viscosity switch	Read length (DNA sample tested)	Advantages/ drawbacks
Thermo-thinning						
HPC/HEC mixture [30]	HPC: 60 HEC: 90–105	~ 42°C	1.5% w/v HPC and 0.4% w/v HEC, 20°C	1000 cP at 20°C, 30 cP at 50°C	NR (dsDNA)	Poor matrix for sequencing separations
pDMA/DEA [30] (70% DEA/30% DMA)	NR	~ 56°C	6% w/v, RT	500 cP at 30°C, 10 cP at 70°C	Estimated to be ~ 130 bases; accuracy, NR	Extremely low loading at 70°C/ gives only 130 base read length
pDMA/DEA [3] (53% DEA/47% DMA)	4000	~ 80°C	7.35% w/v, 44°C	~ 2000 cP at 40°C, ~ 200 cP at 90°C	463 bases in 78 min; accuracy, 97%; 421 bases in 75 min; accuracy, 98.5%	Matrix needs to be heated to 80°C to allow rapid low-pressure loading
Thermo-thickening						
Pluronic polyol F127, $PEO_{99}PPO_{69}PEO_{99}$ [37–39]	12.7	~ 18°C	21–25% w/v, RT	50 cP at 5°C, 250 cP at 20°C	NR (dsDNA)	Has a low η at RT, not tested for sequencing
Pluronic F127, $PEO_{106}PPO_{70}PEO_{106}$ [40–44]	13	~ 23°C	20–30% w/v, RT	~100 cP at 0°C, higher at RT	NR (dsDNA)	Polymer concentrations are extremely high
pNIPA-*g*-PEO [45, 46]	> 10 000	~ 36°C	8% w/v, RT	~ 2500 cP at 31°C, ~ 9500 cP at 36°C	NR (dsDNA)	Not tested for sequencing
LPA-*g*-pNIPA [31]	~ 650–1800	~ 45°C	3–5%, NR	~ 300 cP at RT, ~ 10,000 cP at 60°C	Estimated at 800 bases; accuracy, NR	Unknown separation temperature; necessary control not presented
$PBO_6PEO_{46}PBO_6$ and PBO_{10} $PEO_{271}PBO_{10}$ mixture [48]	2.9 and 13	NR	4% and 4%, RT	~ 2000 cP at 3°C, ~ 24,000 cP at 15°C	NR (dsDNA)	Not tested for sequencing
$C_{16}PEO_8$ [49]	0.6	~ 71°C	7%, 71°C	NR	NR (dsDNA)	Not tested for sequencing

RT, room temperature; NR, not reported; η, viscosity

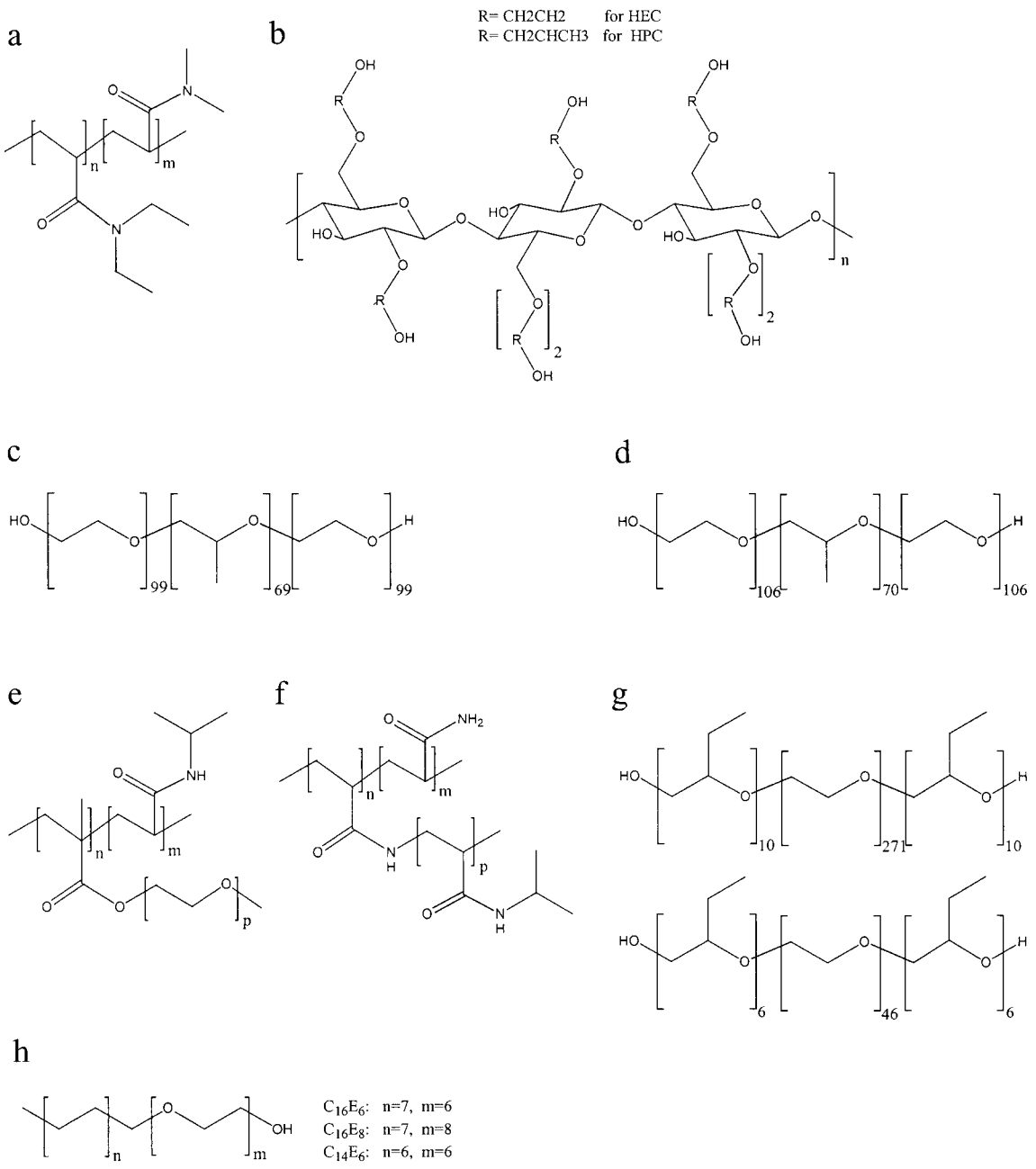

Figure 1. Structures of various synthetic polymer matrices for DNA sequencing with thermo-responsive properties. (a) pDEA/DMA, (b) typical HEC/HPC structure, (c) Pluronic polyol F127, (d) Pluronic F127, (e) pNIPA-g-PEO, (f) LPA-*g*-pNIPA, (g) $PBO_{10}PEO_{271}PBO_{10}$ and $PBO_6PEO_{46}PBO_6$ mixture, (h) $C_{16}E_6$, $C_{16}E_8$, $C_{14}E_6$.

viscosity occurs as a result of this polymer volume phase transition, and allows microchannels to be easily filled under low applied pressure when the solution is heated to an elevated temperature at or just above the LCST.

Interesting preliminary work was published in 1996 with a blended solution of a thermo-responsive polymer, hydroxypropylcellulose (HPC), which has an LCST of 42°C, and a nonthermo-responsive network-stabilizing polymer, HEC. (The structures of HEC and HPC are shown in Fig. 1b.) The polymer solution studied was comprised of 1.4% w/v HPC and 0.5% w/v HEC. When the temperature was raised above the LCST of the HPC polymers, the viscosity of the polymer matrix decreased by more than one order of magnitude, demonstrating the first DNA separation matrix with a thermally controlled "viscosity

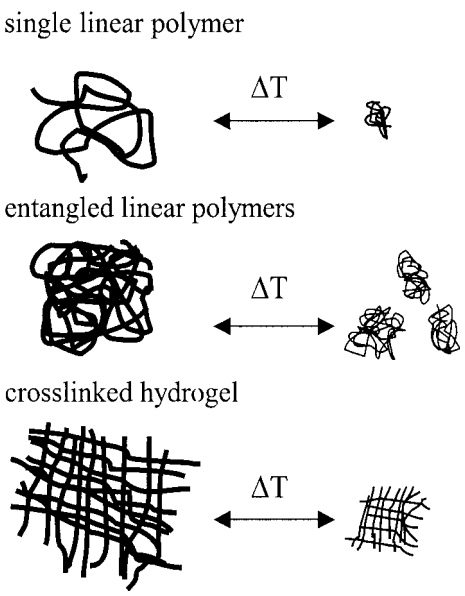

Figure 2. A schematic representation of LCST-exhibiting polymer and hydrogel systems as they undergo a reversible volume phase transition. As the temperature is increased, a single (dilute) linear polymer and entangled linear polymers undergo the collapse transition either as singular or as entangled chains, that can then coalesce into separate polymer microdomains that reach colloidal dimensions, clouding the solution. On the other hand, cross-linked hydrogels undergo the volume phase transition as single monolithic entities [3]. Reprinted from [3], with permission.

switch." These solutions were used for the analysis of dsDNA fragments in a 0.5 X TBE (Tris-borate-EDTA) buffer below the LCST, and gave high-efficiency separations [30].

The first preliminary study showing the feasibility of a "switchable-viscosity" matrix for DNA sequencing separations by capillary electrophoresis was also published in 1996 [30]. An LCST-exhibiting, *N,N*-dialkylacrylamide copolymer of 70% w/w DEA and 30% w/w DMA was designed to have a volume phase transition temperature of $\sim$ 56°C when dissolved at 6% w/v in an electrophoresis buffer containing 7 M urea. Elevating the temperature of the polymer solution to just above the LCST lowered the viscosity by nearly two orders of magnitude, from $\sim$ 500 cP at 25°C to just 10 cP at 70°C. Matrices could be rapidly loaded into capillaries under low applied pressure at the elevated temperature. A performance demonstration of this thermo-responsive matrix at ambient temperature for the separation of the 'T'-termination fragments of a DNA sequencing sample by CE (1-color LIF detection only) showed single-base resolution for DNA up to only 130 bases in length. Although this matrix seemed to have poor resolving power for larger DNA sequencing fragments, this result demonstrated the feasibility of temperature-sensitive polymer matrices for CE, and showed that a further optimization of polymer properties would be required to improve the performance of this type of matrix to make it a viable alternative to LPA for DNA sequencing.

Several years later, a more thorough study was undertaken of related LCST-exhibiting DNA sequencing matrices, comprised of random copolymers with a monomer composition of 53% w/w DEA and 47% w/w DMA, and with optimized average molar mass (M_w $\sim$ 4 MDa) [3]. A 7.35% w/v DNA sequencing matrix based on these pDMA/DEA copolymers yielded a read length of 421 bases in 75 min with 98.5% base-calling accuracy. Raising the temperature of the matrix above the LCST of $\sim$ 80°C precipitously decreased the steady-shear viscosity of the solution by more than one order of magnitude, from 2000 cP to less than 200 cP. While loading the entangled polymer solution into a capillary (50 μm ID, 10 cm long, 50 psi) required $\sim$ 8 min at room temperature, increasing the temperature to just above the LCST reduced the loading time to $\sim$ 500 ms. Both the rheological characterization and pressurized loading time data for this matrix are shown in Fig. 3 [3]. Further optimization of this pDEA/DMA copolymer matrix, to a monomer composition of 42% w/w DEA/58% w/w DMA (a less hydrophobic polymer with a volume phase transition temperature of $\sim$ 95°C), produced a longer read length of 575 bases in 94 min at 98.5% base-calling accuracy (unpublished results). It was clear from this study that DNA sequencing read length is decreased monotonically as the overall hydrophobicity of the copolymer (% DEA monomer) is increased. This phenomenon was later confirmed and studied in more detail, with a comparison of the properties and performance of different *N,N*-disubstituted acrylamide polymer matrices at 44°C [8].

It should be mentioned that the above-cited DNA sequencing results with the 53% w/w DMA/47% w/w DEA copolymer matrix were produced by loading the capillary array at room temperature and then elevating the temperature to 44°C for the sequencing experiment, since 44°C is the maximum achievable temperature in the CAE system used. A novel microchannel electrophoresis instrument, with a good dynamic temperature control in the range of 25–95°C, would be needed to take advantage of this matrix's thermally controlled "viscosity switch."

In a related study, an investigation of the effect of polymer matrix hydrophobicity on DNA sequencing at elevated column temperatures was undertaken [36]. The sequenc-

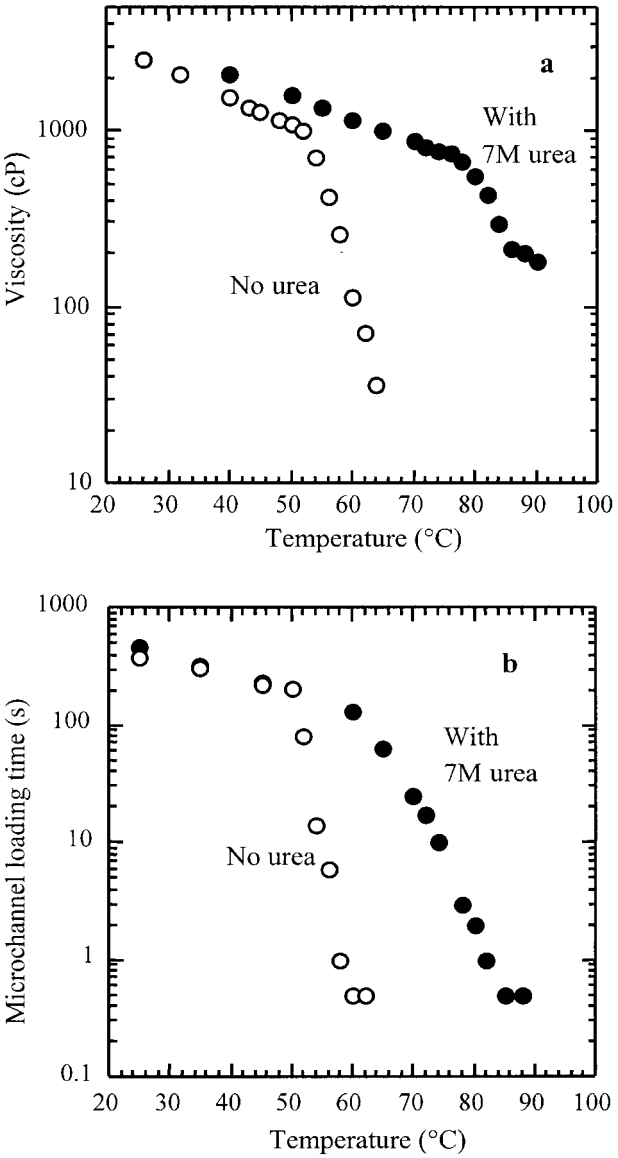

Figure 3. (a) Rheological characterization of thermo-responsive 53% w/w DEA/47% w/w DMA copolymer solutions as a function of temperature. Experiments were run on a Bohlin rheometer with a Couette cell. The shear rate for both samples was 11.6 s^{-1}. Filled circles: 7.35% w/v in 50 mM Tris/50 mM TAPS/2 mM EDTA with 7 M urea. Open circles: 7.35% w/v in 50 mM Tris/50 mM TAPS/2 mM EDTA. (b) Pressurized loading times of 53% w/w DEA/47% w/w DMA copolymer solutions into a 10 cm × 50 μm capillary microchannel under a constant applied pressure of 50 psi at varying temperatures. Filled circles: 7.35% w/v in 50 mM Tris/50 mM TAPS/2 mM EDTA with 7 M urea. Open circles: 7.35% w/v in 50 mM Tris/50 mM TAPS/2 mM EDTA [3]. Reprinted from [3], with permission.

ing performance of hydrophilic LPA was compared with that of pDMA and two different pDMA/DEA random copolymers, one with 30% w/w DMA/70% w/w DEA and the other with 50% w/w DMA/50% w/w DEA. The thermo-responsive properties of the pDMA/DEA matrices were not utilized for capillary loading in this study. A maximum high-accuracy (98.5%) read length for each of these four different polymer matrices was established for a select temperature range. The optimum sequencing temperature was found to decrease with increasing polymer hydrophobicity. Moreover, when DNA sequencing in an LPA matrix was performed at 70–75°C, the optimum temperature for this matrix, a dramatic increase in read length was observed relative to the performance observed at lower, sub-optimum temperatures, presumably because DNA denaturation was facilitated. The increase in read length was not nearly as dramatic at the respective optimum temperatures of the more hydrophobic polymer solutions (pDMA, pDMA/DEA copolymers), which were substantially lower. In fact, the LPA matrix not only gave longer read lengths at the optimum temperature ranges of the pDMA and pDMA/pDEA matrices (which were "below optimum" temperature for LPA), it also gave shorter DNA migration times relative to the more hydrophobic matrices. This study clearly demonstrates that a disadvantage to the use of thermo-responsive polymeric matrices based on polymers with some intrinsic hydrophobic character is the reduction in read lengths that must inevitably result, since hydrophobic matrices form weaker entangled networks that are less able to separate large DNA sequencing fragments [8]. Also, thermo-responsive polymers need to be used in a temperature range that is optimum for each matrix, which is generally lower than the optimum sequencing temperature for LPA. This is a disadvantage since optimal DNA denaturation is accomplished at elevated temperatures.

3.2 Thermo-thickening (thermo-associative) polymer solutions

Compared with thermo-thinning polymer matrices, thermo-thickening matrices display an opposite type of temperature-responsive behavior. These polymer solutions have a low viscosity at room temperature or lower. As the temperature of the solution is increased to the sequencing temperature, the viscosity of the matrix increases. One advantage of these matrices for DNA sequencing is that the polymer solutions can be easily loaded into a microchannel at a lower temperature, where the viscosity is relatively low (*i.e.*, matrix heating is not required for loading). Yet at a typical DNA sequencing temperature (44°C or above), the viscosity and level of entanglement of the matrix increase, and ideally provide good DNA separation properties which the matrix would not exhibit at a lower temperature. Three different types of thermo-thickening matrices have been investigated to

date, including block copolymers with self-associating properties, grafted copolymers that associate hydrophobically at higher temperatures, and blended copolymers that form networks of "flower-like" micelles. These are each discussed below, in turn.

3.2.1 Block copolymers (Pluronics)

Pluronic is a trade name for commercially available triblock surfactants comprised of PEO and poly(propylene oxide) (PPO) segments (BASF Performance Chemicals, Mount Olive, NJ, USA). These low-M_w block copolymers self-associate into micelles, which display a unique temperature-responsive behavior. Upon heating, due to a loss of hydration, the polymeric micelles become more compact and self-assemble into a network with some long-range order, and the viscosity of their solutions increases rapidly. Pluronic polyol F127, with the structure of $PEO_{99}PPO_{69}PEO_{99}$ (Fig. 1c), was first tested as a polymer matrix for the separation of dsDNA fragments in 1997 [37]. In a dilute solution, Pluronic polyol F127 in aqueous buffer forms micelles similar to those formed in water. At high solution concentrations, the micelles tend to pack into crystalline formations with relatively well-structured PPO centers. By using a combination of characterization methods, the gel-like structure was determined to be a face-centered cubic lattice [37]. With a phase transition temperature of around 18°C, the polymer solutions had viscosities of 50 cP at 5°C and 250 cP at 20°C, as shown in Fig. 4 (note that a viscosity of 250 cP is still very low by the standards of DNA sequencing matrices) [37–39]. The separation of a ΦX174/*Hae*III digest over a temperature range from 15°C to 60°C was studied in solutions of this polymer [38]. As the mesh size

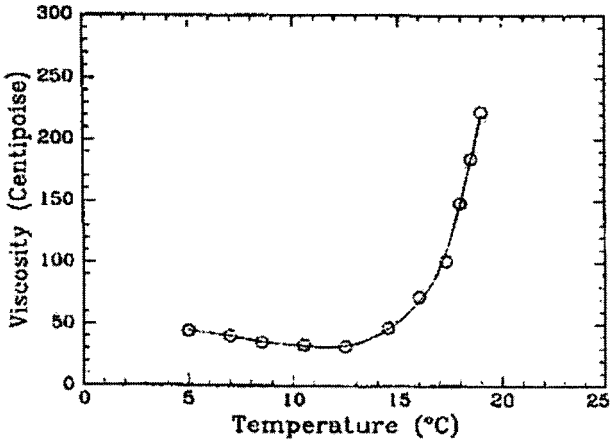

Figure 4. Temperature-dependence of the viscosity of a 21.2% w/v F127 solution in 1× TBE buffer. Reprinted from [38], with permission.

of the separation medium was changed with the increase in temperature, the dsDNA digest could be separated by electrophoresis within 100 s at ambient temperature, using a capillary with an 8 cm effective length and an inner diameter of 50 μm and operating at a field strength of 300 V/cm [39].

Pluronic F127 ($PEO_{106}PPO_{70}PEO_{106}$, structure shown in Fig. 1d) solutions with an M_w of 13 kDa, and with slightly different structures than Pluronic polyol F127, have also been tested extensively as DNA separation matrices [40–43]. A schematic illustration of the Pluronic F127 micellar structure is shown in Fig. 5 [40]. The solutions are isotropic, and flow freely into capillaries at low (refrigerator) temperatures (0–5°C), but rapidly transform into a gel-like, liquid-crystalline phase of spherical micelles at room temperature and above, due to a phase transition at ~ 20°C. The viscosity of a 20% w/v Pluronic F127 solution at 0°C was estimated to be 100 cP. Good resolution of DNA oligonucleotides was obtained in 25% w/w Pluronic F127 at 30°C within 8 min [44]. DNA sequencing has not been demonstrated in these Pluronic matrices; and, it is questionable whether a 0°C loading temperature can be considered as a convenience, since it requires chilling of the solution.

It is also worth mentioning that Pluronic liquid crystals may provide a different DNA sieving mechanism from that of entangled polymer solutions, because the polymer chains aggregate into spherical micelles in aqueous solutions with PPO chains creating a hydrophobic core surrounded by brushes of hydrated PEO tails [41]. The DNA chains presumably prefer to migrate around these hydrophobic cores and through the PEO coronas. The use of higher electric fields for DNA separation appears to affect the gel structure, and necessitates more frequent capillary refilling to refresh the matrix between runs [43]. These results suggest that DNA migration through Pluronic solutions under a high applied potential field might actually disrupt the micellar network, by an unknown mechanism (perhaps related to DNA perturbation of the PPO aggregate cores).

3.2.2 Grafted copolymers

Chu *et al.* [45, 46] designed and synthesized a thermoresponsive comb-like grafted copolymer, poly(*N*-isopropylacrylamide (pNIPA))-*g*-PEO (Fig. 1e) with M_w > 10 MDa, for electrophoretic DNA separations. This copolymer was synthesized by free-radical copolymerization of NIPA and poly(ethylene glycol) monomethyl ether monomethacrylate (with a macromonomer M_w of

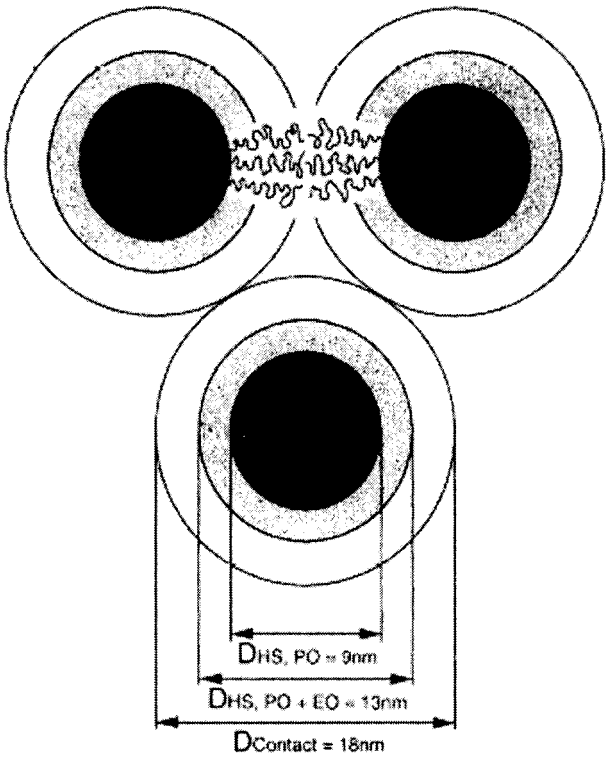

Figure 5. Schematic illustration of Pluronic F127 micelles in the liquid-crystalline phase (20% solution). The central, black region represents the approximate hard-core dimensions of the PPO micelle core. The gray region represents the approximate hard-core dimensions of the PEO micelle brush if hypothetically flattened onto the core surface. White plus gray regions represent the dimensions of the hydrated PEO chains. Individual PEO chains (curly lines) will be more extended than an unperturbed random coil because of crowding near the core surface. Dimensions of the interstitial region depend on the packing geometry and degree of extension of PEO chains into this region. Hard-core radii were calculated based on an aggregation number, n = 54, and an assumed density of 1.0 g/cm^3. The inter-micelle distance of 18 nm shown is based on small angle neutron diffraction studies [40, 50]. Reprinted from [40], with permission.

1000 Da). On average, there was one PEO graft chain per 30 repeating units of pNIPA backbone chain. Pure pNIPA exhibits an LCST-type volume phase transition, driven by hydrophobic collapse and chain association, at 32°C in water [47]. When pNIPA was grafted with PEO chains, this phase transition was manifested in an interesting manner. When the temperature was increased above the transition temperature of the copolymer ($\sim$ 36°C), the copolymer chains collapsed to nanoparticles, with pNIPA inside the core and the hydrophilic PEO chains on the shell. At 8% w/v in a nondenaturing aqueous buffer, the viscosity of the polymer solution was observed to be approximately 2500 cP at 31°C, but to increase to nearly 9500 cP at 36°C. The loading of microchannels with the 2500 cP solution at room temperature is still reasonably challenging (*i.e.*, this is not a very low viscosity). DNA separation results with this matrix were poor at temperatures above the transition temperature of the matrix, hypothetically because the collapsed copolymer chains in the globule state destroyed the chain network and thus the DNA separation ability [46]. However, at lower temperatures the polymers were useful for dsDNA separations. With an 8% w/v pNIPA-*g*-PEO solution in a 1.5 cm long column (100 μm ID) and 2400 V as the running voltage, a ΦX174/*Hae*III dsDNA digest could be separated within 24 s. Molecular architecture factors, such as pNIPA chain length, the NIPA to PEO ratio, and the PEO chain length, have not yet been optimized, and this matrix has not yet been tested for DNA sequencing by capillary electrophoresis.

Sudor *et al.* [31] synthesized a different grafted copolymer with thermo-responsive properties, with a hydrophilic backbone of polyacrylamide and comb-like grafts of the same LCST-exhibiting polymer discussed above, pNIPA [31]. A schematic illustration of this type of grafted thermo-responsive copolymer is shown in Figs. 1f and 6 [31]. These matrices were primarily tested for DNA sequencing applications. DNA sequencing matrices comprised of these grafted copolymers exhibit a relatively low viscosity ($<$ 300 cP) at room temperature. However, increasing the temperature from 20°C to 66°C increases the matrix viscosity from 300 cP to nearly 20 000 cP, as shown in the viscosity *versus* temperature plot shown in Fig. 7 [31]. There was an absence of matrix turbidity at elevated temperatures, and no apparent deleterious interactions with the DNA fragments (*e.g.,* band-broadening) during the DNA sequencing runs. Analysis of the sequencing data showed that if a DNA resolution cutoff of 0.5 was used as a method for crudely determining the length of read, this matrix could purportedly produce a read length of nearly 800 bases in less than 1 h. For good performance, a precisely tuned balance of the percentage and chain-length of the grafted pNIPA chains on the hydrophilic LPA backbone was necessary. The best results were obtained when the backbone of LPA (M_w 1.5–2.0 MDa) was grafted with less than 10% w/w of short pNIPA chains ($M_w \sim$ 10 kDa) [31]. The thermo-thickening properties of the matrix were determined to be too weak if the fraction of LCST-exhibiting grafts was much smaller than 10% w/w. On the other hand, it was found that extensive intermolecular aggregation could lead to network collapse of the polymer chains if the weight fraction of pNIPA chains was too high, as evidenced by a dramatic reduction of the solution viscosity. This thermo-responsive matrix appears to be quite promising for DNA

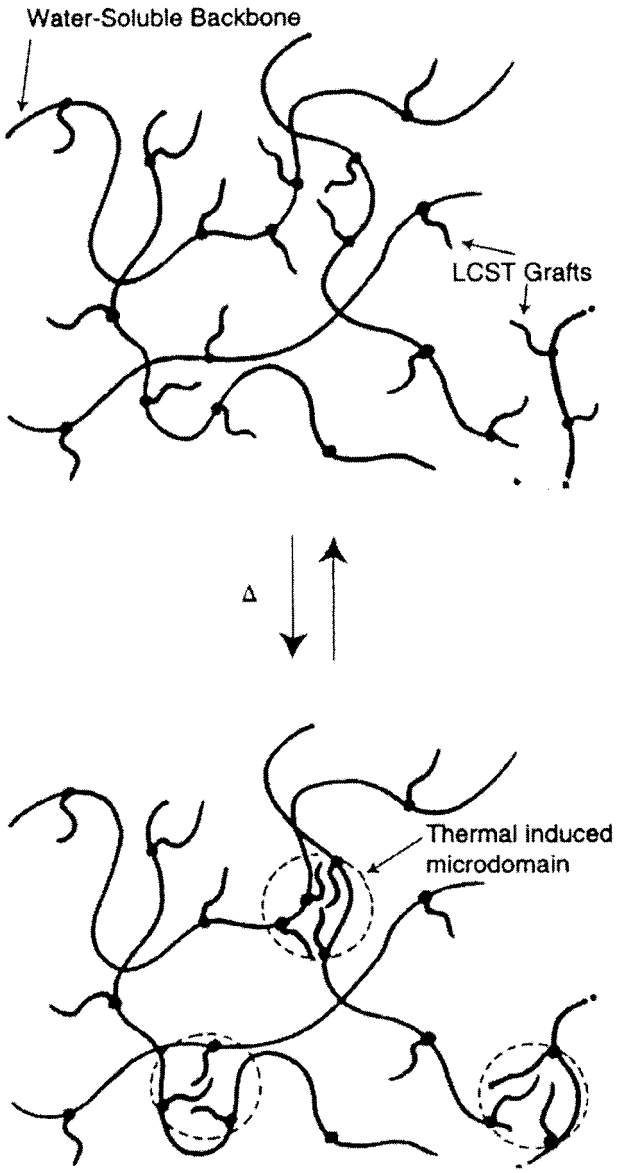

Figure 6. Simplified view of a mechanism for thermo-thickening by associating block copolymers (shown in Fig. 1f). Upon heating, the LCST-exhibiting grafts undergo microphase separation and form micelle-like aggregates that act as transient cross-links driven by hydrophobic interaction. Reprinted from [31], with permission.

sequencing applications. However, no data were presented for what seems like a necessary control experiment, in which the authors would show that a matrix composed of an ungrafted LPA with equivalent M_w to the LPA backbone of the copolymer (2 MDa) does not perform just as well or better for DNA sequencing at 66°C than the grafted copolymers discussed in the article. That is, it was not definitively shown that the hydrophobic association of pNIPA grafts, which increases the matrix viscosity to 20 000 cP, is essential for good DNA sequencing performance.

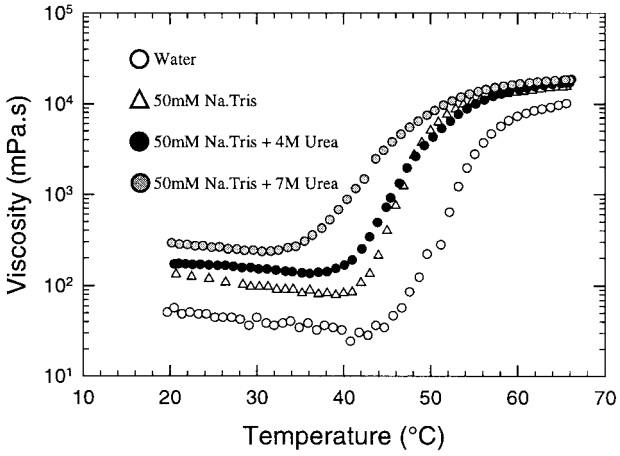

Figure 7. Effects of temperature and sequencing buffer additives on the rheological properties of an LPA-*g*-pNIPA copolymer. Reprinted from [31], with permission.

3.2.3 Block copolymer mixtures

A mixture of 4% w/v $PBO_6PEO_{46}PBO_6$, where PBO is polybutylene oxide, and 4% w/v $PBO_{10}PEO_{271}PBO_{10}$ (Fig. 1g), also shows thermo-thickening properties [48]. This block copolymer mixture was designed to form mixed flower-like micelles in cold, dilute solution, and a homogeneous, gel-like, open network with hydrophobic clusters as cross-linking points at higher polymer concentrations and higher temperatures. Upon heating, the matrix viscosity was found to increase slowly from 2000 cP at 3°C to nearly 24 000 cP at 15°C. Polymer solutions were chilled to 0°C for loading into the capillaries. The mixture was then used to separate dsDNA fragments at room temperature, affording a good separation of a ΦX174/*Hae*III restriction digest under an applied field of 200 V/cm. This novel, self-assembled polymer network has not yet been tested for DNA sequencing. However, the loading temperature of 0°C and run temperature of 15°C do not seem, at first inspection, to be easily compatible with DNA sequencing conditions.

3.2.4 "Dynamic polymers" of self-assembled, nonionic surfactant molecules

Separation of 10-bp DNA ladders at different temperatures was accomplished using matrices comprised of *n*-alkyl PEO oligomers ($C_{16}PEO_6$, $C_{16}PEO_8$, and $C_{14}PEO_6$) [49]. The $C_{16}PEO_8$ solutions studied (see structure in Fig. 1h) exhibit an extremely low viscosity at room temperature. As the temperature of the solution was elevated, the surfactant molecules self-assemble into wormlike micelles that can entangle with each other like polymers. Although the viscosity transition of the solution was not

characterized experimentally, it was apparent that viscosity increased substantially as the wormlike micelles formed a more entangled network in the solution with increasing temperature. When attempts at DNA separation were made at 25°C, the authors observed only poor resolution of a 10 bp DNA ladder. Yet very good resolution was achieved at 71°C, which was determined to be the approximate phase transition temperature for surfactant self-assembly. The DNA-resolving ability of this matrix was lost when the temperature was elevated further to 74°C. These novel matrices have not yet been tested for DNA sequencing separations, to our knowledge.

4 Conclusions

While it has been shown that entangled networks of hydrophilic, high-molar-mass polymers such as LPA are ideal for resolving large DNA fragments, and can provide the longest sequencing read lengths yet observed, the viscometric properties of these solutions are nonideal for microchannel DNA electrophoresis applications. This is because separation matrices comprised of entangled high-molar-mass polymers tend to have very high zero-shear viscosities, making it difficult to initiate their flow into capillaries. However, once flow is initiated by application of high pressure, these matrices can fill the capillaries quite quickly, since viscosity drops dramatically with application of shear. The best sequencing results published to date have been generated by these hydrophilic, shear-thinning polymer solutions. Their disadvantage is that with such high zero-shear viscosities (on the order of 100 000 cP), an enormous initial pressure generally is needed to load the solutions into capillaries or chips. Designing miniaturized DNA electrophoresis systems for high-pressure matrix replacement and robust operation presents a challenge, especially for low-cost plastic microfluidic devices.

The use of thermo-responsive polymer solutions as DNA sequencing matrices has been demonstrated to enable easy loading and replacement of the separation matrix into microchannels. Thermo-thinning solutions can be loaded into capillaries or chips at an elevated temperature, at which the viscosity is dramatically decreased. Subsequently lowering the temperature to a typical DNA sequencing run temperature then allows the solution to become re-entangled, facilitating DNA sequencing separations. Thermo-thickening solutions work in the opposite manner, and have a relatively low viscosity at room temperature or below. Elevating the temperature of these solutions to sequencing temperatures drives hydrophobic association, increasing their viscosities. Some classes of thermo-responsive matrices (both thermo-thinning and thermo-thickening) provide respectable sequencing read lengths, although they are still shorter than the best optimized conventional (nonthermo-responsive) separation matrices such as LPA. This is because the hydrophobic characteristics of the thermo-responsive polymers cause them to form less stable entangled networks then those formed by hydrophilic polymers such as LPA, leading to a decreased ability to resolve large DNA fragments. On the other hand, some of these matrices provide these shorter reads ($\sim$ 400–550 bases) in a short run time ($<$ 75 min) and could be considered immediately useful.

If one considers both the DNA-sieving characteristics of a matrix and the practical issues related to pressurized microchannel loading, there does not appear to be a truly ideal matrix for chip-based DNA sequencing. There are advantages and disadvantages to the use of both shear-responsive and thermo-responsive polymer networks. An ideal polymer would have the excellent DNA-separating abilities of a shear-responsive polymer such as LPA, with a high level of polymer entanglement at DNA sequencing temperatures, and the switchable viscosity of a thermo-responsive polymer, allowing easy low-pressure loading of the solution into microchannels. Perhaps a novel separation matrix, with chemical and physical properties different from polymers or self-assembling oligomers investigated so far, will one day fulfill both requirements.

Received January 24, 2002

5 References

[1] Marshall, E., Pennisi, E., *Science* 1998, *280*, 994–995.

[2] Brennan, M., Zurer, P., *Chem. Engineer. News* 2000, *78*, 11.

[3] Buchholz, B. A., Doherty, E. A. S., Albarghouthi, M. N., Bogdan, F. M., Zahn, J. M., Barron, A. E., *Anal. Chem.* 2001, *73*, 157–164.

[4] Zhou, H., Miller, A. W., Sosic, Z., Buchholz, B., Barron, A. E., Kotler, L., Karger, B. L., *Anal. Chem.* 2000, *72*, 1045–1052.

[5] Song, L., Liang, D., Fang, D., Chu, B., *Electrophoresis* 2001, *22*, 1987–1996.

[6] Fung, E. N., Yeung, E. S., *Anal. Chem.* 1995, *67*, 1913–1919.

[7] Albarghouthi, M. N., Barron, A. E., *Electrophoresis* 2000, *21*, 4096–4111.

[8] Albarghouthi, M. N., Buchholz, B. A., Doherty, E. A. S., Bogdan, F. M., Zhou, H., Barron, A. E., *Electrophoresis* 2001, *22*, 737–747.

[9] Madabhushi, R. S., Vainer, M., Dolník, V., Enad, S., Barker, D. L., Harris, D. W., Mansfield, E. S., *Electrophoresis* 1997, *18*, 104–111.

[10] Liu, S., Shi, Y., Ja, W. W., Mathies, R. A., *Anal. Chem.* 1999, *71*, 566–573.

[11] Schmalzing, D., Tsao, N., Koutny, L., Chisholm, D., Srivastava, A., Adourian, A., McEwan, P., Matsudaira, P., Ehrlich, D., *Genome Res.* 1999, *9*, 853–858.

[12] Salas-Solano, O., Schmalzing, D., Koutny, L., Buonocore, S., Adourian, A., Matsudaira, P., Ehrlich, D., *Anal. Chem.* 2000, *14*, 3129–3137.

[13] Liu, S., Ren, H., Gao, Q., Roach, D. J., Loder, R. T., Armstrong, T. M., Mao, Q., Blaga, I., Barker, D. L., Jovanovich, S. B., *Proc. Natl. Acad. Sci. USA* 2000, *97*, 5369–5374.

[14] Paegel, B. M., Emrich, C. A., Wedemayer, G. J., Scherer, J. R., Mathies, R. A., *Proc. Natl. Acad. Sci. USA* 2002, *99*, 574–579.

[15] Freemantle, M., *Chem. Engineer. News* 1999, *77*, 27–36.

[16] McDonald, J. C., Duffy, D. C., Anderson, J. R., Chiu, D. T., Wu, H., Schueller, O. J. A., Whitesides, G. M., *Electrophoresis* 2000, *21*, 27–40.

[17] Burns, M. A., Johnson, B. N., Brahmasandra, S. N., Handique, K., Webster, J. R., Krishnan, M., Sammarco, T. S., Man, P. M., Jones, D., Heldsinger, D., Mastrangelo, C. H., Burke, D. T., *Science* 1998, *282*, 484–487.

[18] Scherer, J. R., Paegel, B. M., Wedemayer, G. J., Emrich, C. A., Lo, J., Medintz, I. L., Mathies, R. A., *BioTechniques* 2001, *31*, 1150–1154.

[19] Barron, A. E., Sunada, W. M., W., B. H., *Biotechnol. Bioengineer.* 1996, *52*, 259–270.

[20] Han, F., Huynh, B. H., Ma, Y., Lin, B., *Anal. Chem.* 1999, *71*, 2385–2389.

[21] Bashkin, J., Marsh, M., Barker, D., Johnston, R., *Appl. Theor. Electrophor.* 1996, *6*, 23–28.

[22] Madabhushi, R. S., *Electrophoresis* 1998, *19*, 224–230.

[23] Bohdanecky, M., Kovar, J., *Viscosity of Polymer Solutions*, Elsevier Scientific Publishing, Amsterdam 1982.

[24] Goetzinger, W., Kotler, L., Carrilho, E., Ruiz-Martinez, M. C., Salas-Solano, O., Karger, B. L., *Electrophoresis* 1998, *19*, 242–248.

[25] Rodriguez, F., *Principles of Polymer Systems*, Taylor & Francis, Washington 1996.

[26] Ruiz-Martinez, M. C., Berka, J., Belenkii, A., Foret, F., Miller, A. W., Karger, B. L., *Anal. Chem.* 1993, *65*, 2851–2858.

[27] Carrilho, E., Ruiz-Martinez, M. C., Berka, J., Smirnov, I., Goetzinger, W., Miller, A. W., Brady, D., Karger, B. L., *Anal. Chem.* 1996, *68*, 3305–3313.

[28] Salas-Solano, O., Carrilho, E., Kotler, L., Miller, A. W., Goetzinger, W., Sosic, Z., Karger, B. L., *Anal. Chem.* 1998, *70*, 3996–4003.

[29] Buchholz, B. A., Barron, A. E., *Electrophoresis* 2001, *22*, 4118–4128.

[30] Sassi, A. P., Barron, A. E., Alonso-Amigo, M. G., Hion, D. Y., Yu, J. S., Soane, D. S., Hooper, H. H., *Electrophoresis* 1996, *17*, 1460–1469.

[31] Sudor, J., Barbier, V., Thirot, S., Godfrin, D., Hourdet, D., Millequant, M., Blanchard, J., Viovy, J.-L., *Electrophoresis* 2001, *22*, 720–728.

[32] Schild, H. G., Tirrell, D. A., *Langmuir* 1991, *7*, 1319–1324.

[33] Varghese, S., Lele, A. K., Mashelkar, R. A., *J. Chem. Phys.* 2000, *112*, 3063–3070.

[34] Inomata, H., Goto, S., Saito, S., *Macromolecules* 1990, *23*, 4887–4888.

[35] Idziak, I., Avoce, D., Lessard, D., Gravel, D., Zhu, X. X., *Macromolecules* 1999, *32*, 1260–1263.

[36] He, H., Buchholz, B. A., Kotler, L., Miller, A. W., Barron, A. E., Karger, B. L., *Electrophoresis* 2002, *23*, in press.

[37] Wu, C., Liu, T., Chu, B., Schneider, D. K., Graziano, V., *Macromolecules* 1997, *30*, 4574–4583.

[38] Wu, C., Liu, T., Chu, B., *Electrophoresis* 1998, *19*, 231–241.

[39] Liang, D., Chu, B., *Electrophoresis* 1998, *19*, 2447–2453.

[40] Rill, R. L., Locke, B. R., Liu, Y., van Winkle, D. H., *Proc. Natl. Acad. Sci. USA* 1998, *95*, 1534–1539.

[41] Rill, R. L., Liu, Y., van Winkle, D. H., Locke, B. R., *J. Chromatogr. A* 1998, *817*, 287–295.

[42] Rill, R. L., Liu, Y., Ramey, B. A., van Winkle, D. H., Locke, B. R., *Chromatographia Suppl. I.* 1999, *49*, S65-S71.

[43] Epperson, J. D., Dodge, J., Rill, R. L., Greenbaum, N. L., *Electrophoresis* 2001, *22*, 771–778.

[44] Liu, Y., Locke, B. R., van Winkle, D. H., Rill, R. L., *J. Chromatogr. A* 1998, *817*, 367–375.

[45] Liang, D., Song, L., Zhou, S., Zaitsev, V. S., Chu, B., *Electrophoresis* 1999, *20*, 2856–2863.

[46] Liang, D., Zhou, S., Song, L., Zaitsev, V. S., Chu, B., *Macromolecules* 1999, *32*, 6326–6332.

[47] Heskins, M., Guillet, J. E., *J. Macromol. Sci. Chem. A2* 1968, *8*, 1441–1455.

[48] Liu, T., Liang, D., Song, S., Nace, V. M., Chu, B., *Electrophoresis* 2001, *22*, 449–458.

[49] Wei, W., Yeung, E. S., *Anal. Chem.* 2001, *73*, 1776–1783.

[50] Wanka, G., Hoffmann, H., Ulbricht, W., *Macromolecules* 1994, *27*, 4145–4149.