Capillary Electrophoretic Separation of DNA Restriction Fragments in Mixtures of Low- and High-Molecular-Weight Hydroxyethylcellulose

Alexander P. Bünz,[†] Annelise E. Barron,[†,‡] John M. Prausnitz,[§] and Harvey W. Blanch*[,†]

Department of Chemical Engineering and Chemical Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Previous studies (Barron *et al.*, 1993, 1994) have shown that dilute aqueous solutions of hydroxyethylcellulose (HEC) polymers provide an excellent alternative to gel-based DNA separation media for capillary electrophoresis (CE) of DNA restriction fragments (72−23 130 base pairs). DNA separation by CE in HEC solutions is strongly influenced by the average HEC molecular weight as well as by the HEC concentration in the electrophoresis buffer. Here we describe a systematic investigation of the effects of a mixture of low- and high-molecular weight HEC polymers, over a range of concentrations, to form a DNA separation medium with a broad range of polymer chain lengths. Our results show that, relative to separation media containing solely low-molecular-weight or high-molecular-weight HEC polymers, the mixed polymer solutions provide superior separation over the DNA size range of interest, while providing a five-fold viscosity reduction. The addition of a very small amount of high-molecular-weight HEC to a solution of low-molecular-weight HEC leads to a significant improvement in the separation of larger DNA fragments (>603 base pairs), while retaining resolution of smaller DNA fragments. The consequences of these results on the proposed mechanism of DNA separation in un-cross-linked polymer solutions are discussed.

Introduction

Increasing the rate of electrophoretic DNA separations is one important obstacle to be overcome in present international efforts to map and sequence the human genome within prescribed time and funding limits (Guyer and Collins, 1995). Capillary electrophoresis (CE) using un-cross-linked polymer solutions as a DNA separation medium in place of a gel is a relatively new technique which speeds DNA separations by 25-fold in comparison to conventional slab-gel electrophoresis. In this work, we report the use of nongel DNA separation media containing a mixture of low- and high-molecular-weight hydroxyethylcellulose polymers (HEC), which provide significantly improved DNA separations by CE in comparison to solutions containing just low-molecular-weight or just high-molecular-weight polymers.

Slab-gel electrophoresis is presently the most common technique for the separation, identification, and purification of DNA fragments. Agarose or cross-linked poly(acrylamide) gels are commonly used as support matrices for electrophoresis because their rigidity and highly porous structure allow passage of charged analytes, while reducing thermal convection of DNA samples. Furthermore, gels provide size-based separation of DNA molecules, which in free solution exhibit nearly equal electrophoretic mobilities regardless of their chain length (Lerman and Frisch, 1982). Traditional slab-gel electrophoresis, a simple, ubiquitous DNA separation technique, is unfortunately also relatively slow, nonquantitative, manually intensive, and difficult to automate.

There are many practical advantages to performing electrophoretic separations within microbore capillaries (inner diameter (i.d.) 25−100 μm). The technique of electrophoresis in tubes with submillimeter diameters was introduced in 1974 by Virtanen, who separated alkali cations in 200−500 μm i.d. glass tubes. The trend toward miniaturized electrophoresis channels was continued in 1979 when Mikkers *et al.* separated small charged molecules in 200 μm i.d. Teflon tubes. In 1981, Jorgenson and Lukacs were the first to illustrate the application of capillary electrophoresis to bioseparations, demonstrating extremely high resolution and efficiency for the electrophoretic separation of dansylated amino acids in a 75 μm i.d. glass capillary.

The unique transport properties of microbore capillaries make them eminently suitable for electrophoresis. The most significant limitation in slab-gel electrophoresis is Joule heating which results from current flow through the system. Joule heating induces temperature gradients which contribute to band broadening, limiting useful voltage gradients to approximately 5 V/cm for an agarose gel, 8 V/cm for a conventional poly(acrylamide) gel, and 40 V/cm for thin poly(acrylamide) DNA sequencing gels (Sambrook *et al.*, 1992). These limits may be overcome if electrophoresis is carried out in a microbore capillary. Today, a typical capillary used for DNA electrophoresis has a 50−100 μm i.d., fused silica walls about 150 μm thick, and a thin exterior coating (10 μm) of polyimide to provide flexibility. During electrophoresis, Joule heat is produced in the intracapillary volume (~1 μL), but the thick fused silica wall acts as an efficient heat sink, absorbing heat which is then rapidly dissipated from the relatively large surface area of the outer walls. The efficient dissipation of Joule

* Author to whom correspondence is addressed. Telephone: (510) 642-1387. Fax: (510) 642-4778. Email: blanch@cchem.berkeley.edu.

[†] Department of Chemical Engineering.

[‡] Present address: Department of Chemical Engineering, Northwestern University, Technological Institute, 2145 Sheridan Road, Evanston, IL 60208-3120. Telephone: (847) 491-7398. Fax: (847) 491-3728.

[§] Chemical Sciences Division, Lawrence Berkeley Laboratory.

S0888-5885(95)00699-3 CCC: $12.00

heat afforded by the high surface-area-to-volume ratio of a capillary virtually eliminates thermal and gravitational convection and allows electric fields of up to 700 V/cm to be used without significant band broadening, if the capillary is effectively thermostated during electrophoresis (MacGregor and Yeung, 1993). Field strengths of about 300 V/cm are more typically employed, resulting in DNA separations which are about 25 times faster than slab-gel electrophoresis. For these rapid separations at high voltages, efficiencies of at least 100 000 theoretical plates/m are routinely obtained, and with careful optimization, efficiencies as high as 30 million theoretical plates/meter have been achieved for an oligonucleotide separation in a poly(acrylamide) gel-filled capillary (Guttman *et al.*, 1990). Another advantage of CE is its adaptability to automatic, on-column sample loading as well as on-column detection.

In early applications, intracapillary, cross-linked poly(acrylamide) gels were used as DNA separation matrices for CE (Heiger *et al.*, 1990). However, cross-linked intracapillary gels are unstable under the high electric fields which are typically employed in CE and, furthermore, are limited by their structure to the separation of DNA molecules 2000 bp and smaller. However, it was discovered that effective DNA separation media can also be formed by un-cross-linked polymer solutions (Chin and Colburn, 1989; Zhu *et al.*, 1989). Polymer solutions have the advantages of being *replaceable* (when the viscosity is sufficiently low, the separation medium can be readily pumped into the capillary) and remain stable under high electric fields. Furthermore, depending upon polymer molecular weight and concentration, they permit DNA separations over a larger range of base pairs than cross-linked poly(acrylamide) gels. Hence, in recent years polymer solutions have replaced cross-linked gels as the DNA separation medium of choice for CE applications. Advances in the application of capillary electrophoresis to DNA separations were recently reviewed by Barron and Blanch (1995).

CE in polymer solutions provides rapid and efficient separation of DNA restriction fragments up to 23 130 base pairs (bp) in size (Strege and Lagu, 1991, Barron *et al.*, 1994). To date, researchers have used several types of water-soluble polymers in DNA separation media for CE, for example, methylcellulose (Strege and Lagu, 1991; Navin and Morris, 1995), hydroxyethylcellulose (Grossman and Soane, 1991; Barron et al., 1993, 1994, 1995), hydroxypropylcellulose (Baba *et al*., 1993; Mitnik *et al.*, 1995), hydroxypropylmethylcellulose (Kim and Morris, 1994; Figeys *et al.*, 1994), poly(vinyl alcohol) (Kleemiss *et al.*, 1993), liquefied agarose (Bocek and Chrambach, 1991; Klepárnik, *et al.*, 1993, 1995), and linear poly(acrylamide) (Heiger *et al.*, 1990; Maschke *et al.*, 1993; Figeys *et al.*, 1994; Gelfi *et al.*, 1995). Derivatized celluloses appear especially attractive because the low viscosities of cellulosic polymer solutions allow rapid DNA separations.

In this paper we investigate the use of mixed low- and high-molecular-weight HEC solutions as DNA separation media for CE. We will refer to the HEC solutions of interest as *dilute*, meaning that they are at concentrations which are well below their measured entanglement threshold concentrations. The *entanglement threshold concentration* of a polymer solution (Φ^*) is the concentration above which interaction between polymer chains begins to affect bulk solution properties, such as viscosity. Experimentally, Φ^* can be estimated from a log−log plot of solution viscosity *vs* polymer concentration (Barnes, 1989). In a dilute solution there are no strong interactions between solvated polymer molecules, and physical properties change in direct proportion to polymer concentration. Hence, the slope of the log viscosity *vs* log concentration plot is constant at approximately 1.0. If the polymer chains are of sufficient length, inter-chain entanglements can occur at higher concentrations. The formation of an incipient entangled polymer network in solution is evidenced by a significant increase in the slope of the log viscosity *vs* log concentration curve (Barnes, 1989). The entanglement threshold concentration is a unique property of a given polymer/solvent system, which is strongly dependent on the average molecular mass of the polymer sample. The length dependence of Φ^* may range from $N^{-0.8}$ for extremely flexible polymers (de Gennes, 1979) to $N^{-1.2}$ for stiff cellulose derivatives (where N is the number of monomers in the polymer chain) (Barron *et al.*, 1993).

Viovy and Duke (1993) have suggested that using the point of departure from linearity of the viscosity *vs* concentration curve is an ambiguous criterion for the determination of Φ^*, since it depends upon the accuracy with which the experimenter can evaluate the linearity of the data. Instead, they suggest using an equation derived from polymer physics (Broseta *et al.*, 1986) for the calculation of Φ^*:

$$\Phi^* \cong 3M_w/4\pi N_A R_p{}^3 \cong 0.6\eta^{-1} \qquad (1)$$

where M_w is the weight-average molecular weight of the polymer, N_A is Avogadro's number, R_p is the polymer's radius of gyration in solution, and η is the intrinsic viscosity of the polymer in the solvent of interest. The first part of eq 1 is a purely geometrical definition of the overlap threshold, equating Φ^* to the concentration at which the polymers, modeled as spherical coils, statistically touch in solution. Reliable determination of Φ^* by this equation requires accurate knowledge of R_p in various solvent conditions as well as knowledge of the weight-average molecular weight. The second part of eq 1 relies simply on knowledge of the intrinsic viscosity. This obviates the need to determine viscosity as a function of concentration, provided the intrinsic viscosity of the polymer sample in the solvent of interest is already known. However, it is not difficult or ambiguous to determine the point of departure from linearity of the viscosity *vs* concentration data; the viscosity increase upon entanglement is pronounced (see, for example, Barron *et al.*, 1993). In previous work we have used viscosity *vs* concentration data to determine the entanglement threshold concentrations of the HEC 27 000 and HEC 105 000 polymer samples in TBE buffer to be approximately 1.80% (w/w) and 0.37% (w/w), respectively (Barron *et al*., 1993).

Previous studies have shown that dilute, unentangled solutions (*e.g.*, 0.30% HEC, where $\Phi^* \approx 1.80\%$) of low-molecular-weight HEC (M_n 27 000) provide good separation of DNA fragments smaller than 603 bp (Barron *et al.*, 1993, 1994). However, this low-molecular-weight HEC fails to separate larger DNA fragments at any HEC concentration. On the other hand, high-molecular-weight HEC (M_n 105 000) at very dilute concentrations (*e.g.*, 0.025%, where $\Phi^* \approx 0.37\%$) separates large DNA fragments (603−23 130 bp) quite well but gives no resolution of smaller DNA molecules (Barron *et al.*, 1994). Although this high-molecular-weight HEC can be used at higher concentrations (*e.g.*, 0.25%) to provide

good resolution of small as well as large DNA fragments, the time required for loading these higher-viscosity solutions into a microbore capillary, using the 20-psi pressure gradient which is generally available on commercial CE instruments, becomes appreciable. The ideal separation media for CE would maximize the size range of DNA which can be separated, while minimizing the solution viscosity.

In the early 1990s it was believed that electrophoretic DNA separations by CE in polymer solutions could be described by prevailing models of DNA slab-gel electrophoresis (Grossman and Soane, 1991), for example, by the reptation model. Reptation theory for DNA slab-gel electrophoresis is premised on the idea that DNA fragments which are too large to fit through gel pores in a randomly-coiled conformation will migrate end-on, or "reptate", through "tubes" formed by the network of gel pores (Lumpkin and Zimm, 1982; Lumpkin *et al.*, 1985; Slater and Noolandi, 1985). With the hope of extending the applicability of this successful theory, it was proposed that DNA separation by CE in un-cross-linked polymer solutions also occurs by reptative DNA motion, the difference being that the "reptation tubes" were formed by a gellike, entangled polymer network (Grossman and Soane, 1991; Viovy and Duke, 1993; Duke and Viovy, 1994). This approach could be expected to have validity for the case of DNA electrophoresis through high-concentration, fully entangled polymer solutions.

However, experimental results have shown that, contrary to earlier predictions, size-based DNA separation does not occur only in entangled polymer solutions. Using HEC as a model polymer, Barron *et al.* (1994) showed that DNA separation can be accomplished in extremely dilute, unentangled polymer solutions, at polymer concentrations which are up to 2 orders of magnitude below the measured polymer entanglement threshold, or as low as 0.0006% HEC 105 000. In this dilute polymer concentration regime, pore-based models of electrophoretic DNA separation, such as the reptation model, clearly are not applicable. For DNA separations in dilute polymer solutions, Barron *et al.* postulated a new *transient entanglement coupling* separation mechanism based on DNA entanglement and subsequently dragging of uncharged HEC chains during electrophoresis (Barron *et al.*, 1993, 1994). The idea that DNA separation in dilute HEC solutions occurs by transient entanglement coupling mechanism was later supported by the videomicroscopy experiments of Shi *et al.* (1995). Hubert *et al.* (1996) developed a model based on the transient entanglement coupling concept which successfully describes the DNA electrophoresis data of Barron *et al.* which were obtained in short-chain HEC 27 000 solutions, but so far has failed to explain data which were obtained in the long-chain HEC 105 000 solutions.

In the present study, we have investigated the use of aqueous HEC solutions containing a mixture of different molecular weights for the electrophoretic separation of double-stranded DNA ranging from 72 to 23 130 bp in length, showing that this mixture retains the separation capability of both HEC components for the different DNA size ranges. These experiments employ dilute, unentangled polymer solutions. Fung and Yeung (1995) and Chang and Yeung (1995) have reported results for solutions of polymers of different chain lengths for the separation of DNA fragments. However, in these studies poly(ethylene oxide) was used at high concentrations to separate small DNA fragments only (<2200 bp). These workers found that the mixed-polymer matrix, containing equal amounts of poly(ethylene oxide) with different high and low molecular weights, provides comparable resolution, while retaining a significantly lower viscosity, compared to the single-polymer matrices used in their study.

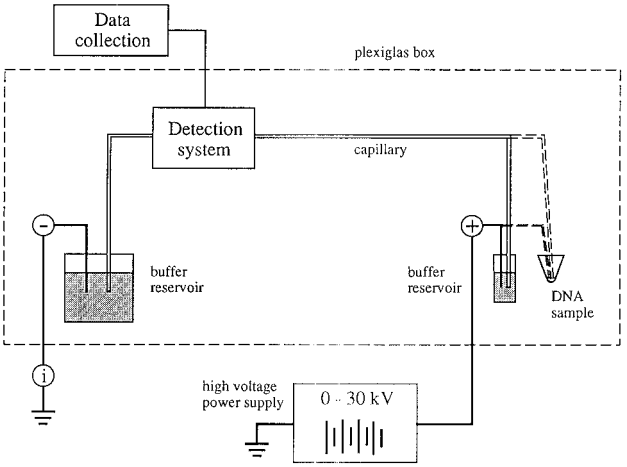

Figure 1. Schematic diagram of the capillary electrophoresis instrument.

Materials and Methods

CE Apparatus. Figure 1 shows a schematic diagram of the CE apparatus. The key feature is a fused-silica capillary with an external coating of polyimide (Polymicro Technologies, Phoenix, AZ) and no internal coating. The capillary has an overall length of 50 cm and a distance between injection end and detector window of 35 cm. The capillary inner diameter is 51, and the outer diameter is 360 μm. The capillary connects the anodic reservoir with the electrically grounded cathodic reservoir; both hold platinum wire electrodes. A high-voltage power supply with a 30 000-V capacity (Gamma High Voltage Research, Ormand Beach, CA) is used to drive electrophoresis. Current is measured over a 1-kΩ resistor in the return circuit of the power supply using a digital multimeter (Model 3465B, Hewlett-Packard, Palo Alto, CA). On-column detection is by UV absorbance at 260 nm using a modified variable-wavelength detector (Model 783, Applied Biosystems, Foster City, CA). Data were either collected using an integrator (Model 3390, Hewlett-Packard, Palo Alto, CA) or acquired and saved for further reduction by a 386 PC equipped with an analog input and digital output (I/O) board (DAS-800 Series board, Keithley Metrabyte, Taunton, MA) connected to the CE apparatus.

Viscometry. To measure the viscosities of the polymer solutions, an automated Ubbelohde-type capillary viscometer (Schott Geräte, Hofheim, Germany) was employed, controlled by a desktop PC and thermostated at 30 °C in a water bath (Model H-1 high temperature bath, Cannon Instrument Co., State College, PA).

Materials. A nonstoichiometric mixture of *λ-HindIII* and ΦX174-*HaeIII* restriction fragments (*λ-HindIII* fragments present at lower concentration) was obtained from Pharmacia LKB Biotechnology (Alameda, CA) at a total concentration of 500 μg/mL. This DNA sample was preheated for 5 min at 65 °C and then immediately placed on ice (the 4361 bp and 23 130 bp fragments in the digest have complementary termini). Mesityl oxide was used as a neutral marker in all electrophoresis runs

to measure electroosmotic velocity (Aldrich Chemical Co., Milwaukee, WI). The buffer used in all experiments was 89 mM tris(hydroxymethyl)aminomethane (Tris), 89 mM boric acid, and 5 mM ethylenediaminetetraacetic acid (EDTA) (TBE) with a pH of 8.15. All buffer reagents were purchased from Sigma Molecular Biology, St. Louis, MO.

Measured amounts of hydroxyethylcellulose (Polysciences, Inc., Warrington, PA) were added to buffer solutions; solutions were vigorously shaken and then mixed for 24 h by tumbling; magnetic stirring sometimes led to incomplete dissolution. Successive dilution was used to make extremely dilute solutions. All HEC concentrations are given on a (w/w) basis. Two different HEC samples were used with manufacturer-specified number-average molecular weights of $M_n \approx 27\,000$ and $M_n \approx 105\,000$ g mol^{-1}. (Hereafter these samples are referred to as HEC 27 000 and HEC 105 000, for brevity.)

Weight-average molecular weights of these two HEC samples were measured in our laboratory by low-angle laser light scattering (Barron, 1995) and were found to be $M_w \approx 139\,000$ and $M_w \approx 1\,315\,000$ g mol^{-1}, respectively. Hence, if one relies on the manufacturer-specified number-average molecular weights, these samples have respective polydispersity indices of $M_w/M_n \approx 5$ and 12, indicating that both samples already contain a very broad range of HEC molecular weights. This is typical for unfractionated cellulose polymers. Hence, our designation of these HEC samples as "low-molecular-weight" and "high-molecular-weight" must be understood to be relative and approximate. Clearly, with $M_w \approx 1\,315\,000$ g mol^{-1}, the so-called "HEC 105 000" polymer sample contains some very long HEC chains, which are not present at an appreciable concentration in the "HEC 27 000" sample having $M_w \approx 139\,000$ g mol^{-1}.

Experimental Procedures

The procedure for preparing each new capillary before its use for electrophoresis is given elsewhere (Barron *et al.*, 1993). Each time a new polymer solution was used, the uncoated inner capillary wall was rinsed first with 1 M NaOH for 10 min, then with 0.1 M NaOH for 10 min, with distilled, deionized water for 10 min, and finally with the electrophoresis buffer (containing dissolved HEC) for 20 min.

Samples were introduced to the anodic end of the capillary by applying a vacuum of 2−4 inHg for a time which depended on the buffer viscosity, to introduce approximately 3 nL of sample for each run. After the sample slug was drawn into the capillary, the anodic end of the capillary was placed back into the electrophoresis buffer, together with the anodic electrode, and the electrophoretic voltage was applied. All experiments were run at 13 282 V (∼265 V/cm for a 50 cm capillary). In all experiments, the capillary was surrounded by convected air at 30.0 ± 0.1 °C, to maintain a constant temperature.

Experiments were performed in uncoated capillaries by *countermigration capillary electrophoresis* (CMCE), a technique which exploits electroosmotic flow as a pump to push DNA analytes past the detector window (Chin and Colburn, 1989). CMCE is schematically diagrammed in Figure 2. During electrophoresis at steady state, a constant electroosmotic flow velocity through the capillary is obtained. The elution time of a neutral marker present in the sample (mesityl oxide)

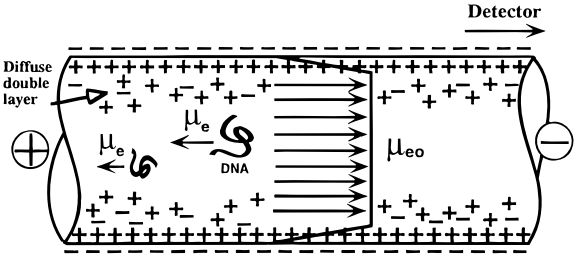

Figure 2. Schematic diagram of "countermigration capillary electrophoresis" for the separation of DNA molecules. DNA migrates by electrophoresis in the anodic direction, with electrophoretic mobility μ_e. Electroosmotic flow, engendered by the movement of the solvated, positively-charged ions in the diffuse double layer, moves with a greater velocity (μ_{eo}) in the cathodic direction, pulling the DNA toward a detector situated at the cathodic end of the capillary. Thus, in the presence of a polymer solution within the capillary (not shown), to separate the DNA based on its size, the largest, most slowly-migrating DNA molecules will be swept past the detecter first, followed by the smaller DNA in order of decreasing size. The thickness of the diffuse double layer is much exaggerated in the diagram.

is used to calculate the average electroosmotic mobility. The negatively-charged DNA would remain at the anodic end of the capillary (where it is injected) were it not drawn toward the UV absorbance detector and the cathode by strong electroosmotic flow. Thus, the largest DNA fragment, which has the smallest electrophoretic mobility in the direction of the anode, will pass the detector first, followed by the smaller ones in order of size, the smallest passing the detector last. The true DNA electrophoretic mobility is calculated by subtracting the electroosmotic velocity from the apparent DNA electrophoretic mobility.

It is also common practice to use capillaries that have their interior walls covalently coated with poly(acrylamide) to eliminate electroosmotic flow. In this case, a DNA sample plug must be introduced to the cathodic end of the capillary, and the negatively-charged DNA molecules will migrate toward the anode driven solely by the voltage gradient. In this operation mode, the smallest DNA fragment passes the detector first and the largest fragment passes the detector last. While the DNA peak separation is generally superior in uncoated capillaries, for the simple reason that electroosmotic flow increases the residence time of the DNA fragments (as demonstrated by Barron *et al.* (1995) for a wide range of HEC concentrations), peak efficiency is often superior in coated capillaries. In any case, the electrophoretic mobilities of the DNA fragments in a given polymer solution are the same whether coated or uncoated capillaries are used (Barron *et al.*, 1995).

Results and Discussion

Figure 3 shows DNA electrophoretic mobility as a function of HEC concentration, as measured for 17 DNA fragments ranging in size from 72 bp to 23 130 bp, in solutions of low-molecular-weight HEC (M_n 27 000 g mol^{-1}). The range in DNA electrophoretic mobilities at a given concentration indicates how well the DNA fragments were separated. Figure 4a illustrates DNA separation in high-molecular-weight HEC (M_n 105 000 g mol^{-1}), under the same electrophoresis conditions used in Figure 3. Note that the mobility scales in Figures 3 and 4a are the same, allowing direct comparison of the peak separation in solutions of the two polymers. A comparison of DNA mobilities in Figures 3 and 4a clearly reveals that much better peak separations for

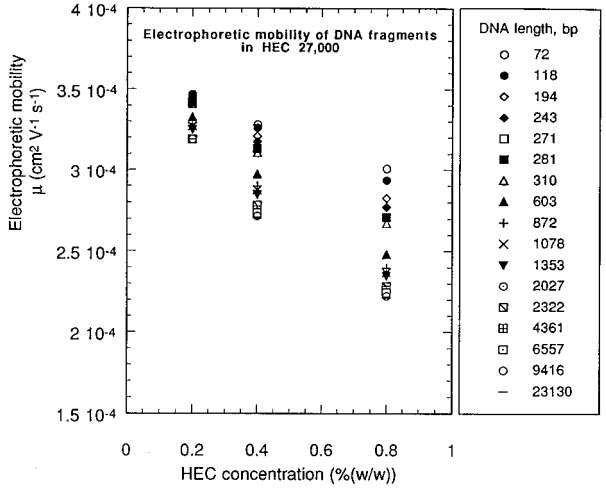

Figure 3. DNA electrophoretic mobility vs HEC concentration ($M_n \approx 27\,000$) for DNA restriction fragments ranging from 72 to 23130 bp at 30 °C. Data points are averaged from three to four individual determinations. Average run-to-run variation in calculated electrophoretic mobilities: ±0.33 %. DNA electrophoretic mobility was calculated by subtracting the electroosmotic mobility, calculated from the elution time of a neutral marker, from the apparent electrophoretic mobility of the DNA fragments, as DNA electrophoretic motion was opposite in direction to the electroosmotic flow used to drive it past the UV absorbance detector.

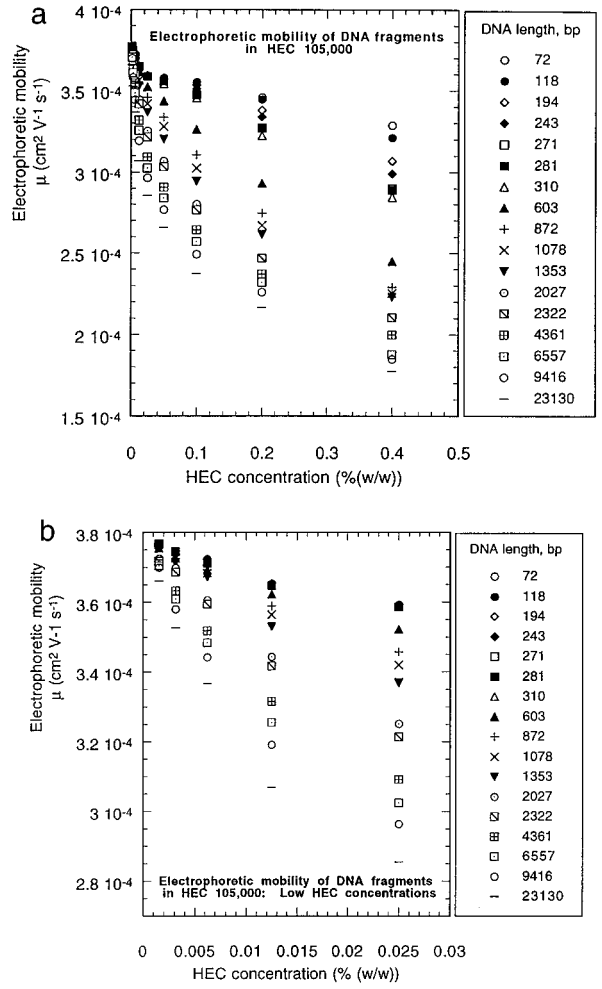

Figure 4. DNA electrophoretic mobility vs HEC concentration for DNA restriction fragments ranging from 72 to 23 130 bp. (a) HEC $M_n \approx 105\,000$. (b) The same data plotted on an expanded scale at low HEC concentrations.

large DNA fragments (larger than 603 bp) are achieved in solutions of the longer HEC polymers, most notably

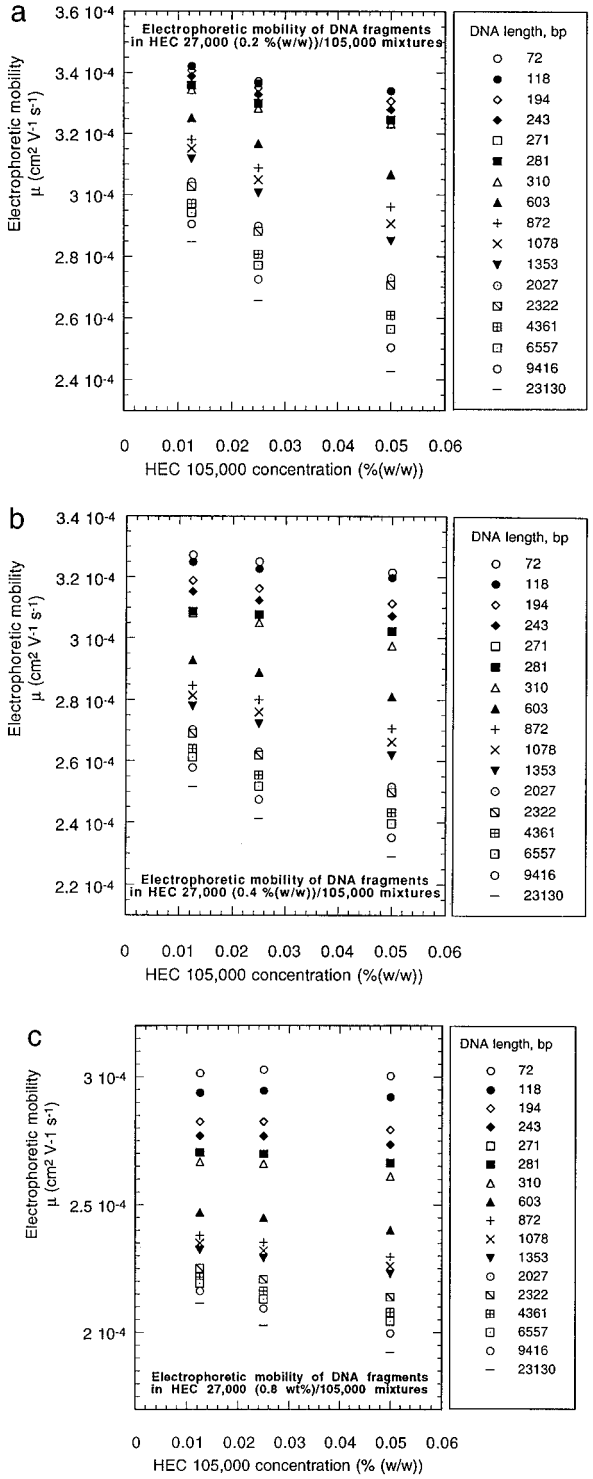

Figure 5. DNA electrophoretic mobility of DNA restriction fragments vs HEC 105 000 concentration for mixed-polymer solutions of HEC 27 000 and HEC 105 000. (a) HEC 27 000 concentration 0.2%. (b) HEC 27 000 concentration 0.4%. (c) HEC 27 000 concentration 0.8%. Data points are averaged from three to four individual determinations. Average run-to-run variation in calculated electrophoretic mobilities: (a) ±0.39%, (b) ±0.34%, (c) ±0.33%.

at low polymer concentrations. Figure 4b shows the dilute concentration regime for the HEC 105 000 sample. It can be seen that, in HEC 105 000 solutions, DNA fragments which are 2027 bp and greater in size can be separated even at 0.005% HEC 105 000. Resolution of DNA fragments which are 603 bp and larger is achievable at the low concentration of 0.01% HEC 105 000.

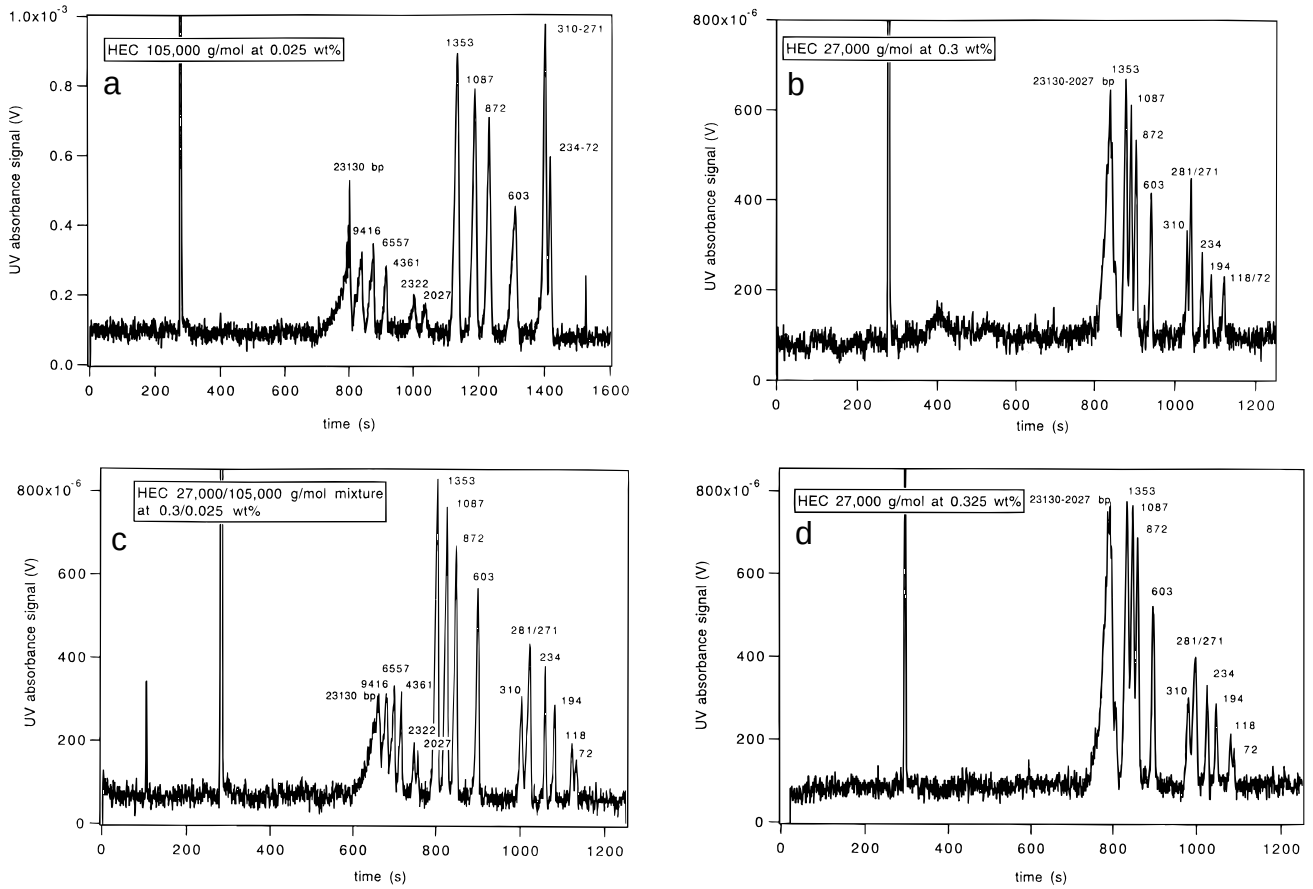

Figure 6. Separation by capillary electrophoresis of *λ-HindIII* and ΦX174-*HaeIII* restriction fragments (in a nonstoichiometric mixture). (a) In 0.025% HEC 105 000. The far left peak corresponds to a neutral marker (mesityl oxide), used to determine the velocity of electroosmotic flow in the capillary. (b) In 0.3% HEC 27 000. (c) In a mixed-polymer solution containing 0.3% HEC 27 000 and 0.025% HEC 105 000. (d) in 0.325% HEC 27 000.

In order to achieve comparable separation of small DNA fragments (less than 603 bp) in the two types of polymer solutions, it is necessary to use HEC 27 000 at a higher concentration than that required for HEC 105 000. This can be seen if one compares the range in electrophoretic mobilities (for DNA 72−310 bp), which is achieved in 0.80% HEC 27 000 (Figure 3), with that obtained at 0.40% HEC 105 000 (Figure 4a). Furthermore, although these two solutions give similar resolution of DNA fragments smaller than 603 bp, large DNA fragments (>603 bp) cannot be separated to baseline resolution using HEC 27 000, regardless of the HEC concentration. Dramatically better large-DNA separation is achieved in solutions of long-chain HEC, even at very dilute HEC 105 000 concentrations.

Parts a−c of Figure 5 show DNA electrophoretic mobilities which are achieved in aqueous solutions containing *mixtures* of HEC 27 000 and HEC 105 000. In each part the HEC 27 000 concentration was held constant at a given value (0.20%, 0.40%, and 0.80%, respectively), while the HEC 105 000 concentration was varied from 0.0125% to 0.05%. These parts illustrate the increase in DNA mobilities obtained as HEC 105 000 concentration is increased. In Figure 5a we see that the addition of a small percentage of high-molecular-weight HEC 105 000 to a dilute HEC 27 000 solution (0.2%) leads to a significant increase in resolution for the larger DNA fragments (>603 bp). An improvement in large-DNA separation is still seen, but to a much lesser extent, when one adds a small amount of HEC 105 000 to a 0.40% HEC 27 000 solution (Figure 5b). When HEC 105 000 is added to a 0.80% HEC 27 000 solutions only a small improvement in large-DNA separation is seen (Figure 5c). From these results it is clear that the greatest improvement in DNA separation can be achieved if long-chain HEC is added to relatively dilute solutions of short-chain HEC. If the short-chain HEC concentration is too high, the effect of the long HEC polymers on large-DNA separation is reduced, because interactions between long-chain HEC polymers and DNA fragments are reduced by the presence of numerous short-chain HEC polymers.

We also observe in Figures 4 and 5 that the presence of low-molecular-weight HEC 27 000, even at just 0.20%, dramatically improves small-DNA separation in dilute HEC 105 000 solutions. To see this, compare Figure 4b, showing no separation of DNA < 603 bp in HEC 105 000 solutions at concentrations lower than 0.025%, with parts a−c of Figure 5, in which good resolution of these smaller DNA fragments is observed.

To study further the separation properties of mixed HEC solutions, an extended concentration range for HEC 105 000 was investigated with the concentration of HEC 27 000 held constant at 0.30%. The electropherograms in parts a−d of Figure 6 exemplify, for selected polymer concentrations, the effect on DNA separation that is observed when HEC samples of different chain lengths are mixed, in comparison to the resolution achieved with solutions of a single polymer size distribution. Figure 6a shows that only DNA larger than 603 bp can be separated in a 0.025% HEC 105 000. On the other hand, Figure 6b shows that the HEC 27 000 solution at 0.30% will separate DNA fragments which are 1353 bp and smaller but gives absolutely no

separation of larger DNA. If one mixes these two polymer samples, at the same concentrations shown in parts a and b of Figure 6, the separation shown in Figure 6c is obtained. Strikingly, the mixed-polymer solution of 0.30% HEC 27 000/0.025% HEC 105 000 almost completely retains the resolution of both the small and large DNA fragments which was possible separately with the respective single-polymer solutions. Excellent large-DNA separation (DNA > 603 bp) is obtained, although only a small percentage of HEC 105 000 was added to the 0.30% HEC 27 000 solution. Furthermore, one observes that the large-DNA separation which is provided by a 0.025% HEC 105 000 solution is refined, *i.e.*, the peaks sharpened considerably, by the presence of the "sea" of HEC 27 000 polymers present at 0.30%.

The separation of small DNA which is obtained in the mixed-polymer solution is also superior to that which was achieved in 0.30% HEC 27 000 alone. In the mixed-polymer solution, the 72 and 118 bp fragments are separated almost to baseline, although they were not resolved in 0.30% HEC 27 000 alone. However, it appears that this is at least partly a simple result of the slightly higher overall polymer concentration (0.325% vs 0.30%): Figure 6d shows the separation of the 72 and 118 bp fragments in a 0.325% solution of HEC 27 000 polymers (no added HEC 105 000).

The type of excellent DNA separation which is obtained over a wide DNA size range in the mixed-polymer solution (Figure 6c) could also be achieved in a more concentrated (0.25%) HEC 105 000 solution, in the absence of any HEC 27 000 polymers (see Barron *et al.*, 1993). However, the viscosity of a 0.25% HEC 105 000 solution is about 6 cP, while the viscosity of the mixed-polymer solution which provides the separation shown in Figure 6c is only 1.35 cP. Hence, a comparable separation is achieved at roughly one-fifth the viscosity of a DNA separation medium employing long-chain polymers alone. The reduced viscosity of the mixed-polymer solution translates directly to a reduced capillary filling (and refilling) time.

In parts a and b of Figure 7, the electrophoretic mobilities of DNA in the mixed solutions containing both HEC 27 000 (at 0.30%) and HEC 105 000 (at concentrations ranging from 0.00156% to 0.40%) are plotted versus the concentration of added HEC 105 000. In Figure 7a, showing the entire HEC 105 000 concentration range, we see the substantial improvement in DNA separation which is achieved when the long-chain HEC is added to 0.30% HEC 27 000, indicating the greater effectiveness of long polymers in separating DNA restriction fragments. Figure 7b focuses just on the low-concentration regime (HEC 105 000 < 0.025%), with HEC 27 000 concentration held constant at 0.30%. There is very little improvement in small-DNA separation in this low-concentration regime but a significant improvement in large-DNA separation (DNA > 603 bp) is observed.

A comparison of the DNA electrophoretic mobility *vs* HEC 105 000 concentration plots which are given in Figure 4a (HEC 105 000 only) and Figure 7a (HEC 105 000 plus 0.30% HEC 27 000) indicates that DNA mobilities are significantly reduced when 0.30% HEC 27 000 is present. This is not surprising, since the mixed-polymer solutions have a higher overall polymer concentration (by 0.30% HEC 27 000) at any HEC 105 000 concentration, leading to lower DNA electrophoretic mobilities.

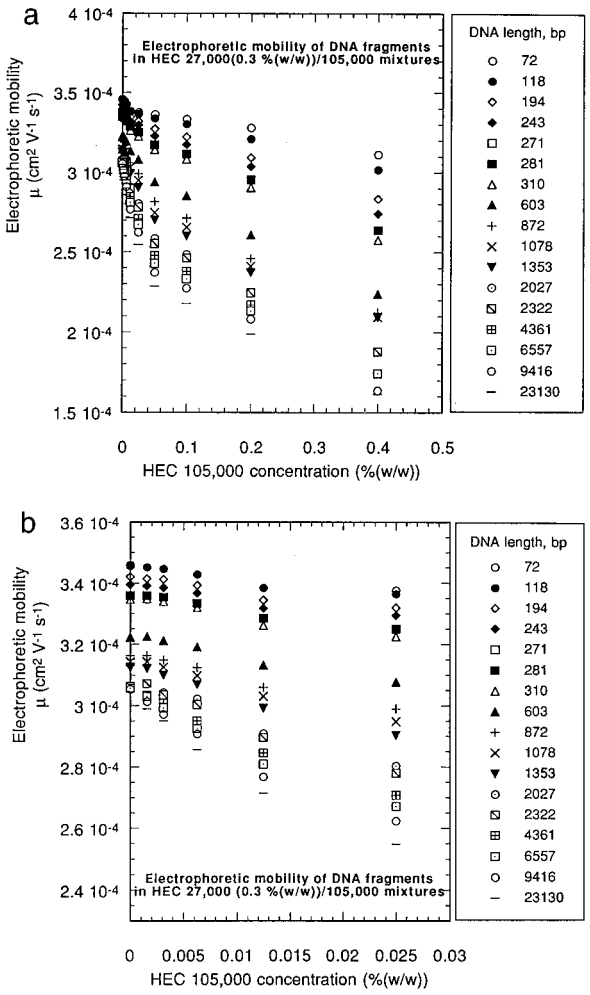

Figure 7. (a) DNA electrophoretic mobility of DNA restriction fragments vs HEC 105 000 concentration for mixed-polymer solutions of HEC 27 000 at 0.3% and HEC 105 000 at various concentrations. Data points are averaged from three to four individual determinations. Average run-to-run variation in calculated electrophoretic mobilities: ±0.58%. (b) The same data plotted on an expanded scale at the lowest HEC concentrations.

However, although all DNA electrophoretic mobilities are reduced in the mixed-polymer solutions (0.3% HEC 27 000/0.00156−0.4% HEC 105 000) compared to the single-polymer solution (0.00156−0.4% HEC 105 000 only), data analysis reveals that the *difference* in DNA electrophoretic mobilities ($\Delta\mu$; a quantity directly proportional to the resolution) shows a more complex behavior. Subtracting the mobility of the 23130 bp fragment from that of the 603 bp fragment gives a good estimate of the "envelope" of separation for large-DNA fragments and similarly for the 310 and 72 bp fragments. It is interesting to see that trends in $\Delta\mu$ *vs* concentration are different for small- and large-DNA fragments, as illustrated in Figure 8, which shows the overall difference in the electrophoretic mobilities of the relatively small (72 bp−310 bp) and large (603 bp−23 130 bp) base pairs as a function of HEC 105 000 concentration. Figure 8 shows that the addition of 0.30% HEC 27 000 polymers to HEC 105 000 solutions invariably improves the resolution of DNA fragments smaller than 310 bp. However, over most of the concentration range, the addition of a sea of small HEC 27 000 polymers has an adverse effect on the separation of DNA fragments larger than 603 bp. Only for HEC 105 000 concentrations lower than 0.006% does the addition of HEC 27 000 widen the envelope of separa-

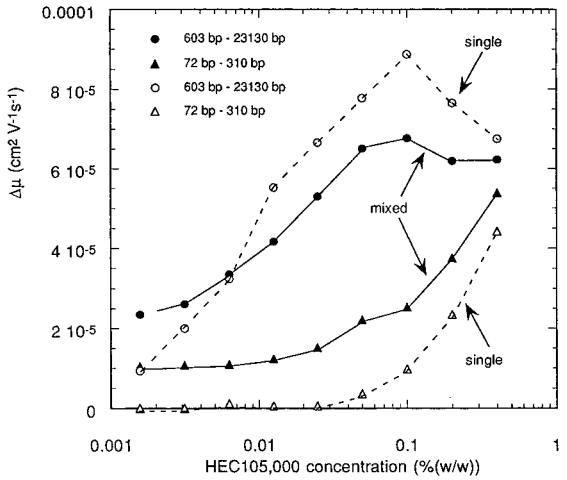

Figure 8. Difference in electrophoretic mobility of DNA restriction fragments for fragments 23 130−610 bp and 310−72 bp vs HEC 105 000 concentration for HEC 105 000 solutions and HEC 27 000 (0.3%)/HEC 105 000 mixtures. Lines are drawn to guide the eye.

tion for larger DNA. These results show that small HEC polymers do not contribute significantly to the separation of large DNA; only relatively long HEC chains can accomplish large-DNA separation. This observation is in contrast to that for separation of small DNA, which can be achieved by both short- and long-chain HEC, provided that they are present at sufficient concentrations.

Conclusions

In this study, we have demonstrated the advantages of mixed-polymer solutions, containing both low- and high-molecular-weight HEC polymers at specific concentrations, for the capillary electrophoretic separation of DNA restriction fragments ranging from 72 to 23 130 bp. One exemplary mixed-polymer solution (0.30% HEC 27 000/0.025% HEC 105 000) provides high-resolution electrophoretic separations, for a broader size range of DNA restriction fragments, at one-fifth the viscosity of a long-chain HEC solution providing the same separation at a higher overall concentration (0.25% HEC 105 000). Our data show that a carefully designed mixture of HEC of low and high average molecular weights permits both large- and small-DNA fragments to be well resolved in a single CE run, with a reduced capillary loading time resulting from the lower viscosity of these mixed-polymer separation media.

Acknowledgment

This work was supported by the National Center for Human Genome Research, NIH (HG01432). For financial support, J.M.P. is grateful to the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy. A.P.B. is grateful for a stipend of the scientific committee of NATO given to him by the Deutscher Akademische Austauschdienst (DAAD). A.E.B. gratefully acknowledges the support of a Dissertation Year Fellowship from the Graduate Division of the University of California, Berkeley.

Literature Cited

Baba, Y.; Ishimaru, N.; Samata, K.; Tsuhako, M. High-resolution separation of DNA restriction fragments by capillary electrophoresis in cellulose derivative solutions. *J. Chromatogr.* **1993,** *653,* 329.

Barnes, H. A.; Hutton, J. F.; Walters, K. *An Introduction to Rheology;* Elsevier Publishers: Amsterdam, The Netherlands, 1989; Chapter 6.

Barron, A. E. Capillary electrophoresis of DNA in un-cross-linked polymer solutions: An experimental and theoretical study. Ph.D. Dissertation, University of California, Berkeley, CA, 1995.

Barron, A. E.; Blanch, H. W. DNA separations by slab gel and capillary electrophoresis: Theory and practice. *Sep. Purif. Methods* **1995,** 24, 1.

Barron, A. E.; Soane, D. S.; Blanch, H. W. Capillary electrophoresis of DNA in un-cross-linked polymer solutions. *J. Chromatogr.* **1993,** *652,* 3.

Barron, A. E.; Blanch, H. W.; Soane, D. S. A transient entanglement coupling mechanism for DNA separation by capillary electrophoresis in ultradilute polymer solutions. *Electrophoresis* **1994,** *15,* 597.

Barron, A. E.; Sunada, W. M.; Blanch, H. W. The use of coated and uncoated capillaries for the electrophoretic separation of DNA in dilute polymer solutions. *Electrophoresis* **1995,** *16,* 64.

Bocek, P.; Chrambach, A. Capillary electrophoresis of DNA in agarose solutions at 40 °C. *Electrophoresis* **1991,** *12,* 1059.

Broseta, D.; Liebler, L.; Lapp, A.; Strazielle, C. Universal properties of semidilute polymer solutions: A comparison between experiments and theory. *Europhys. Lett.* **1986**, *2,* 733.

Chang, H.-T.; Yeung, E. S. Poly(ethylene oxide) for high resolution and high speed separation of DNA by capillary electrophoresis. *J. Chromatogr. B—Biomed. Appl.* **1995,** *669,* 113.

Chiari, M.; Nesi, M.; Righetti, P. G. Capillary zone electrophoresis of DNA fragments in a novel polymer network: Poly(N-acryloylaminoethoxyethanol). *Electrophoresis* **1994,** *15,* 616.

Chin, A. M.; Colburn, J. C. Counter-migration capillary electrophoresis (CMCE) in DNA restriction fragment analysis. *Am. Biotechnol. Lab.* **1989**, *7,* 16.

de Gennes, P.-G. *Scaling Concepts in Polymer Physics;* Cornell University Press: Ithaca, NY, 1979.

Duke, T.; Viovy, J. L. Theory of DNA electrophoresis in physical gels and entangled polymer solutions. *Phys. Rev. E* **1994**, *49,* 2408.

Figeys, D.; Arriaga, E.; Renborg, A.; Dovichi, N. J. Use of fluorescent intercalating dyes POPO-3, YOYO-3 and YOYO-1 for ultrasensitive detection of double-stranded DNA separated by capillary electrophoresis with hydroxypropylmethylcellulose and non-cross-linked poly(acrylamide). *J. Chromatogr. A* **1994**, *669,* 205.

Fung, E. N.; Yeung, E. S. High-speed DNA sequencing by using mixed poly(ethylene oxide) solutions in uncoated capillary columns. *Anal. Chem.* **1995**, *67* (13), 1913.

Gelfi, C.; Orsi, A.; Leoncini, F.; Righetti, P. G. Fluidified poly(acrylamide)s as molecular sieves in capillary zone electrophoresis of DNA fragments. *J. Chromatogr. A* **1995**, *689,* 97.

Grossman, P. D.; Soane, D. S. Experimental and theoretical studies of DNA separations by capillary electrophoresis in entangled polymer solutions. *Biopolymers* **1991,** *31,* 1221.

Guttman, A.; Cohen, A. S.; Heiger, D. N.; Karger, B. L. Analytical and micropreparative ultrahigh resolution of oligonucleotides by poly(acrylamide) gel high-performance capillary electrophoresis. *Anal. Chem.* **1990**, *62,* 137.

Guyer, M. S.; Collins, F. S. How is the Human Genome Project doing, and what have we learned so far? *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92,* 10841.

Heiger, D. N.; Cohen, A. S.; Karger, B. L. Separation of DNA restriction fragments by high performance capillary electrophoresis with low and zero cross-linked poly(acrylamide) using continuous and pulsed electric fields. *J. Chromatogr.* **1990,** *516,* 33.

Hubert, S. J.; Slater, G. W.; Viovy, J. L. Theory of capillary electrophoretic separation of DNA using ultra-dilute polymer solutions. *Macromolecules* **1996**, *29,* 1006.

Jorgenson, J. W., Lukacs, K. C. Free-zone electrophoresis in glass capillaries. *Clin. Chem.* **1981**, *27,* 1551.

Kim, Y.; Morris, M. D. Separation of nucleic acids by capillary electrophoresis in cellulose solutions with mono- and bis-intercalating dyes. *Anal. Chem.* **1994**, *66,* 1168.

Kleemiss, M. H.; Gilges, M.; Schomburg, G. Capillary electrophoresis of DNA restriction fragments with solutions of entangled polymers. *Electrophoresis* **1993**, *14,* 515.

Klepárnik, K.; Fanali, S.; Bocek, P. Selectivity of the separation of DNA fragments by capillary zone electrophoresis in low-melting-point agarose sol. *J. Chromatogr. A* **1993**, *638,* 283.

Klepárnik, K.; Malá, Z.; Doskar, J.; Rosypal, S.; Bocek, P. An improvement of restriction analysis of bacteriophage DNA using capillary electrophoresis in agarose solution. *Electrophoresis* **1995**, *16*, 366.

Lerman, L. S.; Frisch, H. L. Why does the electrophoretic mobility of DNA in gels vary with the length of the molecule? *Biopolymers* **1982,** *21*, 995.

Lumpkin, O. J.; Zimm, B. H. Mobility of DNA in gel electrophoresis. *Biopolymers* **1982**, *21*, 2315.

Lumpkin, O. J.; Déjardin, P., Zimm, B. H. Theory of electrophoresis of polyelectrolytes through gels. *J. Polym. Sci., Polym. Symp.* **1985**, *73*, 67.

MacGregor, D. A.; Yeung, E. S. Optimization of capillary electrophoretic separation of DNA fragments based on polymer-filled capillaries. *J. Chromatogr.* **1993,** *652*, 67.

Maschke, H. E.; Frenz, J.; Belenkii, A.; Karger, B. L.; Hancock, W. S. Ultrasensitive plasmid mapping by high performance capillary electrophoresis. *Electrophoresis* **1993**, *14*, 509.

Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, T. P. E. M. High-performance zone electrophoresis. *J. Chromatogr.* **1979**, *169*, 11.

Mitnik, L.; Salomé, L.; Viovy, J. L., Heller, C. Systematic study of field and concentration effects in capillary electrophoresis of DNA in polymer solutions. *J. Chromatogr. A* **1995**, *710*, 309.

Navin, M. J.; Morris, M. D. Capillary electrophoresis separation techniques and mechanisms in dilute polymer matrices. *J. Chin. Chem. Soc.* **1995**, *42*, 5.

Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual;* Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1992.

Shi, X.; Hammond, R. W.; Morris, M. D. DNA conformation dynamics in polymer solutions above and below the entanglement limit. *Anal. Chem.* **1995**, *34*, 1132.

Slater, G. W., Noolandi, J. On the reptation theory of gel electrophoresis. *Biopolymers* **1985**, *24*, 2181.

Smoluchowski, M. *Z. Phys. Chem.* **1918**, *92*, 129.

Strege, M.; Lagu, A. Separation of DNA restriction fragments by capillary electrophoresis using coated fused silica capillaries. *Anal. Chem.* **1991,** *63*, 1233.

Tietz, D.; Gottlieb, M. H.; Fawcett, J. S.; Chrambach, A. Electrophoresis on un-cross-linked poly(acrylamide): Molecular sieving and its potential applications. *Electrophoresis* **1986,** *7*, 217.

Viovy, J. L.; Duke, T. DNA electrophoresis in polymer solutions: Ogston sieving, reptation and constraint release. *Electrophoresis* **1993**, *14*, 322.

Virtanen, R. Zone electrophoresis in a narrow-bore tube employing potentiometric detection, *Acta Polytech. Scand., Chem. Incl. Metall. Ser.* **1974**, *123*, 1.

Zhu, M.; Hansen, D. L.; Burd, S.; Gannon, F. Factors affecting free zone electrophoresis and isoelectric focussing in capillary electrophoresis. *J. Chromatogr.* **1989**, *480*, 311.

Received for review November 20, 1995
Revised manuscript received April 8, 1996
Accepted April 9, 1996[⊗]

IE950699V

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1996.