Annelise E. Barron
Wade M. Sunada
Harvey W. Blanch

Department of Chemical
Engineering, University of
California, Berkeley, CA

The use of coated and uncoated capillaries for the electrophoretic separation of DNA in dilute polymer solutions

We show that both uncoated and polyacrylamide-coated capillaries provide separation of large DNA restriction fragments (2.0–23.1 kbp) by capillary electrophoresis in dilute cellulosic polymer solutions. Uncoated capillaries, however, provide significantly better resolution of DNA fragments, particularly when ultra-dilute polymer solutions are used. This is because electroosmotic flow in uncoated capillaries increases the residence time of DNA in the capillary, without significantly contributing to band-broadening. At a given field strength and polymer concentration in the buffer, the electrophoretic mobilities of DNA restriction fragments in coated capillaries are virtually identical to those previously measured in uncoated capillaries. It is concluded that the fused silica surface of the capillary does not play a significant role in the mechanism of DNA separation by capillary electrophoresis in uncrosslinked polymer solutions. Thus, the separation of large DNA which has been observed to occur in ultra-dilute polymer solutions arises primarily from entanglement interactions between the cellulosic polymers and DNA restriction fragments which occur within the bulk of the polymer solution.

1 Introduction

The technique of capillary electrophoresis (CE) is evolving rapidly as an increasing number of academic and industrial groups [1] employ it to provide bioseparations which are more rapid, efficient, and sensitive than traditional separation techniques. The use of CE to provide an increase in the rate of routine DNA restriction mapping and sequencing is a particularly important goal, in order to speed current international efforts to map the human genome. To effect DNA separations, microbore capillaries (50–100 μm ID) are filled either with crosslinked polyacrylamide gels [2–12] or with solutions of uncrosslinked polymers, usually linear polyacrylamide [12–31], or hydrophilic cellulose derivatives [22, 24, 30, 32–49]. Others have explored the use of different polymers, including glucomannan [50], galactomannan [38], pullulan [51], polyvinyl alcohol [22, 24, 38], polyethylene glycol [35, 38], polyethylene oxide [52–55], dextran [18], and liquified agarose [22, 56–59]. Chiari *et al.* [29, 60–62] have synthesized a novel acrylamide-based polymer for DNA separations, poly(*N*-acryloylaminoethoxyethanol), which in comparison to polyacrylamide exhibits increased hydrophilicity and greater resistance to hydrolysis at alkaline pH. Gel-filled capillaries can provide excellent size selection of DNA [12, 43, 63], but have limited lifetimes as a result of several difficulties which can arise during inter-capillary gel polymerization and/or during electrophoresis [3–5, 64–66]. The advantage of using polymer solution-filled capillaries, as opposed to gel-filled capillaries, is that polymer solutions (of reasonably low viscosity) can be forced out of the capillaries

under pressure and then replaced, allowing capillaries to be easily re-used. The importance of this advantage is clear if one considers proposals for future capillary electrophoresis DNA sequencers: they might employ an array of 100 or more capillaries in parallel [67–70]. Certainly, one would like to perform the delicate task of setting up and aligning this large array of fine, hair-like capillaries as seldom as possible.

If capillary electrophoresis is to be used for both fine and long-range DNA restriction mapping, its useful separation range should extend up to DNA of 1 Mbp and larger. Guszczynski *et al.* [27] have demonstrated the low-resolution capillary electrophoretic separation of DNA ranging from 10 kbp to 48.5 kbp in uncrosslinked polyacrylamide solutions. However, researchers who have attempted to separate large DNA restriction fragments by steady-field CE in polymer solutions have not yet shown a high-resolution separation of DNA fragments larger than 23.1 kbp (the largest fragment in the commonly used λHindIII restriction digest). Furthermore, severe asymmetry ("tailing") of the 23.1 kbp peak is usually seen, with progressively less tailing observed for smaller DNA peaks; this occurs whether uncoated or polyacrylamide-coated capillaries are used [36, 71–73]. The cause of this peak tailing for large DNA fragments has not been found; it does suggest, however, that a DNA size of 23.1 kbp may be near the upper limit which can be separated by this technique with acceptable resolution for restriction mapping. If it indeed happens that high-resolution steady-field CE in uncrosslinked polymer solutions is limited to DNA smaller than 25 kbp in size, it still has the potential to be a rapid, efficient technique for plasmid mapping [25]. Kim and Morris [74, 75] and Sudor and Novotny [76] have shown that DNA larger than 23 kbp can be separated in dilute polymer solutions upon the application of pulsed fields of a precise frequency. Sudor and Novotny separated DNA as large as 1 Mbp in 180 min in 0.4% (semi-dilute) linear polyacrylamide solution [76], while Kim and Morris have recently reported the separation of DNA as large as 1.6

Mbp in ultradilute (0.002%–0.004%) mixed hydroxyethyl cellulose/polyethylene oxide solutions, in only 12 min [74]. Given that slab gel separations of Mbp-sized DNA often require from 1 to 3 days of pulsed field electrophoresis, these are truly stunning experimental results.

In separating DNA restriction fragments, cellulosic polymer solutions at concentrations of 0.25–1.0% typically are employed, while linear polyacrylamide is used at much higher concentrations (3.0–10.0%T) to produce comparable resolution of DNA restriction fragments (compare Fig. 3 of [36], depicting the separation of a 1-kbp DNA ladder in a 0.5% methyl cellulose solution, to Fig. 1 of [77], depicting the separation of this DNA sample in 4.5%T polyacrylamide solution). It is not yet clear why much greater percentages of polyacrylamide are required for comparable resolution of DNA restriction fragments. Clearly, resolution depends strongly on the physical properties of the polymers; polyacrylamide is an extremely flexible polymer, and hydrophilic cellulose derivatives are typically quite stiff in comparison. What remains unclear is precisely how and why specific polymer properties such as molecular weight, flexibility, hydrophilicity, and polydispersity affect DNA separation. This cannot be fully understood until the mechanism of DNA separation in polymer solutions has been elucidated.

It is well known that in free solution, all DNA molecules > 8 bp in length will migrate at the same, size-independent electrophoretic mobility [78]. However, DNA of different sizes can be separated if electrophoresis is performed in gels or in polymer solutions [79, 80]. The mechanism of DNA separation in uncrosslinked polymer solutions, dilute or concentrated, has not been clearly defined. In agarose or crosslinked polyacrylamide gels, DNA separation is postulated to occur either by Ogston-type sieving [81–84] or by reptation [78, 85], depending on DNA size, field strength, and gel concentration. The Ogston theory [81–84] models DNA as a nondeformable spherical particle, moving through a network of infinitely long, straight gel fibers which form "pores" of some average pore size and pore size distribution. A spherically coiled DNA molecule must diffuse laterally until it finds a pore of sufficient diameter to permit its passage; larger DNA molecules must search for a longer time to find accommodating pores and hence are progressively "sieved" from the smaller ones. On the other hand, the reptation model [78, 85] describes DNA as a snake-like molecule migrating through "tubes" formed by the pores of the gel. Here, the DNA size-dependent friction engendered by diffusion within a solvent-filled tube is postulated to give DNA molecules electrophoretic mobilities which are inversely proportional to their size.

Most researchers interpret their electrophoresis data with the assumption that the mechanism of DNA separation in uncrosslinked polymer solutions is basically the same as that postulated for DNA separation in polyacrylamide or agarose gels [26, 27, 43, 61], an approach that was first proposed by Grossman and Soane [34]. Thus, the trend has been to qualitatively employ pore-based models to interpret capillary electrophoresis data for DNA separation in uncrosslinked polymer solutions. It is assumed that DNA separation occurs because the polymers have formed an entangled network in solution; such a network is often termed a "physical" gel, as opposed to a crosslinked or "chemical" gel [86]. This entangled polymer solution is further assumed to contain "transient pores", similar to the pores of an agarose or polyacrylamide gel. The average size of the transient pores within a polymer solution is thought to determine the range of DNA lengths which can be separated, this average transient pore size having the same implications as the "pore size" of a true gel [34, 87–90]. Viovy and Duke [86, 91] have, for the case of low electric fields and completely entangled polymer solutions, attempted to account for the dynamic nature of the uncrosslinked polymer network, modifying a version of the biased reptation model which includes tube length fluctuations to also include constraint release. Constraint release is postulated to occur when the polymers which form the tube are allowed to reptate away, changing the tube dimensions even while the DNA remains within it.

However, our results [41, 71] suggest that the Ogston and reptation theories for the mechanism of DNA separation in gels may not be sufficient to explain DNA separation in uncrosslinked polymer solutions. We have shown that large DNA (2.0–23.1 kbp) can be separated by CE in polymer solutions which are ultradilute (up to two orders of magnitude in concentration below the entanglement threshold concentration), under a high, steady field (265 V/cm), in uncoated capillaries [71]. When a polymer solution is far below the entanglement threshold concentration, the polymers do not form a physical network and are relatively isolated in solution. Thus, a dilute and unentangled polymer solution cannot be envisioned to form "pores", transient or otherwise. According to both the Ogston and reptation models, no DNA separation should be possible in dilute polymer solutions. To explain our experimental results, we must assume that another mechanism of separation is operative, at the very least in dilute polymer solutions, and possibly in more concentrated polymer solutions as well. We have suggested [41, 71] that this additional mechanism of separation may involve transient entanglement coupling of electrophoretically migrating DNA molecules with the uncharged polymers in the buffer, forcing the DNA to drag and dislocate the polymers. The additional drag forces experienced by DNA molecules due to these transient entanglement interactions could provide DNA separation since they may not scale linearly with DNA size.

Previous studies illustrating DNA separation in dilute polymer solutions [41, 71] have employed uncoated capillaries only. It is relatively rare for capillary electrophoresis researchers to use uncoated capillaries for DNA separations, since it is generally believed that coated capillaries (which have their interior walls covalently coated with polyacrylamide to eliminate electroosmotic flow) give superior performance [24, 36, 92, 93]. Since there have been no other reports of dilute-polymer solution separations, we considered the possibility that the fused silica wall of the uncoated capillaries might play some role in the separation. This question arose even though

any interaction between the DNA and the fused silica may seem unlikely, given that the negatively charged phosphate groups in the DNA molecules should cause them to be repulsed from the negatively-charged fused silica wall. To investigate the importance of the condition of the capillary wall, we compared DNA separations carried out in polyacrylamide-coated and uncoated capillaries. As a result of these studies, we show that at a given polymer concentration, coated capillaries give precisely the same extent and type of DNA separation at a given field strength as that which we have previously observed in uncoated capillaries.

2 Materials and methods

2.1 Instrumentation

The capillary electrophoresis apparatus employed in these studies is described in detail elsewhere [34]. The apparatus employs a single fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) 50 cm in length (35 cm to the detector), with 51 μm ID and 360 μm OD, either uncoated or internally coated with a covalently attached layer of crosslinked polyacrylamide. The capillary connects the anodic reservoir with the electrically grounded cathodic reservoir. A high-voltage power supply with a 30 000 V capacity (Gamma High Voltage Research, Ormand Beach, CA, USA) was used to drive electrophoresis. Current was 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, USA). On-column detection was by UV absorbance at 260 nm, using a modified variable-wavelength detector (Model 783, Applied Biosystems, Foster City, CA, USA). Data were collected using an integrator (Model 3390, Hewlett-Packard).

2.2 Materials

A non-stoichiometric mixture of λ-*Hin*dIII and ΦX174-*Hae*III restriction fragments (λ-*Hin*dIII fragments are present at a lower concentration, so that peaks of the larger DNA fragments in this digest will not dwarf those of the ΦX174-*Hae*III digest) was obtained from Pharmacia LKB Biotechnology (Alameda, CA, USA) at a concentration of 500 μg/mL. In experiments with uncoated capillaries, the time it took a neutral species, mesityl oxide (Aldrich Chemical Co., Milwaukee, WI, USA), to move from the entrance of the capillary to the detection window was used to calculate the neutral marker's average velocity, assumed to be the electroosmotic velocity. The buffer used in all experiments with DNA was 89 mM Tris, 89 mM borate, and 5 mM EDTA, with a pH of 8.15 (all buffer reagents purchased from Sigma Molecular Biology, St. Louis, MO, USA). Measured amounts of hydroxyethyl cellulose (HEC) were added to prefiltered buffer solutions, which were vigorously shaken, and then mixed for 24 h by tumbling (mechanical stirring sometimes led to incomplete dissolution). Successive dilution was used to make extremely dilute solutions. The HEC sample used has a manufacturer-reported number-average molecular mass of $M_n \cong$

90 000—105 000 g/mol, and was obtained from Polysciences, Inc. (Warrington, PA, USA).

2.3 CE

DNA samples containing λ-*Hin*dIII and ΦX174-*Hae*III restriction fragments were pre-heated for 5 min at 65 °C and then stored on ice (λ-*Hin*dIII restriction fragments of 4361 bp and 23 130 bp have cohesive termini). All DNA samples were premixed with a minute amount of mesityl oxide, and injected without dilution. In the electropherograms, peaks were identified by integration of peak areas. A representative plot of peak area as a function of the number of DNA base pairs can be found in [41].

2.3.1 Uncoated capillaries

Each new, uncoated capillary was treated with 1 M NaOH for 3 h and then rinsed with water before use, to etch the fused silica surface clean of adsorbed impurities. Thereafter, before each filling of the capillary with a given polymer solution, the uncoated inner capillary wall was conditioned first with 1 M NaOH for 10 min, then with 0.1 M NaOH for 10 min, and finally with the electrophoresis buffer (containing dissolved HEC) for 15 min. Samples were introduced to the anodic end of the capillary by applying a vacuum of 1–3 in Hg (3386—10159 Pa) for a specific time which depended on the buffer viscosity, to introduce approximately 3 nL (3×10^{-6} cm³) of sample for each run. After the sample was drawn into the capillary, the anodic end of the capillary was replaced in the electrophoresis buffer, together with the anodic electrode, and the electrophoretic voltage of 265 V/cm was applied. The uncoated capillary was enclosed in a plexiglass box and surrounded by convected air at a temperature of 30.0 ± 0.1 °C during all experiments. Absolute electrophoretic mobilities are calculated by subtracting the electroosmotic mobility (determined from the elution time of the neutral marker) from the apparent electrophoretic mobility of the DNA band, since the electroosmotic flow and the DNA move in opposite directions; a mathematical description of this calculation can be found elsewhere [94]. Note that the electroosmosis of the buffer is a bulk flow having a flat velocity profile [95], which exerts an equal force on all DNA molecules in the sample (*i.e.*, the electroosmotic velocity of the DNA is not dependent on molecular size). This is demonstrated by the fact that when electrophoresis is performed in free solution, in the absence of polymers in the buffer, all DNA molecules migrate at the same velocity and elute as a single peak. For an informative discussion of the electroosmotic flow of polymer solutions in fused silica capillaries, see the recent paper on this subject by Bello *et al.* [96].

2.3.2 Coated capillaries

Polyacrylamide-coated capillaries were produced according to the method of Hjertén [92]. When coated capillaries were used, DNA was injected *electrokinetically* at the cathodic end of the capillary by placing both the

capillary and the platinum wire electrode into the DNA sample, and applying a voltage of 265 V/cm for 3—4 s. The capillary and cathodic electrode were then placed in a vial containing the electrophoresis buffer, and the electrophoretic voltage of 265 V/cm was applied. In this study, we will be interested in comparing DNA electrophoretic mobilities measured in both uncoated and coated capillaries. When mobilities are to be directly compared, the issue of temperature is very important. With our CE apparatus, we are only able to perform controlled hydrodynamic injection at the anodic tip of the capillary; hence, for coated capillaries, we had to use electrokinetic injection. This could only be done if the plexiglass box enclosing the capillary was opened. To provide a temperature-controlled enclosure for coated capillaries which would also allow convenient electrokinetic injection would require extensive rebuilding of the CE apparatus. Rather than do this, we chose to perform coated-capillary DNA separations at room temperature (which we monitored), and to correct the electrophoretic mobilities in the coated and uncoated capillaries to a common temperature later (see Section 3).

3 Results and discussion

3.1 Separations in polymer solution

In a recent paper [71], we showed the separation of mixed λ-*Hin*dIII and ΦX174-*Hae*III restriction frag-ments, using dilute polymer solutions in uncoated capillaries. The polymer used for the separation matrix was HEC with a number-average molecular mass of 90 000—105 000 g/mol. We will reproduce three of those electropherograms here, for comparison to electropherograms obtained using solutions of the same HEC polymers and under identical conditions, but in coated capillaries. In general, we find that at a given polymer concentration, field strength and capillary length, dramatically superior resolution of DNA fragments is achieved in uncoated capillaries than in coated capillaries, particularly when the polymer solutions are dilute. For example, Fig. 1a shows the separation of λ*Hin*dIII and ΦX174-*Hae*III restriction fragments in a coated capillary containing 0.15% w/w HEC at a field strength of 265 V/cm; Fig. 1b shows the same separation in an uncoated capillary. The resolution of all DNA fragments is significantly better in the uncoated capillary, simply because the electroosmotic flow increases the DNA residence time in the capillary. (The DNA is "swimming upstream" against the electroosmotic flow which pulls it toward the detector). This effect permits the partial resolution of fragments differing by 10 bp in length (the 271/281 bp fragments), whereas in the coated capillary these fragments are not resolved (Fig. 1a). The entanglement threshold concentration of this particular HEC sample is approximately 0.37% w/w, as determined from viscosity *vs.* concentration data in previous work [41]. Thus, at a concentration of 0.15% w/w the polymers do not form a completely entangled network in solution, and DNA separation
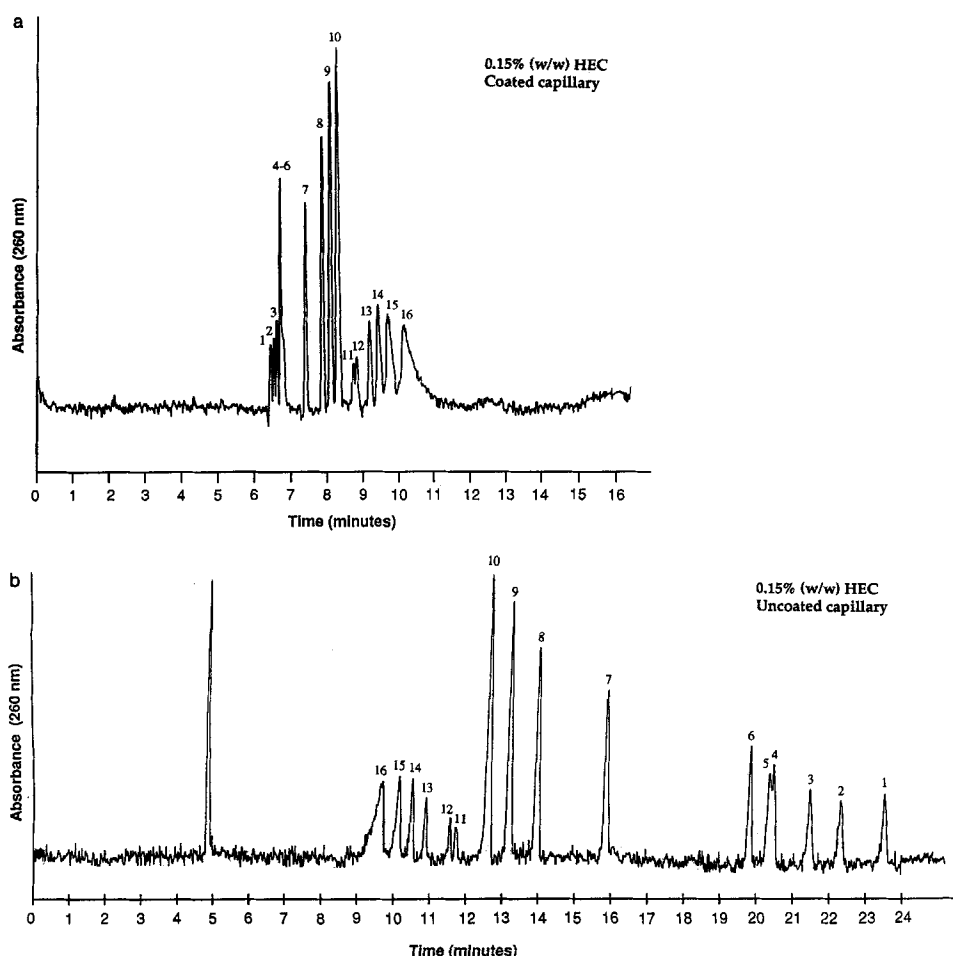

Figure 1. Separation by CE of λ-*Hin*dIII and ΦX174-*Hae*III restriction fragments (in nonstoichiometric mixture) in a solution of 0.15% w/w HEC in TBE buffer (a) in a polyacrylamide-coated capillary, and (b) in an uncoated capillary with mesityl oxide as a neutral marker (appearing as the far left peak). Peak identification: (1) 72 + 118 bp, (2) 194 bp, (3) 234 bp, (4) 271 bp, (5) 281 bp, (6) 310 bp, (7) 603 bp, (8) 872 bp, (9) 1078 bp, (10) 1353 bp, (11) 2027 bp, (12) 2322 bp, (13) 4361 bp, (14) 6557 bp, (15) 9416 bp, (16) 23130 bp. λ-*Hin*dIII restriction fragments of 125 bp and 564 bp are present in such small concentrations that they are not observed. Buffer: 78 mM Tris, 89 mM boric acid, 5 mM EDTA, pH 8.15. Capillaries: 51 µm ID, 50 cm total length (35 cm to detector). Detection was by UV absorbance at 260 nm. The field strength was 265 V/cm. The current was (a) 7.3 µA, and (b) 8.5 µA. For (a), electrokinetic injection was used and the average standard deviation in migration times was 1.57%, *n* = 3. For (b), pressure injection was used and the average standard deviation in absolute electrophoretic mobilities was 0.32%, *n* = 3.

occurs nonetheless. Furthermore, the solution viscosity is only 3.4 cP, allowing for easy filling of the capillary.

3.2 Dilute polymer solutions

More dramatic evidence for dilute polymer solution DNA separations is obtained in solutions with concentrations that are orders of magnitude lower than the entanglement threshold. Figures 2a and b show a comparison between results in coated and uncoated capillaries, in an HEC solution with a concentration of 0.025% w/w, which is 15 times more dilute than the measured entanglement threshold of 0.37% w/w. The viscosity of this solution is only 1.1 cP. Once again, the electroosmotic flow dramatically improves the resolution of the DNA peaks in this dilute polymer solution. While small DNA fragments are not resolved at this concentration, base-line resolution is obtained for all peaks larger than 310 bp.

Figures 3a and b depict the electrophoresis of the λ-HindIII/ΦX174-HaeIII mixture in coated and uncoated capillaries, respectively, in an HEC solution at the extremely low concentration of 0.00125% w/w — just 12 parts per million HEC in TBE. This solution is 300 times more dilute than the concentration required for the formation of an entangled polymer network, and has a viscosity of just 0.94 cP. Yet the DNA restriction fragments of 2.3 kbp, 4.6 kbp, 9.4 kbp, and 23.1 kbp are well-resolved from each other within 17 min in an uncoated capillary (Fig. 3b). However, in a coated capillary filled with the same ultradilute polymer solution, DNA residence time in the capillary is short (about 6 min) because the DNA mobilities are close to their free-solution value, and observable DNA separation is limited to two (reproducible) shoulders on the one large peak. We note that even in the uncoated capillary, much better resolution of these large DNA fragments can be obtained in more concentrated HEC solutions (we found in earlier work that the optimum HEC concentration
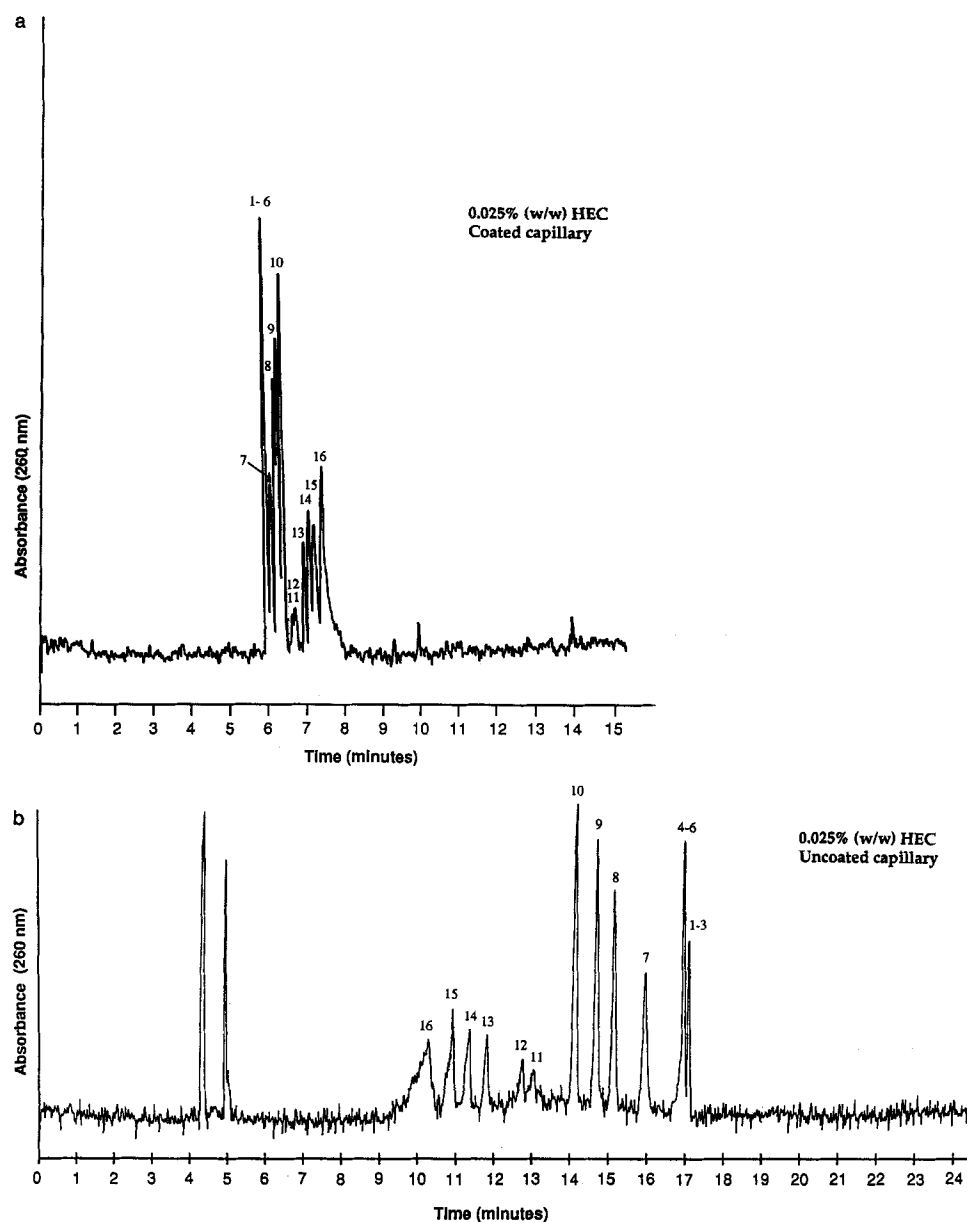

Figure 2. Separation by CE of λ-HindIII and ΦX174-HaeIII restriction fragments (in nonstoichiometric mixture) in a solution of 0.025% w/w HEC in TBE buffer in (a) a polyacrylamide-coated capillary, and (b) an uncoated capillary with mesityl oxide as a neutral marker (appearing as the far left peak; second peak from left is an impurity present in DNA sample). Peak identification: (1) 72 + 118 bp, (2) 194 bp, (3) 234 bp, (4) 271 bp, (5) 281 bp, (6) 310 bp, (7) 603 bp, (8) 872 bp, (9) 1078 bp, (10) 1353 bp, (11) 2027 bp, (12) 2322 bp, (13) 4361 bp, (14) 6557 bp, (15) 9416 bp, (16) 23130 bp. λ-HindIII restriction fragments of 125 bp and 564 bp are present in such small concentrations that they are not observed. Buffer, capillary dimensions, and detection methods, same as Fig. 1. The field strength was 265 V/cm. The current was (a) 7.0 μA, and (b) 7.2 μA. For (a), electrokinetic injection was used and the average standard deviation in migration times was 1.39%, *n* = 3. For (b), pressure injection was used and the average standard deviation in absolute electrophoretic mobilities was 0.41%, *n* = 5.
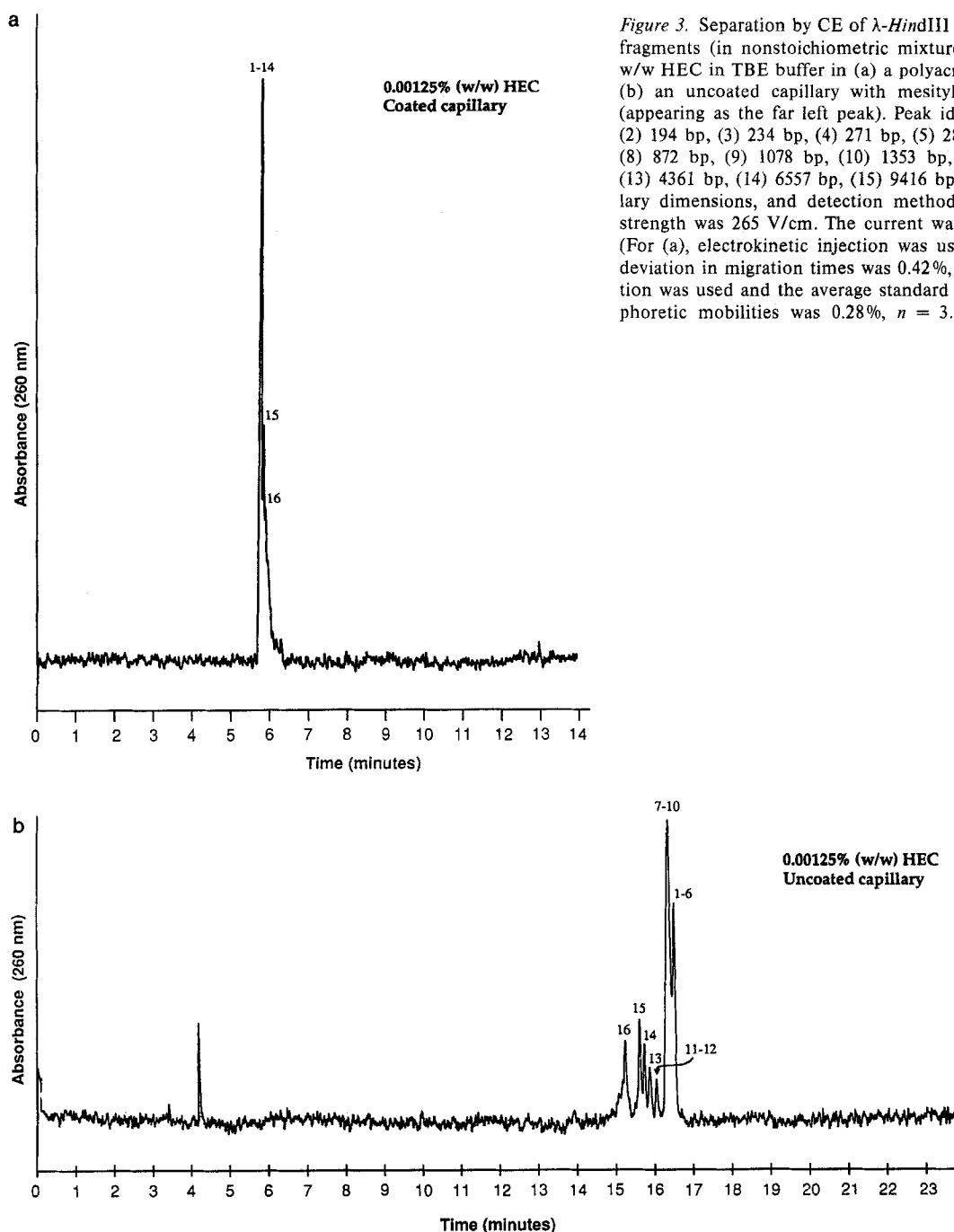
a

1-14

0.00125% (w/w) HEC
Coated capillary

Absorbance (260 nm)

15

16

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Time (minutes)

Figure 3. Separation by CE of λ-*Hind*III and ΦX174-*Hae*III restriction fragments (in nonstoichiometric mixture) in a solution of 0.00125% w/w HEC in TBE buffer in (a) a polyacrylamide-coated capillary, and (b) an uncoated capillary with mesityl oxide as a neutral marker (appearing as the far left peak). Peak identification: (1) 72 + 118 bp, (2) 194 bp, (3) 234 bp, (4) 271 bp, (5) 281 bp, (6) 310 bp, (7) 603 bp, (8) 872 bp, (9) 1078 bp, (10) 1353 bp, (11) 2027 bp, (12) 2322 bp, (13) 4361 bp, (14) 6557 bp, (15) 9416 bp, (16) 23130 bp. Buffer, capillary dimensions, and detection methods, same as Fig. 1. The field strength was 265 V/cm. The current was (a) 7.0 µA, and (b) 7.2 µA. (For (a), electrokinetic injection was used and the average standard deviation in migration times was 0.42%, $n = 3$. For (b), pressure injection was used and the average standard deviation in absolute electrophoretic mobilities was 0.28%, $n = 3$.

b

7-10

0.00125% (w/w) HEC
Uncoated capillary

Absorbance (260 nm)

1-6

15

16 14

13

11-12

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

Time (minutes)

for resolution of DNA larger than 2 kbp is about 0.05% HEC [71]).

3.3 Residence time

The results shown in Fig. 1, 2, and 3 demonstrate that uncoated capillaries of the same effective length as coated capillaries can provide superior DNA separation if uncrosslinked polymer solutions are used, because in uncoated capillaries electroosmotic flow serves to increase DNA residence time within the capillary. This increase in residence time becomes more pronounced as the polymer concentration is decreased and the DNA electrophoretic mobilities increase. This is because when fewer dissolved polymers are present to pose as obsta-

cles to DNA motion, the DNA can "swim upstream" more rapidly, delaying its elution past the detector for a longer time and increasing the "effective length" of the capillary. The corollary of this is that in concentrated polymer solutions, in which DNA mobilities are lower, the enhancement in DNA peak resolution will be less dramatic. If quite concentrated polymer solutions are used (*e.g.*, 1.0% HEC [24]) it is well-known that excellent DNA resolution can be obtained in the absence of electroosmotic flow. The enhancement of DNA residence time and peak resolution which can be gained by the use of uncoated capillaries will be more useful in the case of dilute and semi-dilute polymer solutions, which flow easily through the capillary by electroosmosis.

3.4 CE in absence of HEC

Figure 4 shows the electropherogram obtained when λ-*Hind*III/ΦX174-*Hae*III restriction fragments are injected into a coated capillary containing TBE buffer only, *i.e.*, 0% HEC. No separation is obtained. The same lack of resolution in free solution is seen in an uncoated capillary (data not shown). This result shows that it must be interactions between the HEC polymers and the DNA which provide DNA separation, even at ultradilute HEC concentrations.

3.5 Electrophoretic mobilities

It is interesting to compare the measured electrophoretic mobilities of the DNA restriction fragments in the coated and uncoated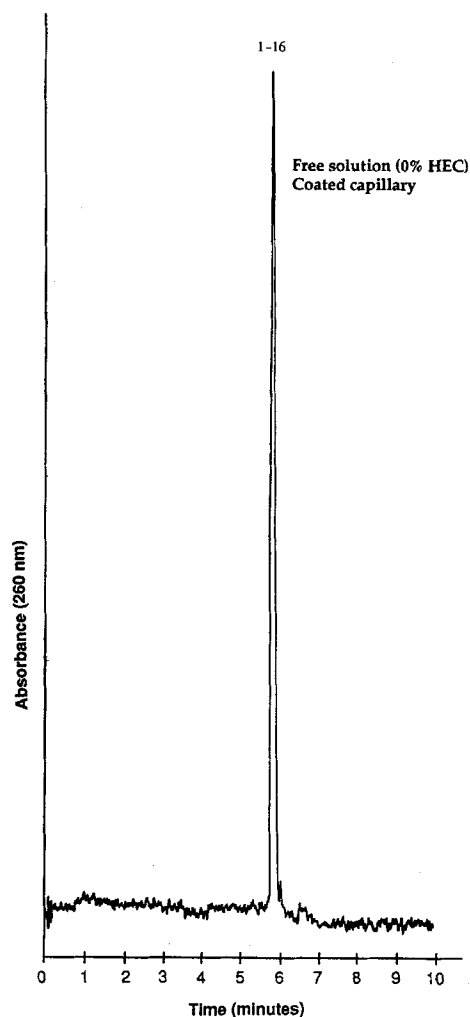 capillaries, in order to determine whether there is any difference in the forces experienced by the DNA when electroosmotic flow is eliminated. Consider first the two separations shown in Fig. 1, in 0.15% w/w HEC solutions. For coated capillaries (Fig. 1a), the DNA electrophoretic mobilities are easily calculated, simply using the migration times of the DNA t_m, the length of capillary to the detection window L_d, and the known field strength, E, of 265 V/cm: $\mu = L_d/t_m E$ in the units of cm²/V s. It is not much more difficult to calculate DNA mobilities for the separation in uncoated capillaries (Fig. 1b): one simply subtracts the electroosmotic mobility, calculated from the migration time of the neutral marker, from the apparent electrophoretic mobility of a given DNA restriction fragment. In this case, the DNA electrophoretic mobility obtained is a negative number (of which we will take the absolute value) since the DNA is "swimming upstream", away from the detector. The same calculations of electrophoretic mobility can be carried out for the separations in Fig. 2 and for Fig. 3b. For the coated-capillary separation in Fig. 3a, in 0.00125% w/w HEC solution, the difference in the elution times of the DNA peaks (except for the 23.1 kbp and the 9.4 kbp shoulders) could not be visually discerned in a coated capillary (due to the short retention time in the capillary). However, DNA larger than 2 kbp could be resolved in a coated capillary in more concentrated solutions of 0.002%, 0.003%, 0.004%, and 0.005% w/w HEC. For the purposes of comparing the electrophoretic mobilities of the DNA in coated and uncoated capillaries at 0.00125%, then, we extrapolated the measured DNA electrophoretic mobilities at these four concentrations back to 0.00125% w/w HEC. An example of the linear fit to this data, used for extrapolation, is shown in Fig. 5 for several of the large DNA fragments.
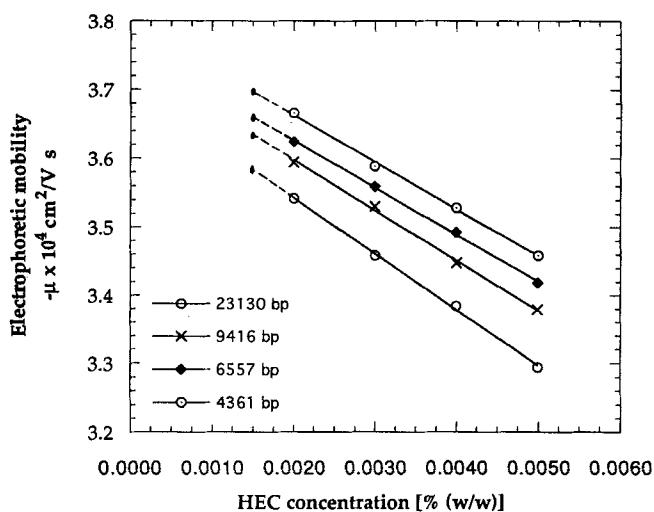

Figure 4. CE of λ-*Hind*III and Φ174-*Hae*III restriction fragments in TBE buffer (*i.e.*, 0% HEC) in a polyacrylamide-coated capillary. This figure demonstrates that no separation is possible in the absence of dissolved polymers in the electrophoresis buffer. Peak identification: (1) 72 + 118 bp, (2) 194 bp, (3) 234 bp, (4) 271 bp, (5) 281 bp, (6) 310 bp, (7) 603 bp, (8) 872 bp, (9) 1078 bp, (10) 1353 bp, (11) 2027 bp, (12) 2322bp, (13) 4361 bp, (14) 6557 bp, (15) 9416 bp, (16) 23130 bp. Buffer, capillary dimensions, and detection methods, same as Fig. 1. The field strength was 265 V/cm. The current was 7.0 µA. Electrokinetic injection was used. The standard deviation in the migration time was 0.49%, *n* = 3.

Figure 5. A sample plot showing the extrapolation of the electrophoretic mobilities of representative DNA restriction fragments from 0.005% back to 0.00125% w/w HEC, by fitting a straight line to the measured electrophoretic mobilities at 0.002%, 0.003%, 0.004%, and 0.005% w/w HEC. The same type of extrapolations were used to determine the mobilities of all DNA restriction fragments in the λ-*Hind*III and ΦX174-*Hae*III mixture at 0.00125% w/w HEC. This extrapolation had to be performed because the DNA peaks (for DNA larger than 2 kbp) were too close to be resolved in a coated capillary filled with a 0.00125% w/w HEC solution (see Fig. 3a) but could be resolved at concentrations of 0.002% and higher.

3.6 Effect of temperature

To perform an exact comparison of the DNA electrophoretic mobilities in coated and uncoated capillaries, a temperature correction must be made. This can be clearly seen in Fig. 6, which shows the raw data for the DNA electrophoretic mobilities in 0.15% HEC, calculated as described above for both uncoated and coated capillaries. It can be seen that the sigmoidal curves formed by the mobility data are very similar in shape; however, the mobilities in the uncoated capillary are systematically higher. This is due to the fact that a higher temperature prevailed in the uncoated capillary. While uncoated capillaries were surrounded during electrophoresis by air under forced convection at 30°C, the coated capillaries were exposed to room temperature (~ 26°C) and so were cooled by natural convection only (see explanation in Section 2.3). Electrophoretic mobility is strongly dependent upon temperature (increasing about 2% per 1°C increase [97]), primarily because of the effect of temperature on buffer viscosity. The temperature dependence of liquid viscosity takes the form [98]

$$\frac{1}{\eta} = A \exp(-B/T) \qquad (1)$$

where η is the buffer viscosity, A and B are constants, and T is the temperature in °K. Qualitatively, then, as temperature increases, buffer viscosity will decrease and hence electrophoretic mobility will increase. The ratio of
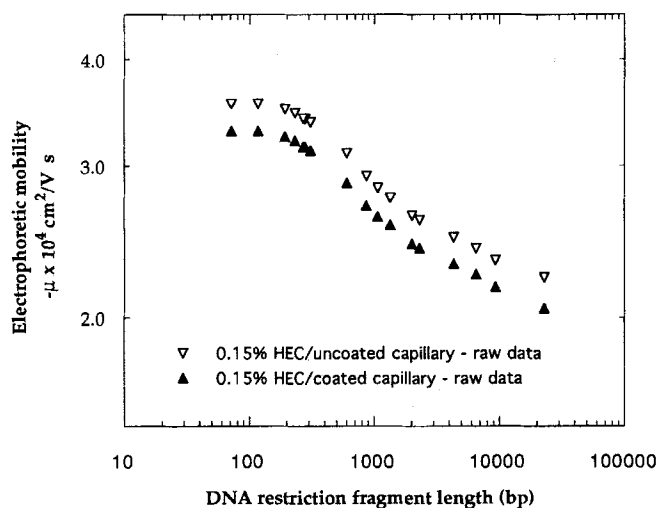

Figure 6. A log-log plot comparing the measured electrophoretic mobilities (uncorrected for differences in temperature) of λ-*Hind*III and ΦX174-*Hae*III restriction fragments, as a function of DNA size, in polyacrylamide-coated and uncoated capillaries. The HEC concentration in the capillary was 0.15% in both cases. Electrophoresis was performed at 265 V/cm, and both uncoated and coated capillaries had the dimensions of 51 mM ID, 50 cm total length, and 35 cm to detector. It can be seen that the shapes of the curves formed by the data points is similar in both cases; the offset between them is attributable to a difference in run temperature between the coated and uncoated capillaries (as described in the text, the uncoated capillaries were thermostatted by forced air convection at 30°C, while the coated capillaries were surrounded by stagnant air at room temperature (~ 26°C)). In order to superimpose these curves (as shown in Fig. 7), we performed a temperature correction to the mobilities in the coated capillaries, which is described in Section 3.6.

electrophoretic mobilities at two different temperatures, T_1 and T_2, is given by [99]

$$\frac{\mu_{e1}}{\mu_{e2}} = \exp\left[-\left(\frac{B}{T_1} - \frac{B}{T_2}\right)\right] \qquad (2)$$

The value of B for an aqueous solution has been determined to be 1820°K by Burgi *et al.* [99]. This expression can be used to correct DNA mobilities measured at one temperature to their expected values at another temperature. However, we do not know the exact inner temperatures of the coated and uncoated capillaries during electrophoresis (only their external temperatures), and calculating the internal temperature exactly in the two different cases of forced convection of surrounding air at 30°C (for uncoated capillaries) and natural convection of air at 26°C (for coated capillaries) is rather an involved procedure [100]. We therefore decided to forego making an exact temperature correction. Instead, we made two assumptions and performed an approximate, semi-empirical correction to adjust the electrophoretic mobilities in the coated and uncoated capillaries to the same temperature. We began by assuming that the internal temperature of the uncoated capillary, which was cooled by forced convection during electrophoresis, was close to the external temperature of 30°C. Then, using Eq. (2), we guessed the internal temperature of the uncooled, coated capillary during electrophoresis, choosing the value which provided the best superposition of the electrophoretic mobility data for the two data sets. Thus, the correction was made using one adjustable parameter. The internal temperature guess which provided the best superposition was 29°C; this is a reasonable temperature rise for an external temperature of 26°C [101]. With this value, we used Eq. (2) to correct the DNA mobilities in the coated capillaries, at HEC concentrations of 0.15%, 0.025%, and 0.00125%, from their measured values to what they would be at 30°C. By making this minor temperature correction, we were able to virtually superimpose the curves of electrophoretic mobility *vs.* DNA size taken at all three polymer concentrations in coated and uncoated capillaries.

This superposition is shown in Fig. 7, in which DNA electrophoretic mobilities in the uncoated capillaries and in polyacrylamide-coated capillaries are plotted on a log-log scale as a function of DNA size for the three different HEC concentrations shown in Fig. 1 through 3. Not only are the sigmoidal shapes of the μ *vs.* DNA size curve similar, but in fact the values of the electrophoretic mobilities in the coated and uncoated capillaries are virtually identical once this minor temperature correction was made.

3.7 Mechanism of separation

Our data show that the presence of a covalent, hydrophilic polyacrylamide coating on the capillary wall has no significant effect on DNA electrophoretic mobilities in polymer solutions. Based on this result, we conclude that the dilute-polymer solution separation of large DNA restriction fragments which was originally observed in uncoated capillaries [71], and which is illustrated in Fig.
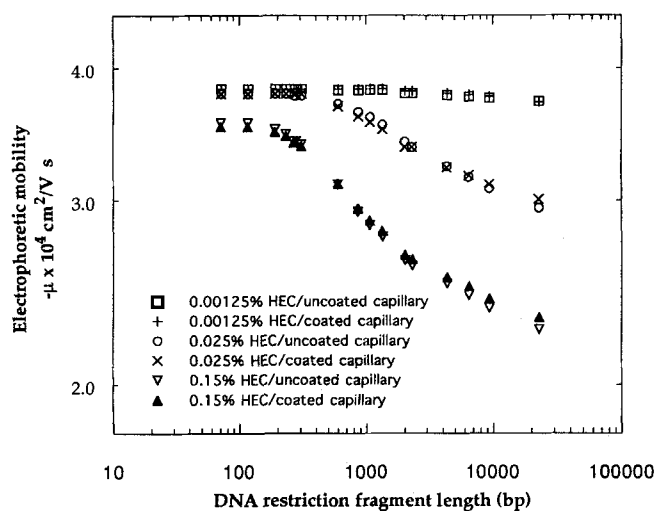
Figure 7. A log-log plot comparing the electrophoretic mobilities of λ-*Hind*III and ΦX174-*Hae*III restriction fragments, as a function of DNA size, in polyacrylamide-coated and uncoated capillaries (corrected for the difference in temperature). The mobilities in coated and uncoated capillaries are practically equal at all three HEC concentrations of 0.00125%, 0.025%, and 0.15% w/w HEC; the shapes of the mobility vs. DNA size curves are the same as well. Electrophoresis was performed at 265 V/cm, and both uncoated and coated capillaries had the dimensions of 51 μm ID, 50 cm total length, and 35 cm to detector. We have corrected for the difference in run temperature for uncoated and coated capillaries, using one adjustable parameter (see Section 3.6).

3b, cannot be attributed to any specific interactions of the DNA with the fused silica surface. Given this, DNA separation in ultradilute polymer solutions must be attributable to DNA/HEC interactions which occur in the bulk of the polymer solution. A polymer solution which is 15 times (Fig. 2) or 300 times (Fig. 3) more dilute than the entanglement threshold concentration is certainly unentangled, and hence the HEC polymers do not form a polymer network. We cannot attribute DNA separation to a mechanism of separation which relies on the presence of "pores" in a polymer network (transient or otherwise), nor to one which relies on the concept of a "tube" formed by such pores. The Ogston sieving model [81–84] and the reptation model [78, 85] thus fail to explain such dilute polymer-solution DNA separation. We suggest that in dilute solutions, large DNA fragments may transiently entangle with the uncharged HEC polymers in the buffer (even though the HEC polymers are not entangled with each other). If DNA molecules are forced to drag and deform HEC polymers during electrophoresis, they must experience additional drag, which could be nonlinearly dependent on DNA size. For a more detailed discussion of this proposed mechanism of separation, see our recent paper [71].

3.8 Advantages of uncoated capillaries

There are advantages to using uncoated capillaries for DNA separations. One advantage is that, as we have shown, under the same electrophoresis conditions uncoated capillaries can provide far better DNA peak separation than coated capillaries of the same dimensions. An uncoated capillary can be automatically filled,

cleaned, and refilled, and can be used for weeks or even months — we only retire a capillary if it is accidentally broken. Also, it is undeniably simpler to omit the three-hour process of coating the capillaries with polyacryl-amide. Furthermore, the coating process may not always be effective; it depends on the specific properties of the fused silica capillaries which one is using [93]. A partially coated capillary can have *local* sections of electroosmotic flow, causing serious band broadening. Electroosmotic flow in an uncoated capillary, which is uniform, measurable, and has a relatively flat velocity profile, does not significantly contribute to band broadening.

3.9 Electroosmotic flow

The interactions of the capillary wall with DNA analytes and with the hydrophilic polymers in the buffer are not perfectly understood. In a coated capillary, electroosmotic flow is prevented because the viscosity of that dense polyacrylamide layer near the capillary wall is infinite, not because the negative charge of the capillary surface is completely masked [92]. Although a close approach to the capillary surface is prevented by the layer of polyacrylamide, DNA is still repulsed by the negative surface charge. On the other hand, in an uncoated capillary filled with a polymer solution at pH 8, the fused silica surface is virtually completely ionized and highly negatively charged [95]. There is some evidence, however, that the cellulosic polymers may adsorb dynamically on the fused silica surface [96, 102]; the extent to which they adsorb is probably dependent on the polymer properties of the specific cellulose derivative (*e.g.*, hydroxypropylmethyl cellulose *vs*. HEC). Bello *et al.* [96] studied the electroosmosis of methyl cellulose solutions in fused silica capillaries. They found that the electroosmotic mobility of the polymer solution through the capillary was progressively lower with each successive injection of a neutral marker, suggesting that a "dynamic coating" of methyl cellulose was being adsorbed onto the capillary wall. Even so, the electroosmotic mobility of the methyl cellulose solutions tended to a plateau value which was much higher than the value which would be predicted by the classical von Smoluchowski equation, given the viscosity of the bulk polymer solution. Bello *et al.* [96] convincingly argue that the discrepancy between the measured and predicted values of the electroosmotic mobility results from non-Newtonian behavior of the polymer solution near the capillary wall. As a result of a very high shear rate in the electrical double layer, the polymer molecules undergo an orientation or conformation change which results in a reduced fluid viscosity in this region, relative to the bulk.

Using HEC solutions, we have observed the same trends that Bello *et al.* [96] observe for methyl cellulose solutions, even though HEC has somewhat different polymer properties, being more stiff and extended in solution and less hydrophobic. That is, the electroosmotic mobility of an HEC solution through the capillary is slightly reduced from run to run, approaching a plateau value after an hour or so (Barron, A. E., Blanch, H. W., unpublished observations, 1994). However, for HEC solutions the suppression of electroosmotic flow *which*

occurs with successive runs is small, almost unnoticeable. For example, if the elution time of a neutral marker being driven by electroosmotic flow at 265 V/cm in a capillary with a 35 cm effective length was 4.97 min during the first run, it might be 4.98 min 20 minutes later and 4.99 min another hour after that. The electrophoretic mobilities of DNA molecules, calculated by subtracting the electroosmotic mobility from the apparent DNA mobilities, are not affected by the formation of this "dynamic coating". Also, in our observation, the electroosmotic mobility of HEC solutions is not a strong function of HEC concentration, at least for relatively low-viscosity solutions. For example, at 265 V/cm in an uncoated capillary with a 35 cm effective length, the elution time of a neutral marker is approximately the same — about 5 min — for all HEC solutions having viscosities less than 60 cP, regardless of the average HEC molecular mass. This observation is consistent with the finding of Bello *et al.* [96] that the electroosmotic mobility is primarily determined by the conformation of the polymers which lie in the electrical double layer, and not the viscosity of the bulk solution.

We have emphasized the larger-than-predicted electroosmotic flow velocity of polymer solutions in uncoated capillaries in order to stress what is clearly seen in Fig. 1, 2, and 3: over a wide range of polymer concentrations, the electroosmotic flow present in uncoated capillaries can be used as an efficient "pump" to drive DNA fragments past the detector window, at the same time significantly improving peak spacing and DNA resolution. Without exception, the peak spacing is better in uncoated than in coated capillaries. In fact, the separation of DNA in ultradilute polymer solutions would probably never have been discovered in coated capillaries, since in order to observe the resolution shown in Fig. 3b one would require a coated capillary with an effective length of more than 90 cm; capillaries of this length are not generally used. Furthermore, the average percent standard deviation in electrophoretic mobility/migration time is superior in the uncoated capillaries (see captions to Fig. 1 through 3 for the values). The trade-off is a possible loss in peak sharpness attributable to the band broadening which occurs during the analytes' increased residence time in the capillary, or due to any slight disturbances in the electroosmotic flow profile. Whether electroosmotic flow is a desirable pump and separating aid for the DNA mixture of interest will depend upon the application.

3.10 Impact of ultradilute solutions

The steady-field DNA separations which we have shown in ultradilute polymer solutions are primarily of theoretical interest, since, in general, better resolution can be obtained in more concentrated solutions. (We note, however, that ultradilute HEC solutions have recently been shown to be useful for the rapid separation of very large DNA (20 kbp–1.6 Mbp) by pulsed field capillary electrophoresis [74]). We would like to emphasize, however, that this does not mean that the polymers must be entangled in order to provide good resolution of larger DNA molecules. In fact, we found in a previous study

[71] that the resolution of DNA larger than 600 bp diminishes dramatically as the polymer concentration is raised above the measured entanglement threshold. The high-resolution separation of DNA smaller than 500 bp, however, does demand either crosslinked gels [9, 12, 63] or very concentrated polymer solutions [12]; in this case (and only in this case) the formation of a dense polymer network does seem to be necessary.

4 Concluding remarks

We have shown that it is possible to use CE to separate large DNA restriction fragments (2.0–23.1 kbp) under a high-voltage, steady field in dilute HEC solutions, using coated capillaries as well as uncoated capillaries. DNA peak separation is dramatically superior in uncoated capillaries, however, because electroosmotic flow increases the DNA residence time in the capillary. At a given field strength and HEC concentration, the electrophoretic mobilities of DNA restriction fragments are the same regardless of whether an uncoated or a coated capillary is used. This lends credence to our earlier suggestion that the mechanism of DNA separation in ultra-dilute polymer solutions is attributable to DNA/HEC interactions in the bulk of the HEC solution, and not to any specific interactions of the DNA with the capillary wall. Furthermore, we suggest that the use of uncoated capillaries with strong, constant electroosmotic flow may be a desirable approach for the improvement of peak spacing for hard-to-separate DNA fragments.

5 References

[1] *Directory of Capillary Electrophoresis,* Elsevier Science Publishers, Amsterdam 1994.

[2] Cohen, A. S., Paulus, A., Karger, B. L., *Chromatographia* 1987, *24*, 15–24.

[3] Swerdlow, H., Gesteland, R., *Nucleic Acids Res.* 1990, *18*, 1415–1419.

[4] Drossman, H., Luckey, J. A., Kostichka, A. J., D'Cunha, J., Smith L. M., *Anal. Chem.* 1990, *62*, 900–903.

[5] Luckey, J. A., Drossmann, H., Kostichka, A. J., Mead, D. A., D'Cunha, J., Norris, T. B., Smith, L. M., *Nucleic Acids Res.* 1990, *18*, 4417–4421.

[6] Cohen, A. S., Najarian, D. R., Karger, B. L., *J. Chromatogr.* 1990, *516*, 49–60.

[7] Lux, J. A., Yin, H. F., Schomberg, G., *J. High Res. Chromatogr.* 1990, *13*, 436–437.

[8] Baba, Y., Matsuura, T., Wakamoto K., Tsuhako, M., *Chem. Letters* 1991, *371*–374.

[9] Baba, Y., Matsuura, T., Wakamoto, K., Morita, Y., Nishitsu, Y., Tsuhako, M., *Anal. Chem.* 1992, *64*, 1221–1225.

[10] Huang, X. C., Stuart, S. G., Bente, P. F. I., Brennan, T. M., *J. Chromatogr.* 1992, *600*, 289–295.

[11] Bruin, G. J. M., Wang, T., Xu, X., Kraak, J. C., Poppe, H., *J. Microcol. Sep.* 1992, *4*, 439–448.

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

[13] Tietz, D., Gottlieb, M. H., Fawcett, J. S., Chrambach, A., *Electrophoresis* 1986, *7*, 217–220.

[14] Heiger, D. N., Cohen, A. S., Karger, B. L., *J. Chromatogr.* 1990, *516*, 33–48.

[15] Guszczynski, T., Chrambach, A., *Biochem. Biophys. Res. Comm.* 1991, *179*, 482–486.

[16] Liu, J., Dolnik, V., Hsieh, Y.-Z. Novotny, M., *Anal. Chem.* 1992, *64*, 1328–1336.

[17] Wu, D., Regnier, F. E., *J. Chromatogr.* 1992, *608*, 349–356.

[18] Ganzler, K., Greve, K. S., Cohen, A. S., Karger, B. L., Guttman, A., Cooke, N. C., *Anal. Chem.* 1992, *64*, 2665–2671.

[19] Garner, M. M., Chrambach, A., *Electrophoresis* 1992, *13*, 176–178.

[20] Pulyaeva, H., Wheeler, D., Garner, M. M., Chrambach, A., *Electrophoresis* 1992, *13*, 608–614.

[21] Rampino, N. J., Chrambach, A., *J. Chromatogr.* 1992, *596*, 141–149.

[22] Chrambach, A., Aldroubi, A., *Electrophoresis* 1993, *14*, 18–22.

[23] Kuypers, A. W. H. M., Willems, P. M. W., van der Schans, M. J., Linssen, P. C. M., Wessels, H. M. C., de Bruijn, C. H. M. M., Everaerts, F. M., Mensink, E. J. B. M., *Chromatogr. Biomed. Appl.* 1993, *621*, 149–156.

[24] Kleemiß, M. H., Gilges, M., Schomburg, G., *Electrophoresis* 1993, *14*, 515–522.

[25] Maschke, H. E., Frenz, J., Belenkii, A., Karger, B. L., Hancock, W. S., *Electrophoresis* 1993, *14*, 509–514.

[26] Paulus, A., Hüsken, D., *Electrophoresis* 1993, *14*, 27–35.

[27] Guszczynski, T., Pulyaeva, H., Tietz, D., Garner, M. M., Chrambach, A., *Electrophoresis* 1993, *14*, 523–530.

[28] Carson, S., Cohen, A. S., Belenkii, A., Ruiz-Martinez, M. C., Berka, J., Karger, B. L., *Anal. Chem.* 1993, *65*, 3219–3226.

[29] Gelfi, C., Orsi, A., Righetti, P. G., Brancolini, V., Cremonesi, L., Ferrari, M., *Electrophoresis* 1994, *15*, 640–643.

[30] Figeys, D., Arriaga, E., Renborg, A., Dovichi, N. J., *J. Chromatogr.* 1994, *669*, 205–216.

[31] Heller, C., Viovy, J.-L., *Appl. Theor. Electrophoresis* 1994, *4*, 39–41.

[32] Zhu, M., Hansen, D. L., Burd, S., Gannon, F., *J. Chromatogr.* 1989, *480*, 311–319.

[33] Chin, A. M., Colburn, J. C., *Am. Biotech. Lab., News Edition* 1989, *7*, 16.

[34] Grossman, P. D., Soane, D. S., *J. Chromatogr.* 1991, *559*, 257–266.

[35] Schwartz, H. E., Ulfelder, K., Sunzeri, F. J., Busch, M. P., Brownlee, R. G., *J. Chromatogr.* 1991, *559*, 267–283.

[36] Strege, M., Lagu, A., *Anal. Chem.* 1991, *63*, 1233–1236.

[37] Oefner, P. J., Bonn, G. K., Huber, C. G., Nathakarnkitkool, S., *J. Chromatogr.* 1992, *265*, 331–340.

[38] Dolnik, V. Novotny, M., *J. Microcol. Sep.* 1992, *4*, 515–519.

[39] MacCrehan, W. A., Rasmussen, H. T., Northrup, D. M., *J. Liq. Chromatogr.* 1992, *15*, 1063.

[40] McCord, B. R., Jung, J. M., Holleran, E. A., *J. Liq. Chromatogr.* 1993, *16*, 1963–1981.

[41] Barron, A. E., Soane, D. S., Blanch, H. W., *J. Chromatogr.* 1993, *652*, 3–16.

[42] Guttman, A., Nolan, J. A., Cooke, N., *J. Chromatogr.* 1993, *632*, 171–175.

[43] Baba, Y., Ishimaru, N., Samata, K., Tsuhako, M., *J. Chromatogr.* 1993, *653*, 329–335.

[44] Clark, B. K., Sepaniak, M. J., *J. Microcol. Sep.* 1993, *5*, 275–282.

[45] Zhu, H., Clark, S. M., Benson, S. C., Rye, H. S., Glazer, A. N., Mathies, R. A., *Anal. Chem.* 1994, *66*, 1941–1948.

[46] Butler, J. M., McCord, B. R., Jung, J. M., Allen, R. O., *BioTechniques* 1994, *17*, 4–7.

[47] Sumita, C., Baba, Y., Hide, K., Ishimaru, N., Samata, K., Tanaka, A., Tsuhako, M., *J. Chromatogr.* 1994, *661*, 297–303.

[48] Ueno, K., Yeung, E. S., *Anal. Chem.* 1994, *66*, 1424–1431.

[49] McGregor, D. A., Yeung, E. S., *J. Chromatogr.* 1994, *680*, 491–496.

[50] Izumi, T., Yamaguchi, M., Yoneda, K., Isobe, T., Okuyama, T., Shinoda, T., *J. Chromatogr.* 1993, *652*, 41–46.

[51] Nakatani, M., Shibukawa, A., Nakagawa, T., *J. Chromatogr.* 1994, *672*, 213–218.

[52] Shieh, P. C. H., Hoang, D., Guttman, A., Cooke, N., *J. Chromatogr.* 1994, *676*, 219–226.

[53] Guttman, A., Shieh, P. H. C., Lindahl, J., Cooke, N., *J. Chromatogr.* 1994, *676*, 227–231.

[54] Benedek, K., Thiede, S., *J. Chromatogr.* 1994, *676*, 209–217.

[55] Guttman, A., Shieh, P., Hoang, D., Horváth, J., Cooke, N., *Electrophoresis* 1994, *15*, 221–224.

[56] Boček, P., Chrambach, A., *Electrophoresis* 1991, *12*, 1059–1061.

[57] Boček, P., Chrambach, A., *Electrophoresis* 1992, *13*, 31–34.

[58] Tietz, D., Chrambach, A., *Electrophoresis* 1992, *13*, 286–294.

[59] Klepárník, K., Fanali, S., Boček, P., *J. Chromatogr.* 1993, *638*, 283–292.

[60] Chiari, M., Micheletti, C., Nesi, M., Fazio, M., Righetti, P. G., *Electrophoresis* 1994, *15*, 177–186.

[61] Chiari, M., Nesi, M., Righetti, P. G., *Electrophoresis* 1994, *15*, 616–622.

[62] Nesi, M., Righetti, P. G., Patrosso, M. C., Ferlini, A., Chiari, M., *Electrophoresis* 1994, *15*, 644–646.

[63] Cohen, A. S., Najarian, D. R., Paulus, A., Guttman, A., Smith, J. A., Karger, B. L., *Proc. Natl. Acad. Sci. USA* 1988, *85*, 9660–9663.

[64] Swerdlow, H., Wu, S., Harke, H., Dovichi, N. J., *J. Chromatogr.* 1990, *516*, 61–67.

[65] Yin, H. F., Lux, J. A., Schomburg, G., *J. High Resol. Chromatogr.* 1990, *13*, 624–627.

[66] Swerdlow, H., Dew-Jager, K. E., Grey, R., Dovichi, N. J., Gesteland, R., *Electrophoresis* 1992, *13*, 475–483.

[67] Huang, X. C., Quesada, M. A., Mathies, R. A., *Anal. Chem.* 1992, *64*, 967–972.

[68] Huang, X. C., Quesada, M. A., Mathies, R. A., *Anal. Chem.* 1992, *64*, 2149–2154.

[69] Mathies, R. A., Huang, X. C., *Nature* 1992, *359*, 167–169.

[70] Clark, S. M., Mathies, R. A., *Anal. Biochem.* 1993, *215*, 163–170.

[71] Barron, A. E., Blanch, H. W., Soane, D. S., *Electrophoresis* 1993, *15*, 597–615.

[72] Hebenbrock, K., Schügerl, K., Freitag, R., *Electrophoresis* 1993, *14*, 753–758.

[73] Pariat, Y. F., Berka, J. Heiger, D. N., Schmitt, T., Vilenchik, M., Cohen, A. S., Foret, F., Karger, B. L., *J. Chromatogr.* 1993, *652*, 57–66.

[74] Kim, Y., Morris, M. D., *Anal. Chem.*, 1995, in print.

[75] Kim Y., Morris, M. D., *Anal. Chem.* 1994, *66*, 3081–3085.

[76] Sudor, J., Novotny, M. V., *Anal. Chem.* 1994, *66*, 2446–2450.

[77] Chiari, M., Nesi, M., Righetti, P. G., *J. Chromatogr.* 1993, *652*, 31–39.

[78] Lerman, L. S., Frisch, H. L., *Biopolymers* 1982, *21*, 995–997.

[79] Olivera, B. M., Baine, P., Davidson, N., *Biopolymers* 1964, *2*, 245–257.

[80] Bode, H.-J., *FEBS Lett.* 1976, *65*, 56–58.

[81] Ogston, A. G., *Trans. Faraday Soc.* 1958, *54*, 1754–1757.

[82] Rodbard, D., Chrambach, A., *Proc. Nat. Acad. Sci. USA* 1970, *65*, 970–977.

[83] Chrambach, A., Rodbard, D., *Science* 1971, *172*, 440–451.

[84] Rodbard, D., Chrambach, A., *Anal. Biochem.* 1971, *40*, 95–134.

[85] Slater, G. W., Rousseau, J., Noolandi, J., Turmel, C., Lalande, M., *Biopolymers* 1988, *27*, 509–524.

[86] Duke, T., Viovy, J.-L., *Phys. Rev. E.,* 1994, *49*, 2408–2416.

[87] Serwer, P., *Electrophoresis* 1983, *4*, 375–382.

[88] Righetti, P. G., *J. Biochem. Biophys. Methods* 1989, *19*, 1–20.

[89] Holmes, D. L., Stellwagen, N. C., *Electrophoresis* 1991, *12*, 253–263.

[90] Righetti, P. G., Caglio, S., Saracchi, M., Quaroni, S., *Electrophoresis* 1992, *13*, 587–595.

[91] Viovy, J.-L., Duke, T., *Electrophoresis* 1993, *14*, 322–329.

[92] Hjertén, S., *J. Chromatogr.* 1985, *347*, 191–198.

[93] Hjertén, S., Kubo, K., *Electrophoresis* 1993, *14*, 390–395.

[94] Grossman, P. D., Colburn, J. C., Lauer, H. H., *Anal. Biochem.* 1989, *179*, 28–33.

[95] Grossman, P. D., Colburn, J., *Capillary Electrophoresis: Theory and Practice,* Academic Press, San Diego 1992.

[96] Bello, M. S., de Besi, P., Rezzonico, R., Righetti, P. G., Casiraghi, E., *Electrophoresis* 1994, *15*, 623–626.

[97] Coxon, M., Binder, M. J., *J. Chromatogr.* 1974, *101*, 1–16.

[98] Daniels, F., Alberty, R. A., *Physical Chemistry,* John Wiley & Sons, Inc., New York 1975.

[99] Burgi, D. S., Salomon, K., Chien, R.-L., *J. Liq. Chromatogr.* 1991, *14*, 847–867.

[100] Bello, M. S., de Besi, P., Righetti, P. G., *J. Chromatogr.* 1993, *652*, 317–328.

[101] Grushka, E., McCormick, R. M., Kirkland, J. J., *Anal. Chem.* 1989, *61*, 241–246.

[102] Belder, D., Schomburg, G., *J. High Res. Chromatogr.* 1992, *15*, 686–693.