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Poly-*N*-hydroxyethylacrylamide (polyDuramide): A novel, hydrophilic, self-coating polymer matrix for DNA sequencing by capillary electrophoresis

A replaceable polymer matrix, based on the novel monomer *N*-hydroxyethylacrylamide (HEA), has been synthesized for application in DNA separation by microchannel electrophoresis. The monomer was found by micellar electrokinetic chromatography analysis of monomer partitioning between water and 1-octanol to be more hydrophilic than acrylamide and *N,N*-dimethylacrylamide. Polymers were synthesized by free radical polymerization in aqueous solution. The weight-average molar mass of purified polymer was characterized by tandem gel permeation chromatography-multiangle laser light scattering. The steady-shear rheological behavior of the novel DNA sequencing matrix was also characterized, and it was found that the viscosity of the novel matrix decreases by more than 2 orders of magnitude as the shear rate is increased from 0.1 to 1000 s^{-1}. Moreover, in the shear-thinning region, the rate of change of matrix viscosity with shear rate increases with increasing polymer concentration. Poly-*N*-hydroxyethylacrylamide (PHEA) exhibits good capillary-coating ability, *via* adsorption from aqueous solution, efficiently suppressing electroosmotic flow (EOF) in a manner comparable to that of poly-*N,N*-dimethylacrylamide. Under DNA sequencing conditions, adsorptive PHEA coatings proved to be stable and to maintain negligible EOF for over 600 h of electrophoresis. Resolution of DNA sequencing fragments, particularly fragments >500 bases, in PHEA matrices generally improves with increasing polymer concentration and decreasing electric field strength. When PHEA is used both as a separation matrix and as a dynamic coating in bare silica capillaries, the matrix can resolve over 620 bases of contiguous DNA sequence within 3 h. These results demonstrate the good potential of PHEA matrices for high-throughput DNA analysis by microchannel electrophoresis.

Keywords: Capillary coating / Capillary electrophoresis / DNA sequencing / Dynamic coating / Poly-*N*-hydroxyethylacrylamide EL 4853

1 Introduction

The sequencing of the first human genome has been accomplished on an accelerated schedule, thanks to recent advances in the development of high-speed, high-throughput, automated DNA sequencing technologies, in particular capillary electrophoresis (CE). Among the growing number of different genetic analysis techniques, DNA sequencing remains the gold standard for accuracy. Projects in *de novo* sequencing of additional human and nonhuman genomes, as well as comparative sequencing of closely related genomes and local sequencing to assess variation within genomes, will continue to require new advances in high-throughput and cost-effective DNA analysis technologies.

Current DNA sequencing technology is based on the electrophoretic separation of fluorescently labeled DNA fragments, produced by the Sanger cycle sequencing reaction, in polymeric sieving matrices. Until 1998, DNA sequencing was predominantly performed by slab-gel electrophoresis. However, CE is now being accepted widely as an effective, high-speed method for DNA sequencing and fragment analysis [1]. Compared to the slab format, microchannel electrophoresis offers the advantages of higher speed of analysis, improved peak efficiency, and the possibility of full automation. Moreover, the use of a fluid polymer solution rather than cross-linked gels in the capillaries allows automated replacement of the sieving matrix between runs and extends the capillary

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Abbreviations: AAM, acrylamide; **DEA**, *N,N*-diethylacrylamide; **DMA**, *N,N*-dimethylacrylamide; **FWHM**, full width at half maximum; **GPC**, gel permeation chromatography; **HEA**, *N*-hydroxyethylacrylamide; **LPA**, linear polyacrylamide; **MALLS**, multiangle laser light scattering; **PDMA**, linear poly-*N,N*-dimethylacrylamide; **PHEA**, poly-*N*-hydroxyethylacrylamide (polyDuramide); **TTE**, Tris-TAPS-EDTA buffer

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lifetime. Although CE provides faster separation than slab gels, it is necessary to operate multiple capillaries in parallel to compete with the throughput of slab-gel electrophoresis systems, which can run up to 96 samples side by side. Capillary array electrophoresis (CAE) instruments, accommodating 96 capillaries or more, provide a practical solution to overcome these throughput challenges [2, 3]. Thus, CAE has become the dominant technique in DNA sequencing centers [4, 5].

The next generation of automated, high-throughput DNA sequencing instruments may very well be based on microfluidic devices or "microchips". Microfluidic devices offer the possibility of a five- to tenfold increase in DNA sequencing rate over CAE instruments [6, 7] primarily because of the narrow sample zone and highly controlled injections that are attainable with these devices. With narrow sample zones, glass microfluidic devices can deliver reasonably long DNA sequencing reads *(e.g.,* 550 bases in under 27 min) [8–10].

The different formats of DNA electrophoresis, *i.e.,* slab gel, capillary and microchip, have a common requirement for a polymeric separation medium for DNA analysis, typically termed a "separation matrix". In order to achieve electrophoretic size-based separation of DNA fragments, the molecules must have a size-dependent electrophoretic mobility. In free solution, DNA molecules exhibit a constant charge to frictional coefficient ratio regardless of their size. Hence, the electrophoretic mobility of DNA in free solution is independent of fragment size [11, 12]. In the presence of an entangled polymer matrix, the apparent molecular friction coefficient of DNA increases with its molecular size, resulting in an electrophoretic mobility that decreases with increasing DNA chain length. Currently, CAE instruments employ DNA separation matrices composed of viscous solutions of entangled, water-soluble polymers (as recently reviewed [13]). The polymer matrix is replaced from capillary arrays with a fresh solution before each sequencing run through the application of high pressure. A range of polymer types have been used for DNA sequencing by CE, the most practically useful of which so far are linear polyacrylamide (LPA) and poly-*N,N*-dimethylacrylamide (PDMA). The best sequencing performance to date is that of LPA matrices, which can produce up to 1000 bases of contiguous sequence in about 1 h [14] and 1300 bases in 2 h [15], if employed with highly optimized polymer molar mass distribution, separation matrix formulation, sample preparation and cleanup, and base-calling algorithms. Much lower sequencing rates are more common in commercial CAE instruments such as the MegaBACE 1000 (600 bases in 2 h) [16] and the ABI PRISM 3700 (550 bases in 2–3 h) [17]. The use of less-optimized polymer matrices as well as the quality of real genomic DNA samples, in particular a lower purity of sequencing reaction products and the tendency for anomalous migration of some fragments due to formation of DNA secondary structure, all contribute to the shorter read lengths achieved in a practical sequencing environment.

Although LPA has a high sieving capacity for DNA fragments, it suffers a few drawbacks, one of which is the high viscosity of LPA solutions. Although high molar mass LPA ($>$ 10 MDa) solutions show pronounced shear-thinning behavior, such that LPA solution viscosities decrease when the applied shear force is increased, the zero-shear viscosities are high *(e.g.,* 120 000 cP) [18, 19]. Hence, LPA matrices require the application of high pressure to initiate microchannel flow and to fill capillary arrays within a reasonable time. Requirement for high-pressure matrix replacement may significantly contribute to the building and maintenance costs of the instrument, while the need to replace high-viscosity solutions also increases turnaround time and decreases system robustness. For this reason, LPA matrices may be difficult to implement for automated replacement from chip microchannels because of the inherent difficulty of applying positive pressure to microfluidic devices, especially those made from plastic [20].

Another drawback to the use of LPA solutions for DNA analysis is the need to suppress electroosmotic flow (EOF) in the microchannel (which typically is glass, sometimes plastic). This is typically accomplished *via* coating the wall by covalent silanol derivatization, a time-consuming and tedious process that represents a practical obstacle to the development of robust approaches to biomolecule separations by microchip techniques. A variety of covalently attached polymeric wall coatings for capillary and microfluidic devices have been reported in the literature, including polyacrylamide [21–23], poly-*N*-acryloylaminoethoxyethanol [24], and polyvinyl alcohol [14, 15]. Preparation of covalently coated capillaries increases capillary cost and limits its lifetime to that of the coating layer, which is subject to degradation by hydrolysis as well as by fouling. Moreover, covalently bound coating layers require *in situ* synthesis, the conditions of which are difficult to control inside the microchannel, presenting a risk of wall coating inhomogeneity. It is therefore desirable to obviate the need for covalently attached wall coatings with adsorptive polymer coatings that can be prepared and regenerated *via* simple protocols, prolong microchannel life, and thus reduce sequencing cost while also increasing separation efficiency.

The drawbacks of LPA matrices, as well as commercial interest in novel formulations, prompted the search for other separation media that can circumvent these shortcomings, while maintaining or improving the resolution

of DNA fragments. Lower-viscosity, self-coating polymer matrices, such as PDMA, polyethylene oxide (PEO) and polyvinylpyrrolidone (PVP) have been introduced for DNA sequencing in capillaries. At a given polymer molar mass and concentration, solutions of these polymers have a significantly lower zero-shear viscosity than that of LPA. Low-viscosity polymer solutions can be more easily pumped into microchannels and replaced using a practically achievable and robust pressurizing system.

Another important issue in DNA sequencing by CE is the requirement for capillary inner surface modification to suppress EOF. Polymer matrices such as PDMA and PVP have been shown to eliminate the need for a covalent wall coating because these polymers can function as stable "dynamic" capillary coatings, which adhere to the silica surface by physical adsorption and reduce EOF to negligible levels [25]. The driving forces for polymer adsorption and binding on the surface is still a matter of controversy in the literature, and so far the search for a self-coating matrix has been empirical. Even while they are water-soluble, polymers such as PDMA and PVP are slightly hydrophobic. Water acts as a somewhat "poor" solvent for these polymers, and hydrophobic interactions with the hydrophobic siloxane groups of the skeleton structure of the silica surface may favor polymer adsorption [26]. Chiari *et al.* [27] have shown that hydrogen bonding between polymer chains and surface silanols may play a role in the adsorption mechanism and stability of some polymer coatings. Thus, the hydrophilic-hydrophobic balance of the polymer, its potential for hydrogen bonding with the wall, and the nature of the solvent apparently dictate the adsorption properties of the polymer.

The adsorptive polymer matrices PDMA and PVP have been successful in circumventing some of the limitations of the LPA matrices, in particular the need for the formation of a covalent wall coating. However, none of these matrices have demonstrated a DNA sieving capacity as high as that of LPA. The best 4-color sequencing performance reported using PDMA resolved 600 bases in 2 h [25]. Recently, Song *et al.* [28] optimized the performance of a high-molar-mass PDMA matrix and reported single-color DNA sequencing up to 800 bases in 96 min. Shorter read lengths were produced by PVP matrices [29, 30]. The main reason for the lower sieving capacity of PDMA, PEO, and PVP compared to LPA is that these matrices are more hydrophobic than LPA. In a previous study, we have shown that polymer hydrophobicity has a negative impact on DNA sequencing performance and attainable read length [19]. An increase in polymer hydrophobicity results in the adoption, on average, of a more compact and dense coil configuration of the polymer in water, adversely affecting the mechanical robustness and extent of entanglement of the polymer network it forms. Furthermore, hydrophobic interactions of the polymer chains with the hydrophobic DNA-labeling fluorescent dyes can lead to band broadening and loss of resolution of DNA fragments [31]. Thus, the use of more hydrophilic polymers can be expected to improve the resolution of DNA sequencing fragments and to extend the attainable read length. Song *et al.* [32] successfully combined the hydrophilicity and sieving capacity of LPA with the self-coating ability of PDMA through the synthesis of several formulations of copolymers of acrylamide (AAM) and DMA. Under optimized single-color DNA sequencing conditions, 700 bases were separated in bare capillaries in 67 min.

In addition to polymer chemistry, variables such as polymer molar mass, matrix composition, electric field strength, and electrophoresis temperature have significant impact on the performance of a given polymer matrix for DNA analysis [14, 15, 33–38]. Regardless of polymer structure, most polymer matrices exhibit a similar dependence of DNA sequencing performance upon these variables. It has been shown that a relatively low concentration of high-molar-mass polymer chains is needed to separate large DNA fragments, whilst a higher overall concentration of polymer chains helps to improve the resolution of small DNA fragments. The use of low electric field strength generally improves the resolution of DNA fragments by delaying the onset of biased reptation, thus extending the read length at the expense of longer run time. Increasing the temperature of analysis can reduce separation time significantly and also helps to denature DNA fragments and melt DNA secondary structures, thus minimizing the occurrence of band compressions and improving resolution, provided that the entangled polymer network retains its robustness at elevated temperatures.

In this study, we report the synthesis and characterization of a novel, hydrophilic, replaceable, self-coating polymer matrix, poly-*N*-hydroxyethylacrylamide (PHEA), for application in DNA sequencing by CE. The hydrophilicity of the novel monomer is compared to those of AAM and DMA. The synthesis and characterization of molar mass distribution of PHEA are described. Polymer solution rheological behavior and the capillary coating ability of the novel polymer are characterized. Finally, we evaluate the functional performance of the PHEA matrix for DNA sequencing in LPA-coated and bare silica capillaries using a commercially available CAE instrument.

2 Materials and methods

2.1 Chemicals

N-Hydroxyethylacrylamide (HEA), trade name Duramide , was obtained from BioWhittaker Molecular Applications (Walkersville, MD, USA). AAM, urea, Tris, EDTA, *N*-tris(hy-

droxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), and sodium hydroxide were from Amresco (Solon, OH, USA). DMA was from Monomer-Polymer and Dajac Labs (Feasterville, PA, USA). V-50 initiator (2,2'-azobis (2-amidinopropane) dihydrochloride) was from Wako Chemical USA (Richmond, CA, USA). Benzyl alcohol, cetyltrimethylammonium bromide, and sodium azide were from Aldrich (Milwaukee, WI, USA). Boric acid, Immobiline p*K* 9.3, lithium hydroxide monohydrate, and 2-(*N*-morpholino) ethanesulfonic acid were from Sigma (St. Louis, MO, USA). Sodium chloride, sodium hydroxide, monobasic sodium phosphate monohydrate, methanol, 1-octanol, and hydrochloric acid were from Fisher Scientific (Pittsburgh, PA, USA).

2.2 Monomer hydrophilicity

Hydrophilicities of HEA, AAM, and DMA were compared by determining their equilibrium partition coefficients in water/1-octanol according to Gelfi *et al.* and Chiari *et al.* [39, 40]. The partition coefficient is defined as the ratio between the molarity of a given compound in the organic phase *vs.* the aqueous phase at equilibrium. Each monomer was dissolved (50 mM) in 5 mL water saturated with 1-octanol. Five mL of the aqueous solution was shaken with 5 mL of 1-octanol for 5 min in a separating funnel. The two phases were allowed to separate by gravity for 1 h. Then, the water phase was collected and centrifuged for 90 min at 4000 rpm. The clarified solution was diluted to about 1 mM monomer with sodium borate buffer, pH 9.0, and supplemented with 20 mM cetyltrimethylammonium bromide and 0.5 mM Immobiline p*K* 9.3 as an internal standard. Micellar electrokinetic chromatography (MEKC) was performed to quantify the amount of monomer in the aqueous phase [39, 40] before and after partitioning, allowing estimation of the partition coefficient. MEKC runs were carried out on a Beckman P/ACE 5000 instrument (Beckman Coulter, Palo Alto, CA, USA) using a 75 μm ID fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with 57 cm total length and 50 cm effective length. All runs were in the anodic direction, at 15 kV. Samples were loaded for 5 s by hydrodynamic injection and detected by UV at 254 nm. The partitioning experiments for each monomer, as well as the quantification of the monomer by MEKC, were each repeated 3 times. Average values and standard deviation from the average are reported.

2.3 Polymer synthesis

Homopolymers were synthesized from HEA, AAM, and DMA by free radical polymerization in 5% w/w aqueous solution at 47°C. Polymerization was initiated by 0.02% w/w V-50 and allowed to proceed overnight. Prior to initiation, the polymerization solution was deoxygenated by continuous bubbling of nitrogen gas through the solution for 2 h. The polymer was purified by dialysis against deionized water using Spectra/Por cellulose ester dialysis membranes (Spectrum, Gardena, CA, USA), with a molecular-mass cutoff of 100 kDa. The polymer was recovered from the dialyzed solution by lyophilization (Labconco, Kansas City, MO, USA).

2.4 Polymer molar mass distribution

To determine weight-average molar mass of the synthesized polymers, samples were fractionated by gel permeation chromatography (GPC) prior to on-line multi-angle laser light scattering (MALLS) and refractive index detection. The GPC system is comprised of 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. Effluent from the GPC system flows directly into a DAWN DSP Laser Photometer and Optilab DSP Interferometric Refractometer connected in series (both, Wyatt Technology, Santa Barbara, CA, USA). Each sample (100 μL) was injected into the tandem GPC-MALLS system at a polymer concentration of $\sim$ 0.5 mg/mL. The flow rate through the columns was 0.35 mL/min and the mobile phase consisted of 100 mM NaCl, 50 mM NaH_2PO_4, and 200 ppm NaN_3. Tandem GPC-MALLS data were processed using ASTRA software from Wyatt Technology. ASTRA was used to calculate the weight-average molar mass, polydispersity index, and weight-average radii of gyration of the analyzed polymers. The error associated with each of the calculated values was less than 1%. All analyses were repeated 3 times and the standard deviation was less than 1%, indicating the high accuracy and reproducibility of the estimated values.

2.5 Rheological characterization

A temperature-controlled rotational Bohlin VOR rheometer (Cranbury, NJ, USA) equipped with a cone-plate geometry (diameter 30 mm, angle 2.5°) was used to determine the steady-shear viscosity of 4, 5, 6 and 7% w/v PHEA matrices at different rates of applied shear. PHEA solutions were prepared in DNA sequencing buffer consisting of 1 × TTE (*i.e.*, 50 mM Tris, 50 mM TAPS, 2 mM EDTA) and 7 M urea. All viscosity measurements were taken at 25°C.

2.6 Capillary coating and suppression of EOF

The dynamic coating ability of each polymer studied was assessed by first measuring the EOF in an uncoated capillary, and then in the polymer-coated capillary. A

Beckman P/ACE 5000 instrument was used to determine the mobility of EOF in fused-silica capillaries. A 57 cm total length, 50 cm effective length, and 50 μm ID capillary was used for the estimation of EOF. All EOF measurements were carried out at 44°C. To measure EOF in an uncoated capillary, the bare capillary was filled with background electrolyte (BGE), 23 mM lithium hydroxide, and 32 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5 [41]. A neutral marker, 0.1% benzyl alcohol in methanol, was injected into the capillary by hydrodynamic pressure followed by the application of a 100 V/cm electric field. The migration time of the marker peak was recorded and used to calculate the mobility of EOF, μ_{eo}, in the uncoated capillary. To determine the ability of a polymer to suppress EOF as an adsorptive coating, the bare capillary was coated with LPA, PDMA or PHEA according to the following protocol: the capillary was washed with 0.1 M HCl for 15 min, followed by a 15 min water wash. The capillary was then flushed with 0.1% w/v polymer solution for 15 min. Then the method of Williams and Vigh [41] was applied to determine the EOF coefficient in noncovalently polymer-coated capillary. The capillary was flushed with BGE for 5 min. Next, a solution of benzyl alcohol marker was injected by hydrodynamic pressure for 1 s. The marker band was pushed inside the capillary by BGE under pressure for 90 s. A second marker band was injected for 1 s and pushed by BGE for 90 s. A separation voltage of 100 V/cm was then applied for 5 min, during which time the neutral marker would migrate towards the cathode. After the electric field had dropped to zero, a third marker band was injected for 1 s. Finally, the capillary was flushed with BGE to move the three marker bands past the UV detector set at 254 nm, and the migration time of each band was recorded. μ_{eo} was then calculated as explained in [41]. The stability of the PHEA coating was evaluated by measuring the variation in EOF after the capillary had been flushed with DNA sequencing buffer containing 0.1% w/v PHEA and an electric field of 100 V/cm had been applied at 44°C. EOF measurement was carried out every 10 h interval in BGE buffer as detailed previously.

2.7 DNA sequencing

The performance of polymer matrices for DNA sequencing was tested using a MegaBACE 1000 CAE instrument (Molecular Dynamics, Sunnyvale, CA, USA) equipped with 6 × 16 fused-silica capillary arrays (75 μm inner diameter, 64 cm total length, 40 cm effective length) that were either covalently coated with LPA or left uncoated. Prior to injecting the sequencing matrix into an uncoated array, the capillaries were washed with 0.1 M HCl, water, and then with 0.1% w/v PHEA solution in water. Polymer matrices were prepared by dissolving the polymer at the desired concentration by slow stirring in DNA sequencing buffer. PHEA solutions of concentrations up to 6% w/v were loadable into the capillaries, whereas more concentrated solutions were too viscous to be loaded under a 1000 psi applied pressure. MegaBACE Sequencing Standards (Amersham Pharmacia Biotech, Piscataway, NJ, USA) consisting of M13mp18 DNA sequencing reaction products labeled with energy transfer dye primers were used. Sequencing matrices were loaded into the capillaries under a pressure of 1000 psi for 200 s, followed by a relaxation time of 20 min and prerun electrophoresis for 5 min at 140 V/cm. After electrokinetic sample injection at 94 V/cm for 60 s, electrophoresis of DNA was performed at 44°C. In order to achieve the optimal performance of the PHEA matrix for DNA sequencing, we systematically investigated the effect of polymer concentration and electric field strength on the DNA sequencing performance. Laser-induced fluorescence (LIF) data were collected, analyzed and translated into called DNA sequence using the MegaBACE 1000 DNA Sequencing Software Version 2.0 .

2.8 Data analysis

Raw LIF data of the T-track of the sequencing reaction products were extracted from the MegaBACE sequencing software. Single T-peaks were subjected to a Gaussian fit using PeakFit Version 4.06 (SPSS Inc., Chicago, IL, USA), from which the full width at half-maximum (FWHM) was estimated for each peak. Peak spacing was estimated as the average spacing between the centers of a given T-peak and the peaks on both sides of that T-peak. The plot of FWHM *vs.* DNA fragment size was modeled by a second-order polynomial and the peak spacing curve was modeled by an exponential function. The selected functions best modeled the experimental data [19, 25, 34] and yielded the lowest sum of squares of errors. The fitted functions were used to calculate the resolution, R_s, of the peaks using the following equation [22, 25]:

$$R_s = 0.59\,\frac{x_2 - x_1}{\mathrm{FWHM}} \qquad (1)$$

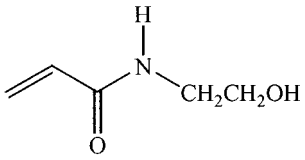
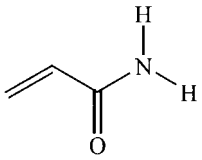
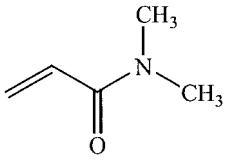
where x_i is the center of peak *i*.

3 Results and discussion

3.1 Monomer hydrophilicity

Table 1 shows the chemical structures of the monomers HEA, AAM, and DMA. Table 2 compares the partition coefficient in water/1-octanol of the acrylamide-based

Table 1. Chemical structure of AAM, DMA and HEA

Monomer	Chemical structure
HEA	
AAM	
DMA	

monomers. Results indicate that the novel monomer, HEA, on the whole is more hydrophilic than AAM, which in turn is substantially more hydrophilic than DMA. Whereas this does not necessarily translate directly to greater hydrophilicity of PHEA than LPA, given that these measurements were made for the monomers and not the polymers, it gives strong evidence that good hydrophilicity of PHEA is to be expected. It has been shown that hydrophilicity is a highly desired property of matrices for DNA and protein analysis [19, 27]. Our results are consistent with and in good agreement with the acrylamide-based monomer hydrophobicity scale reported by the Righetti group [39, 40, 42]. HEA and the monomer *N*-acryloylaminopropanol have similar chemical structures, and also have similar equilibrium partition coefficients in water/1-octanol system. In aqueous phase, the hydroxyl group at the end of the side chain of HEA becomes hydrated by forming hydrogen bonds with water molecules [43]. This favorable hydration shields the hydrophobic alkyl chain from the access of water and locally breaks up hydrophobic hydration, enhancing the overall hydrophilicity of the monomer.

Table 2. Partition coefficient in water/1-octanol of HEA, AAM and DMA

Monomer	Partition coefficient[a)]
HEA	0.11 ± 0.02
AAM	0.17 ± 0.03
DMA	0.50 ± 0.02

a) Reported values represent the average ± standard deviation of three runs.

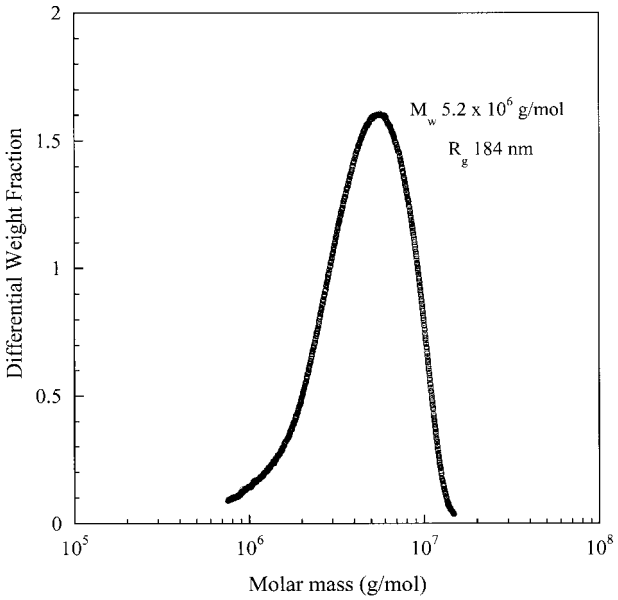


Figure 1. Molar mass distribution of PHEA as characterized by GPC-MALLS.

3.2 Polymer molar mass characterization

Polymers synthesized in the course of this study were characterized by tandem GPC-MALLS to determine their molar mass distributions. The molar mass distribution of PHEA used throughout this study is shown in Fig. 1. Table 3 compares the physical characteristics of the synthesized PHEA, LPA and PDMA and shows that the coil density of PHEA chains, calculated as the ratio of the polymer molar mass to its coil volume, is smaller than that of both LPA and PDMA polymers, when measured under similar conditions. This is perhaps the most useful measure of polymer hydrophilicity, though some influence of polymer side-chain chemical bulk may also contribute to this value. The relatively low coil density of PHEA indicates that the polymer adopts a more open and extended structure in aqueous solution than LPA and PDMA, indicating that water-PHEA interaction, *i.e.* hydration, is favorable over inter- or intrapolymer interactions,

Table 3. Physical characteristics of PHEA, LPA, and PDMA, estimated by GPC-MALLS

Polymer	Weight-average molar mass[a)] (MDa)	Polydispersity index[a)]	Measured R_g[a)] (nm)	Polymer coil density (Da/nm^3)
PHEA	5.2	1.39	184	0.20
LPA	5.3	1.52	168	0.27
PDMA	4.4	1.59	141	0.37

a) Reported values represent the average ± standard deviation of three runs.

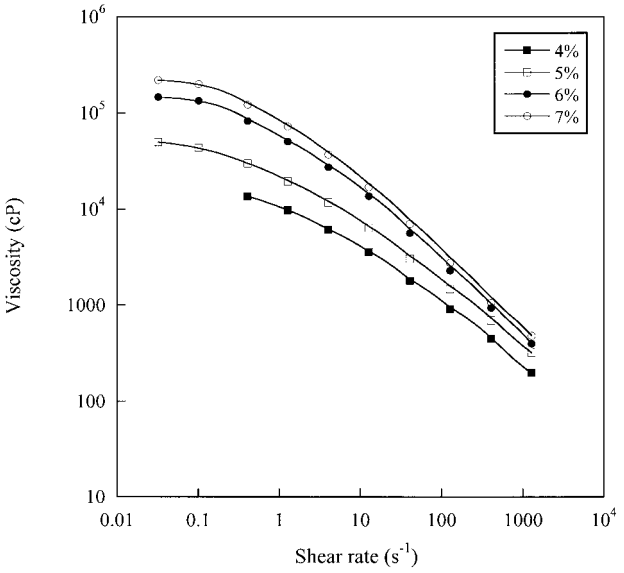


Figure 2. Effect of shear rate on the viscosity of PHEA solutions in DNA sequencing buffer (1 × TTE with 7 M urea) at different concentrations (w/v): (■) 4%, (□) 5%, (●) 6% and (○) 7%.

which reflects the hydrophilic nature of PHEA. Thus, the hydrophilicity of PHEA appears to be greater than that of LPA and PDMA, which is consistent with the hydrophilicity scale established in the monomer partitioning experiments.

3.3 Rheological behavior of PHEA solutions

Figure 2 demonstrates the dependence of the viscosity of different solutions of PHEA on polymer concentration and shear rate. The rheological behavior of PHEA solutions is consistent with the widely observed behavior of semi-dilute polymer solutions [44]. At low shear rates, PHEA solutions approximate the behavior of Newtonian fluids, the viscosity of which is virtually constant and independent of shear rate. Non-Newtonian behavior is observed through the resolution of chain entanglements at higher shear rates, which induces the so-called "shear-thinning" response. For a given shear rate, the viscosity of PHEA solution increases with increasing polymer concentration. Furthermore, the rate of change of solution viscosity with shear rate (given by the slope of the curve in the shear-thinning region) increases with increasing polymer concentration. Generally, the viscosity of a polymer solution increases with increasing polymer concentration due to an increase in polymer chain entanglements in the solution, increasing the resistance to flow. Increasing the rate of applied shear on an entangled polymer solution increases the shear force, that deforms the polymer coils and dismantles entanglements between polymer chains. Thus, entanglement density is reduced and solution viscosity decreases, resulting in the observed shear-thinning behavior [45]. At high PHEA concentration, there are initially more polymer chain entanglement points to be disrupted by the application of shear than there are at lower concentration. Thus, the extent of shear-thinning is more significant at higher polymer concentration.

3.4 Capillary coating and suppression of EOF

Electrophoretic separation of DNA in silica or glass microchannels requires passivation of the inner channel wall by a viscous, neutral polymer coating to suppress EOF and minimize analyte-wall interactions. Although covalent coatings have been applied successfully, adsorptive coatings are more attractive technologically since they are easier to prepare and oftentimes can be regenerated under certain conditions. The ability of a polymer to adsorb to and coat the microchannel wall can be estimated from the extent of reduction of EOF in the channel after exposure to dilute solutions of the polymer. Table 4 compares the EOF in an uncoated capillary with that in capillaries dynamically coated with LPA, PDMA and PHEA of similar molar masses. Note the different orders of magnitude in values of μ_{eo}. The result for PHEA is very interesting as it shows that PHEA is essentially as efficient as PDMA in suppressing EOF, whereas LPA is not suitable, as expected, for adsorptive capillary coating. The ability of PDMA and PHEA, but not LPA, to adsorb and coat the capillary wall raises the question of how and why polymers adsorb onto the silica surface. Polymer adsorption on silica may involve two types of interactions: (i) hydrogen bonding between hydrophilic, hydrogen bond-forming groups on the polymer and the silanol groups on the silica surface, and (ii) hydrophobic interactions between polymer segments and the hydrophobic siloxane groups of the skeleton structure of the silica surface [26]. The extent to which these interactions take place is influenced by competition or affinity of water molecules and polymer hydrophilic groups for the silanol groups.

Table 4. Comparison of EOF mobility in uncoated and dynamically coated capillaries as a function of polymer type

Capillary coating	μ_{eo}[a)] ($\times 10^8$ m^2/Vs)
No coating	4.177 ± 0.226
LPA	3.148 ± 0.201
PDMA	0.075 ± 0.027
PHEA	0.070 ± 0.011

a) Reported values represent the average ± standard deviation of three runs.

In aqueous phase, hydrophilic groups such as hydroxyls and amides on the polymer chain compete with water molecules to form hydrogen bonds with the silanol groups. For the polymer to form a stable coating on the silica surface, it must have a higher affinity for the silica than do the water molecules. Another factor that contributes to polymer adsorptive properties is the solubility of the polymer in the solvent from which it is adsorbed. If the polymer is somewhat hydrophobic, then water may be a relatively "poor" solvent for the polymer, and hence the polymer will have a greater tendency to leave the solvent phase and adsorb on the wall, which has some hydrophobic regions due to the siloxane groups. Polymer adsorption on the silica wall may provide a thermodynamic advantage (a free energy decrease) for the polymer-solvent system, due to the release of the water of hydrophobic hydration and an overall increase in entropy. Thus, in order for a polymer to form a wall coating, it must have hydrophilic hydrogen bonding groups as well as hydrophobic groups to adsorb on the hydrophilic/hydrophobic silica wall. The extent and stability of hydrogen bonds and hydrophobic interactions control the stability of the coating. For a stable system at equilibrium, the Gibbs free energy tends to a minimum. Both hydrophilic and hydrophobic interactions influence the free energy of the system.

The stability of the PHEA adsorptive coating was evaluated by measuring the EOF variation in a capillary under conditions similar to those employed in DNA sequencing analyses (*i.e.*, 1 × TTE, 7 M urea, 44°C, 100 V/cm). Figure 3 shows that the PHEA coating maintained the EOF at very

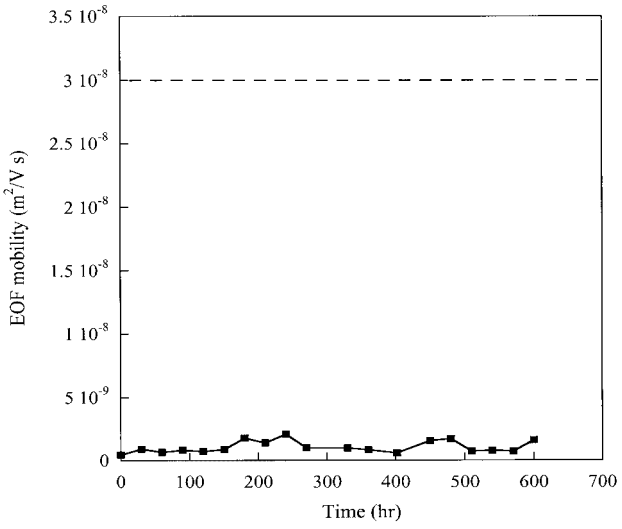


Figure 3. Variation of EOF mobility in PHEA noncovalently coated capillaries with time of electrophoresis. Analyses were conducted in DNA sequencing buffer (1 × TTE with 7 M urea, pH 8.4, 44°C) containing 0.1% w/v PHEA. Dashed line represents the EOF mobility in an uncoated silica capillary.

low levels for over 600 h of electrophoresis under these conditions. In real sequencing runs, the lifetime of the coating might be shorter due to the susceptibility of the polymer coating to fouling by impurities in DNA sequencing samples. Nonetheless, our results indicate that the PHEA coating, which is quite simple to prepare, has a long durability and extends the capillary lifetime, which would significantly contribute to the reduction of overall capillary cost.

3.5 DNA sequencing in PHEA matrices

First, the DNA separation performance of PHEA matrices was investigated in an LPA-coated capillary array on the MegaBACE 1000 . Tables 5a and b summarize the effect of polymer concentration and electric field strength on DNA sequencing read lengths, at 98.5% base-calling accuracy, and on the required run times, respectively. Depending upon the particular set of conditions, read lengths produced by PHEA matrices ranged from 445 to 750 bases, resolved within 1–5 h. The structurally similar linear poly-*N*-acryloylaminopropanol produced a maximum DNA read length of 385 bases [37]. A performance comparable to that of commercial DNA sequencing matrices is obtained using a 6% w/v PHEA matrix at

Table 5. Effect of PHEA concentration and electric field strength on DNA sequencing in terms of a) read length at 98.5% accuracy, b) run times, and c) DNA sequencing read length at resolution 0.59, as determined from crossover plots

a) Read length at 98.5% accuracy

	Field strength (V/cm)			
PHEA conc. (% w/v)	40	117	94	70
4	445	550	560	620
5	570	620	660	700
6	620	680	735	750

b) Run time (min)

	Field strength (V/cm)			
PHEA conc. (% w/v)	40	117	94	70
4	65	83	167	219
5	93	155	168	224
6	109	185	245	304

c) Read length at 0.59 resolution

	Field strength (V/cm)			
PHEA conc. (% w/v)	40	117	94	70
4	460	620	700	830
5	680	700	800	880
6	750	780	970	1070

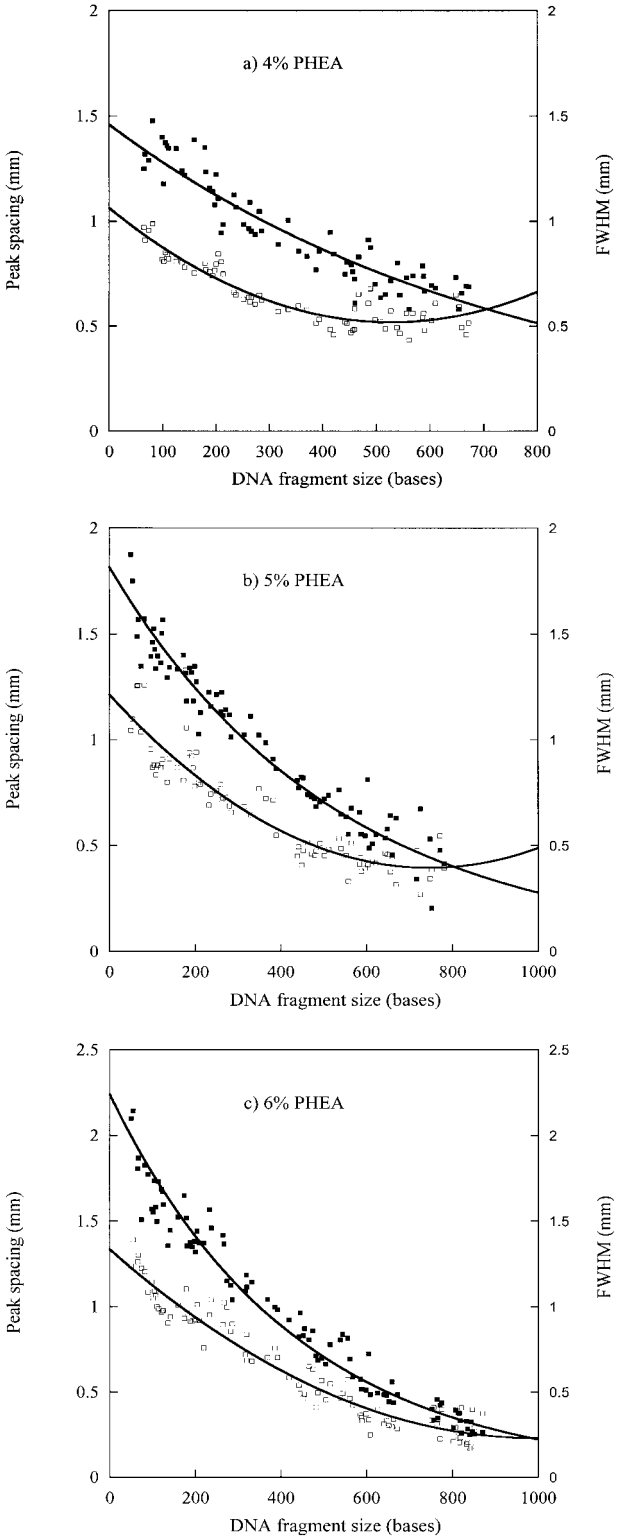


Figure 4. Crossover plot of (■) peak spacing and (□) FWHM *vs.* DNA fragment size for the T-track of the sequencing reaction products, separated at 94 V/cm, 44°C, 75 μm ID and 40 cm effective length LPA-coated capillary, using different PHEA matrix concentrations: (a) 4%, (b) 5%, and (c) 6% w/v.

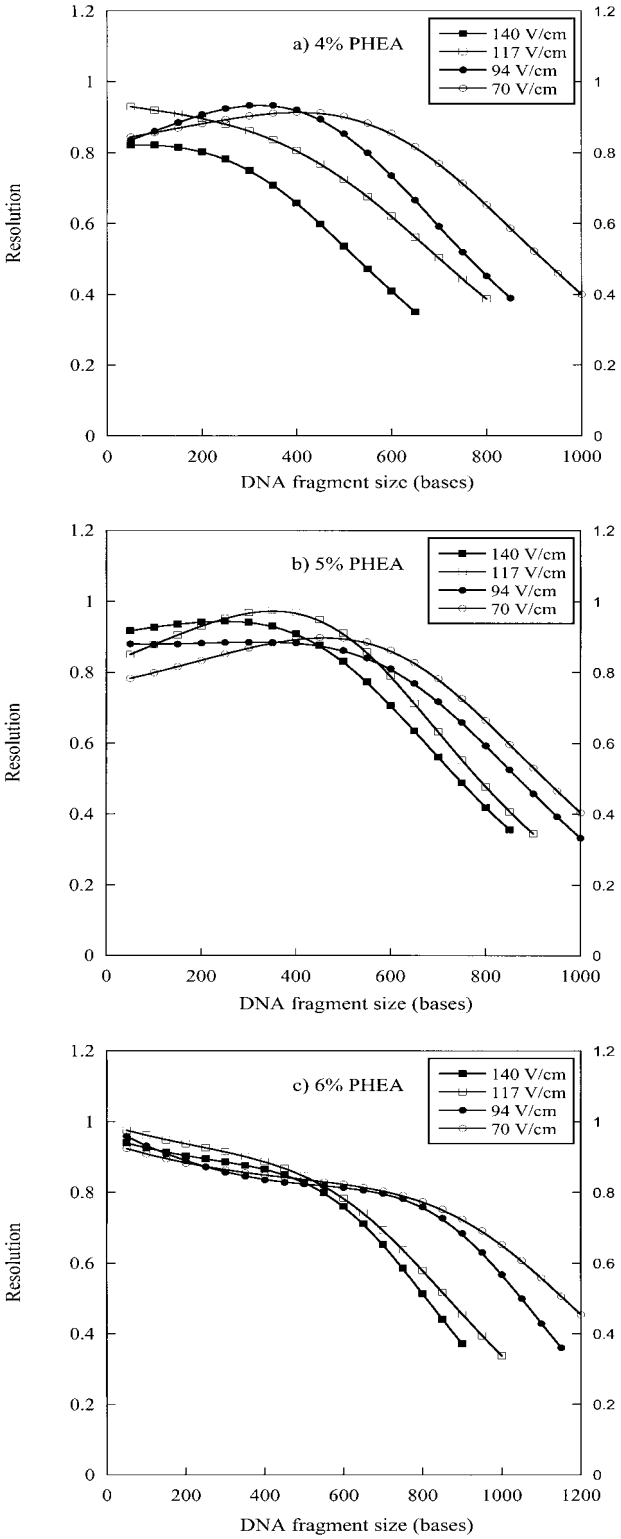


Figure 5. Effect of electric field strength, (■) 140, (□) 117, (●) 94 and (○) 70 V/cm, on resolution of DNA fragments obtained in different PHEA matrix concentrations including (a) 4%, (b) 5%, and (c) 6% w/v. Separations carried out in 75 μm ID and 40 cm effective length LPA-coated capillary at 44°C.

140 V/cm, producing 620 bases in less than 2 h. Under different sets of conditions, longer read lengths were obtained at the expense of longer run times. Table 5c shows the read lengths as estimated from crossover plots (*i.e.* plots of peak spacing and FWHM *vs.* DNA fragment size), which correspond to the DNA size at which peak spacing and FWHM are equal, a point at which the resolution equals 0.59. This is a practically useful resolution for current commercial base-calling softwares to accurately process DNA sequencing data.

Figures 4a–c show a representative set of crossover plots of the DNA sequencing runs, demonstrating the typically scattered data points and the fitted curves of peak spacing and FWHM plots used to estimate the read length. For a given PHEA matrix and set of electrophoretic conditions, we find that read lengths from crossover plots are much longer than those reported by the base-calling software. The base-calling program used for PHEA matrices investigated in this study was optimized for LPA matrices, so it is possible that the base-called read lengths are underestimated for the PHEA matrices. Thus, more DNA bases may be called accurately with base-calling software that is optimized and well-trained for PHEA matrices.

Over the ranges studied, increasing PHEA concentration and likewise, decreasing electric field strength, each has the effect of increasing read length and run time. The dependence of DNA resolution on fragment size at different applied field strengths is shown in Fig. 5. High values of resolution ($R > 0.8$) are maintained for smaller DNA fragments, then the resolution drops monotonically with increasing DNA size. Readable resolution (> 0.5) is maintained for longer DNA fragments with decreasing electric field strength and with increasing polymer concentration. Generally, good resolution of electrophoresing DNA molecules can be obtained as long as the DNA-polymer interactions responsible for the size-based separation of DNA molecules do not extensively disrupt the polymer-polymer entanglements and hence the polymer network [46]. An increase in polymer concentration increases the extent of polymer chain entanglements, accompanied by an in

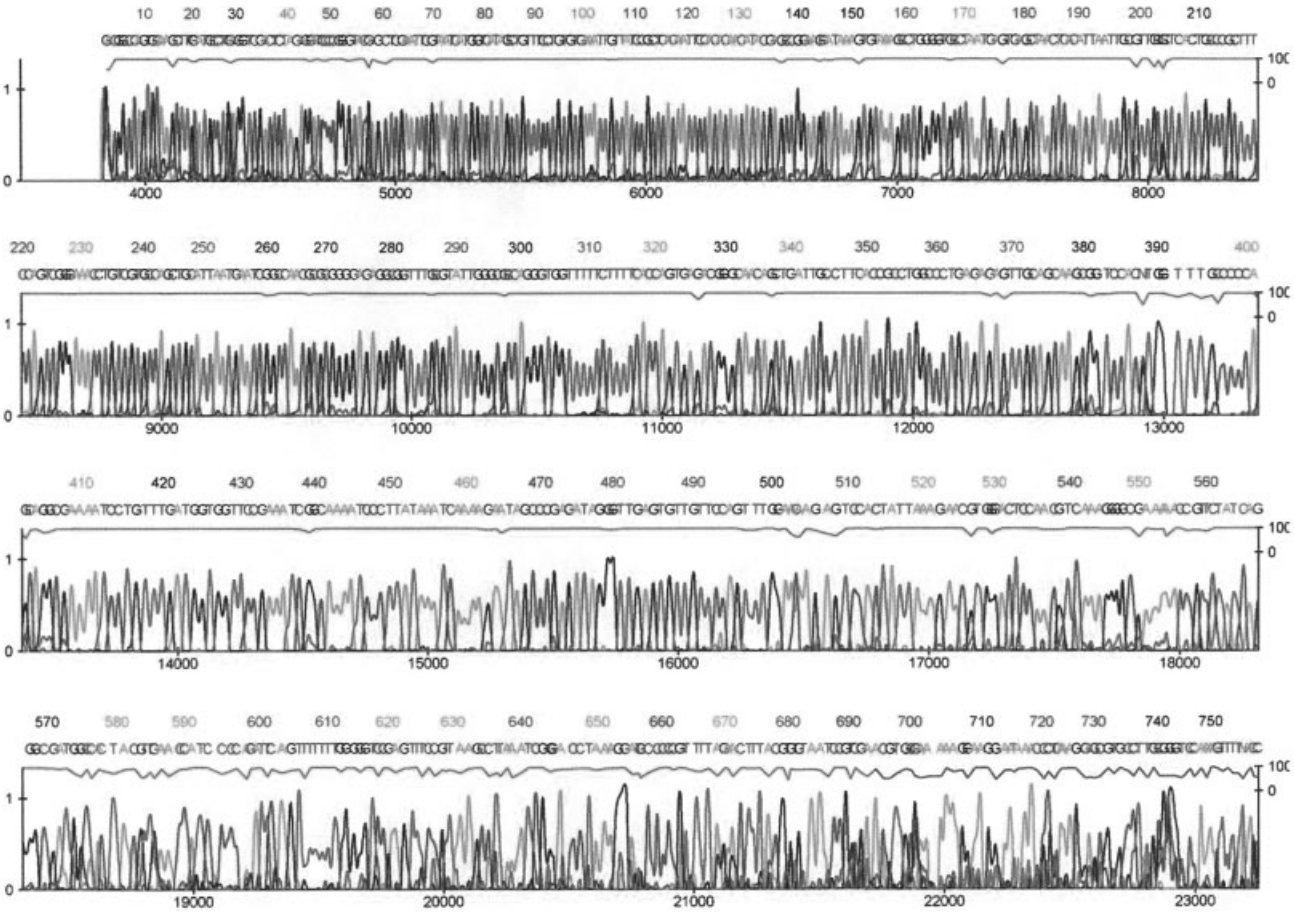


Figure 6. DNA sequencing electropherogram of M13 MegaBACE sequencing standards resolved in 6% w/v PHEA sequencing matrix at 94 V/cm, 44°C, 75 μm ID and 40 cm effective length capillary adsorptively coated with PHEA.

crease in polymer solution viscosity (Fig. 2), and hence an increase in the robustness of the polymer network during DNA migration. Moreover, increasing the polymer concentration increases the frequency of DNA-polymer interactions that lead to slower migration of DNA. The ability of a migrating DNA fragment to cause polymer chains to disengage from the entangled network is proportional to DNA fragment size and migration speed. Thus, the more concentrated and robust the polymer network, the larger the DNA fragment size that will disrupt the polymer matrix by dragging of the polymer chains, hence allowing good resolution of DNA fragments to be extended to longer DNA fragment sizes. An increase in electric field strength speeds up the migration of DNA fragments through the entangled polymer matrix. For a given DNA fragment size, the faster migration of the DNA fragment, the stronger the DNA-polymer interactions, and the easier it is for these interactions to disrupt polymer-polymer entanglements in the network. Thus, the faster the DNA migration, the smaller the DNA fragment size that will begin to adversely affect the robustness and integrity of the polymer network, and the sooner a loss of resolution will start to occur that limits the read length.

Figure 6 shows a DNA sequencing electropherogram obtained using 6% w/v PHEA solution both as a DNA sequencing matrix and as capillary coating in a bare fused-silica capillary. Under these conditions, 620 bases of DNA were resolved at 98.5% accuracy in about 3 h of electrophoresis time. For the same run, a crossover plot (Fig. 7) predicts that a longer read length (700 bases) should be achievable with a better-trained base-caller, highlighting good potential for improvement in PHEA sequencing performance through optimization of the base-calling software. Hence, it seems that further optimization of matrix formulation, run temperature and electrophoretic variables may improve the DNA sequencing performance of PHEA matrices to >700 bases per run.

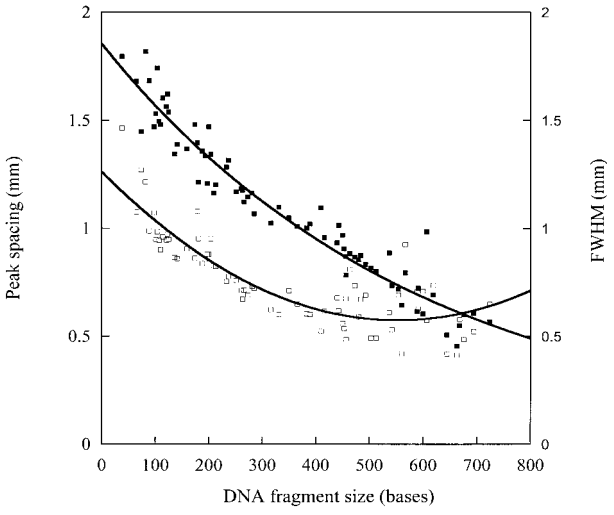


Figure 7. Crossover plot of (■) peak spacing and (□) FWHM *vs.* DNA fragment size for the T-track of the sequencing reaction products, obtained in PHEA matrix (conditions as specified in Fig. 6).

4 Concluding remarks

We have synthesized and characterized PHEA (polyDuramide) as a novel polymer that is useful for DNA separation by capillary electrophoresis. This new polymer offers a unique combination of two properties, hydrophilicity and adsorptive capillary-coating ability, that are important for improving separation efficiency and reducing capillary cost, respectively. We have demonstrated the suitability of PHEA matrices for DNA sequencing by electrophoresis in both coated and uncoated silica capillaries. Further improvement in the sequencing performance can be achieved by optimization of variables such as polymer molar mass distribution, separation temperature and base calling software. Ongoing research in our laboratory is investigating the potential use of PHEA for other types of biomolecular separations.

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