Title

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Author

Bunz, A.P.

Publication Date

1995-10-01

LBL-38031
UC-401
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

CHEMICAL SCIENCES DIVISION

Submitted to Industrial and Engineering Chemistry Research

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A.P. Bünz, A.E. Barron, J.M. Prausnitz, and H.W. Blanch

October 1995

Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

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Alexander P. Bünz, Annelise E. Barron, John M. Prausnitz and Harvey W. Blanch

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

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This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

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Alexander P. Bünz, Annelise E. Barron, John M. Prausnitz* and Harvey W. Blanch
Department of Chemical Engineering, University of California, Berkeley, and
*Chemical Sciences Division, Lawrence Berkeley Laboratory,
University of California, Berkeley, CA 94720

Abstract

Previous studies (Barron et al., 1993, 1994) have shown that dilute aqueous solutions of hydroxyethyl cellulose (HEC) provide an excellent medium for capillary electrophoretic separation of DNA restriction fragments (72 - 23,130 base pairs). DNA resolution is strongly affected by the average HEC molecular weight, as well as by the HEC concentration in the electrophoresis buffer. We used low-molecular weight HEC ($M_n \approx 27{,}000$, $M_w \approx 139{,}000$ g mol $^{-1}$) and high-molecular weight HEC ($M_n \approx 105{,}000$. $M_w \approx 1{,}315{,}000$ g mol $^{-1}$), both separately and in mixtures, to study the impact of HEC molecular weight on the separation of small and large DNA restriction fragments. Our results show that, relative to the single-polymer solutions, the mixed-polymer solutions provide superior separation for the entire DNA range. Addition of a small amount of larger HEC to a solution containing smaller HEC leads to an improvement in separation of the larger DNA fragments (> 603 base pairs) while resolution for the smaller DNA fragments is retained.

Introduction

Increasing the rate of electrophoretic DNA separations is one of the major obstacles to be overcome in present international efforts to map and sequence the human genome. Employing current techniques, it will not be possible to sequence the entire genome without prohibitive time and cost (Chang and Yeung, 1995). Capillary electrophoresis (CE) is a relatively new DNA separation technique which promises to speed the task of sequencing and restriction mapping by 25-fold. In this work, we report DNA separations by CE with an aqueous, non-gel medium that contains both low and high-molecular-weight hydroxyethyl cellulose (HEC) polymers.

Slab-gel electrophoresis is the most common contemporary technique for the separation, identification and purification of DNA fragments. Important applications of slab-gel electrophoresis include DNA sequencing, restriction mapping, polymerase chain-reaction (PCR) analysis, restriction fragment length polymorphism (RFLP) analysis, and Southern blotting. Crosslinked gels are commonly used as support matrices for electrophoresis because their rigid and highly porous structure allows passage of charged analytes, but also dramatically reduces thermal and gravitational convection. In addition, the gel structure provides size-based separation of DNA molecule, which in free solution exhibit nearly equal electrophoretic mobilities regardless of their chain length (Lerman and Frisch, 1982). Traditional slab-gel electrophoresis, a simple and ubiquitous DNA separation technique, is unfortunately also slow, non-quantitative, manually intensive, and difficult to automate.

There are many practical advantages to CE, wherein electrophoretic separation is carried out within a microbore capillary (i.d. 50-100 μm) (Barron and Blanch, 1995). In early applications, crosslinked polyacrylamide gels were used as DNA separation matrices for CE (Heiger et al., 1990). However, there are some major drawbacks to the use of crosslinked gels in the capillary geometry. The routine synthesis of high-quality capillary gels is difficult, due to spatial inhomogeneities in the polymerized gel and

incomplete polymerization. Also, crosslinked capillary gels break down under the high electric fields typically used in CE, and hence are only useful for a few runs. The severity of this drawback is clear if one considers proposals for future capillary electrophoresis DNA sequencers: they might employ an array of 100 or more capillaries in parallel (Huang et al., 1992). The instability of gel-filled capillaries would render this type of apparatus impractical. Therefore, despite the fact that extremely high resolution of small DNA molecules (such as oligonucleotides and DNA sequencing fragments) can be achieved by CE in crosslinked polyacrylamide gels, alternative DNA-sieving matrices are required to take full advantage of the easy automation possible with CE. Uncrosslinked polymer solutions, employed as DNA separation matrices in capillaries, overcome some of the above problems (Chin and Colburn, 1989, Zhu et al., 1989). The primary advantages of polymer solutions are that they are replaceable (*i.e.,* solutions with sufficiently low viscosities can be readily pumped into the capillary), and they remain stable at much higher temperatures and electric field strengths than those that can be applied to crosslinked capillary gels.

Capillary electrophoresis using uncrosslinked polymer solutions provides a promising technique for rapid and efficient separation of DNA restriction fragments up to 23,000 base pairs (bp) in size (Strege and Lagu, 1991, Barron et al., 1994). To date, researchers have used semi-dilute solutions of several water-soluble polymers as DNA separation media for CE, including methyl cellulose (Strege and Lagu, 1991), hydroxyethyl cellulose (HEC) (Grossman and Soane, 1991), hydroxypropyl cellulose (Baba et al., 1993), liquefied agarose (Bocek and Chrambach, 1991), linear polyacryloylaminoethoxyethanol (Chiari et al., 1994), and linear polyacrylamide (Heiger et al., 1990, Tietz et al., 1986). Derivatized celluloses appear especially attractive because the low viscosities of cellulose solutions allow rapid DNA separations.

Previous studies (Barron et al., 1993, 1994) have shown that dilute solutions (e.g., 0.30 % (w/w)) of low-molecular-weight HEC (*e.g.* M_n 27,000) provide good separation

of DNA fragments smaller than 600 base pairs. However, 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 dilute concentrations (*e.g.*, 0.025 %(w/w)) separates large DNA fagments (> 600 base pairs) very well but gives no resolution of smaller DNA molecules. Although this high-molecular-weight HEC can be used at semi-dilute concentrations to provide good resolution of small as well as large DNA fragments, the time required for loading these higher-viscosity solutions into the capillary is larger than that required for dilute solutions of low-molecular weight HEC. Ideally, one would maximize the size range of DNA which can be separated, while minimizing the solution viscosity.

To model size-dependent DNA electrophoretic mobilities, current electrophoresis theories assume that DNA fragments "reptate" through either a crosslinked gel, or a fully entangled polymer network. Barron and coworkers (1993) were the first to suggest a different mechanism, *transient entanglement coupling*, to explain the separation of DNA observed at dilute and ultra-dilute HEC concentrations. According to this model, individual DNA strands entangle with one or more HEC polymers and drag them along, effectively increasing the molecular friction factor of the DNA molecules. The probability of DNA-HEC entanglement is postulated to increase with the length of the DNA chain, yielding a size-dependent separation for DNA up to 23,000 bp. For DNA electrophoresis in concentrated HEC solutions, recent epifluorescence videomicroscopy studies (Shi et al., 1995; Navin and Morris, 1995) support entanglement theories.

In this work, we investigate 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, postulating that this mixture will retain the separation capability of both HEC components for the different DNA size ranges. These experiments employ dilute 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, polyethylene oxide was used at high concentrations to separate small DNA fragments only (< 2200 bp) in these studies. It was found that the mixed-polymer matrix containing equal amounts of polyethylene oxide with different molecular weights provides comparable resolution, while retaining a much lower viscosity, compared to the single-polymer matrices used in their study.

Experimental Procedure

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 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 electrodes. A high-voltage power supply with a 30,000-V capacity (Gamma High Voltage Research, Ormand Beach, CA) was used to drive electrophoresis. Current was measured over a 1-kW resistor in the return circuit of the power supply using a digital multimeter (Model 3465B, Hewlett-Packard, Palo Alto, CA). On-column detection was 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, USA) 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

desk-top PC and thermostatted at 30°C in a water bath (Model H-1 High Temperature Bath, Cannon Instrument Co., State College, PA).

Materials

A non-stoichiometric 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 minutes at 65°C to prevent reannealing of restriction fragments of 4361 and 23,130 bp and was then immediately placed on ice. Mesityl oxide was used as a neutral marker in all experiments 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 hydroxyethyl cellulose (Polysciences, Inc., Warrington, PA) were added to buffer solutions; solutions were vigorously shaken, and then mixed for 24 hours by tumbling because magnetic stirring sometimes led to incomplete dissolution. Successive dilution was used to make dilute solutions. 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.) The weight-average molecular weights of these HEC samples, measured by low-angle laser light-scattering, are $M_w \approx 139{,}000$ and $M_w \approx 1{,}315{,}000$ g/mol. respectively.

Experimental Procedures

The procedure for preparing each new capillary before it use for electrophoresis is given elsewhere (Barron et al., 1994). Each time a new polymer solution was used, the

uncoated inner capillary wall was rinsed first with 1 M NaOH for 10 minutes, then with 0.1 M NaOH for 10 minutes, with distilled, deionized water for 10 minutes, and finally with the electrophoresis buffer (containing dissolved HEC) for 20 minutes.

Samples were introduced to the anodic end of the capillary by applying a vacuum of 2 to 4 in Hg (7773 - 13546 Pa) for a time which depended on the buffer viscosity, to introduce approximately 3 nL (3×10^{-6} cm^3) 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). In all experiments, the capillary was surrounded by convected air at 30.0 ± 0.1°C, to maintain a constant temperature.

At steady-state, a constant *electroosmotic flow velocity* is obtained. 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.

It is also common practice to use capillaries that have their interior walls coated with covalently-attached polyacrylamide to eliminate electroosmotic flow. In this case, samples have to be introduced to the cathodic end of the capillary and the negatively charged DNA molecules migrate toward the anode, driven solely by the voltage gradient. Therefore, in this operation mode, the smallest DNA fragment pass the detector first and the largest fragment pass the detector last. While the DNA peak separation is superior in uncoated capillaries, in which electroosmotic flow increases the residence time of the DNA fragments, the actual electrophoretic mobilities of the DNA fragments are the same whether coated or uncoated capillaries are used (Barron et al., 1995).

Results and Discussion

Figures 2 and 3 show the electrophoretic separation of DNA fragments in polymer solutions of low- and high-molecular-weight HEC (M_n 27,000 and M_n 105,000 g mol^{-1}, respectively); the electrophoretic mobility is plotted as a function of HEC concentration. Earlier work (Barron et al., 1994) has shown that, to achieve comparable separations of small DNA fragments (< 603 bp), it is necessary to use HEC 27,000 at higher concentrations than those required for HEC 105,000. This effect of molecular weight and concentration is seen for separations in 0.40% HEC 105,000 (Fig. 4) and 0.80% HEC 27,000 (Fig. 3), respectively; both of these conditions give similar resolution of DNA fragments smaller than 603 bp. However, the large DNA fragments (> 603 bp) cannot be separated to baseline resolution using HEC 27,000, regardless of concentration. The longer-chain HEC can separate fragments larger than 603 bp at concentrations well below the HEC- entanglement threshold concentration of 0.37% (Barron et al., 1994), *i.e.* in dilute HEC solutions in which no continuous polymer network is thought to exist.

Figures 4 a - c show DNA electrophoretic mobility in aqueous solutions containing mixtures of HEC 27,000 and HEC 105,000. For each diagram the HEC 27,000 concentration was held constant (at 0.20, 0.40, and 0.80%, respectively), while the HEC 105,000 concentration varied from 0.0125 to 0.05%.

Comparison of Figure 4 with Figure 2 indicates that addition of a small amount of high-molecular-weight HEC 105,000 to a solution containing HEC 27,000 leads to a significant increase in resolution for the larger DNA fragments (> 603 bp). This increase is most pronounced for the mixture with HEC 27,000 at 0.20% (Fig. 4 a).

At the same time, the presence of low-molecular-weight HEC (27,000) in a solution containing HEC 105,000 improves the separation of the smaller DNA fragments (< 603 bp) significantly, even at 0.20%; however, the low-molecular-weight HEC does not have a significant affect on the separation of the larger DNA fragments. The one exception is for the highest HEC 27,000 concentration of 0.80%, where the 2027/2322 bp fragments

are no longer baseline-resolved (compare Fig. 4 a - c with Fig. 3).

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%. Our previous data have indicated that the optimum separation of DNA restriction fragments is obtained at HEC-27,000 concentrations between 0.20 and 0.40%. The electropherograms in Figures 6 a - d show, 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. As indicated in Figure 5 c, it is striking that the mixed-polymer solution of 0.30%/0.025% (HEC 27,000/105,000) almost completely retains the resolution of both the small and large DNA fragments which was possible separately with the respective single-polymer solutions. Comparison with Figure 5 d shows that the smallest fragments (118/72 bp) are separated in the mixed-polymer solution, due to the increase in total polymer concentration when HEC 105,000 is added to the HEC 27,000 solution. In Figure 5 d the same total polymer concentration (0.325% HEC 27,000) yields the same resolution for the 118/72 bp fragments.

In Figures 6 a - b, the electrophoretic mobility of DNA in the solutions containing both HEC 27,000 (0.30%) and HEC 105,000 (0.00156 - 0.40%) is plotted versus the HEC 105,000 concentration. The same observations pertaining to Figures 4 a - c apply. Comparison of Figure 4 with Figure 6 indicates that 0.30% HEC 27,000 is the best choice for separating all DNA fragments when the polymer solutions also contains HEC 105,000.

Comparing Figure 6 with Figure 3 indicates that for all concentrations, DNA electrophoretic mobilities are reduced when the mixed-polymer solution is used instead of the HEC-105,000 solution. This reduction is most pronounced at low concentrations of HEC 105,000, where the presence of HEC 27,000 is the prime determinant of DNA electrophoretic mobility. To investigate the origin of this effect, we measured the

absolute viscosities of both the mixed- and the single-polymer solutions for analyzing the electrophoretic mobility data. Results are shown in Table 1.

Although all DNA electrophoretic mobilities are reduced in the mixed-polymer solutions (0.3% HEC 27,000, 0.0016% - 0.4% HEC 105,000) compared to the single-polymer solution (0.0016% - 0.4% HEC 105,000 only), the *difference* in DNA electrophoretic mobilities ($\Delta\mu$; a quantity directly proportional to the resolution) shows a more complex behavior. Trends in $\Delta\mu$ *vs.* concentration are different for small and large DNA fragments as illustrated in Figure 7 that shows the overall difference in electrophoretic mobilities of the relatively small (72 bp-310 bp) and large (603 bp-23130 bp) as a function of HEC 105,000 concentration. 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 fragments, and similarly for the 310 bp and 72 bp fragments. Figure 7 shows that the addition of 0.3% 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 separation 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.

According to the classical derivation (Smoluchowski, 1918), the electrophoretic mobility μ of a charged molecule is inversely proportional to η, the viscosity of the medium through which the molecule moves. However, it is not clear that the reduction in DNA electrophoretic mobilities, seen in mixed HEC 27,000 / HEC 105,000 solutions, as compared to HEC 105,000 solutions, can be accounted for solely by differences in bulk

viscosities of these HEC solutions. Thus, we chose to normalize the electrophoretic mobilities with the viscosity of the buffer (*i.e.* the polymer-free solution) by multiplying by a ratio of the measured viscosity of the polymer solution and the viscosity of the buffer. This normalization should allow us to compare the differences in DNA electrophoretic mobilities in the single- and mixed-polymer solutions independent of the effects of bulk solution viscosity. The viscosity-normalized mobilities therefore reflect the strength of the entanglement interactions between the DNA and the HEC polymers which lead to DNA separation, since it is well known that a change in solution viscosity alone does not provide DNA separation.

We find that viscosity-normalized DNA electrophoretic mobilities in the mixed-HEC-27,000 / HEC-105,000 solutions (at a given HEC 105,000 concentration) are systematically *higher* than normalized mobilities in solutions of HEC-105,000 alone (data not shown). That is, at a given HEC-105,000 concentration, DNA molecules are *more* retarded by entanglement interactions in the *absence* of added HEC-27,000 polymers. This leads to an interesting observation about the mechanism of DNA separation in uncrosslinked polymer solutions. Surprisingly, the presence of a 'sea' of small HEC-27,000 polymers appears to *dampen* the DNA entanglement interactions with longer HEC which provide DNA separation. This effect cannot be attributed to the existence of an entangled network of the HEC-27,000 chains at 0.3%; the entanglement threshold concentration of this HEC sample is 1.8%. Instead, this reduction in viscosity-scaled DNA mobilities may result from the effect of higher polymer concentrations on the conformations which DNA molecules assume during electrophoresis. Further investigations are required to fully understand this phenomena.

Conclusions

In this study, we demonstrate the advantages of mixed-polymer solutions containing hydroxyethyl cellulose of different average chain lengths in separating DNA restriction

fragments. These mixed-polymer solutions can provide high-resolution electrophoretic separation for a broader molecular-weight range of DNA restriction fragments at lower viscosities than those possible with solutions containing only a single polymer. Our data show that a mixture of HEC of varying molecular weights permits large and small DNA fragments to be resolved. Because the maximum chain length of currently commercially available HEC is limited, it is unlikely that dilute HEC solutions will enable electrophoretic separation with a DC field of DNA fragments larger than 48 kbp. However, other water-soluble polymers of higher-molecular-weight may provide further progress in high-efficiency electrophoretic separation processes for charged biomolecules.

Acknowledgments

This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy, under Contract No. DE-AC03-76SF00098.

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Figure Captions

Figure 1. Schematic diagram of the capillary electrophoresis instrument.

Figure 2. 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 3 - 4 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 3. DNA electrophoretic mobility vs. HEC concentration for DNA restriction fragments ranging from 72 to 23130 bp. (a) HEC $M_n \approx 105{,}000$. (b) The same data plotted on an expanded scale at the lowest HEC concentrations.

Figure 4. 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 3 - 4 individual determinations. Average run-to-run variation in calculated electrophoretic mobilities:(a) ± 0.39 %, (b) ± 0.34 %, (c) ± 0.33 %.

Figure 5. Separation by capillary electrophoresis of λ-*HindIII* and ΦX174-*HaeIII* restriction fragments (in non-stoichiometric 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.

Figure 6. (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 3 - 4 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

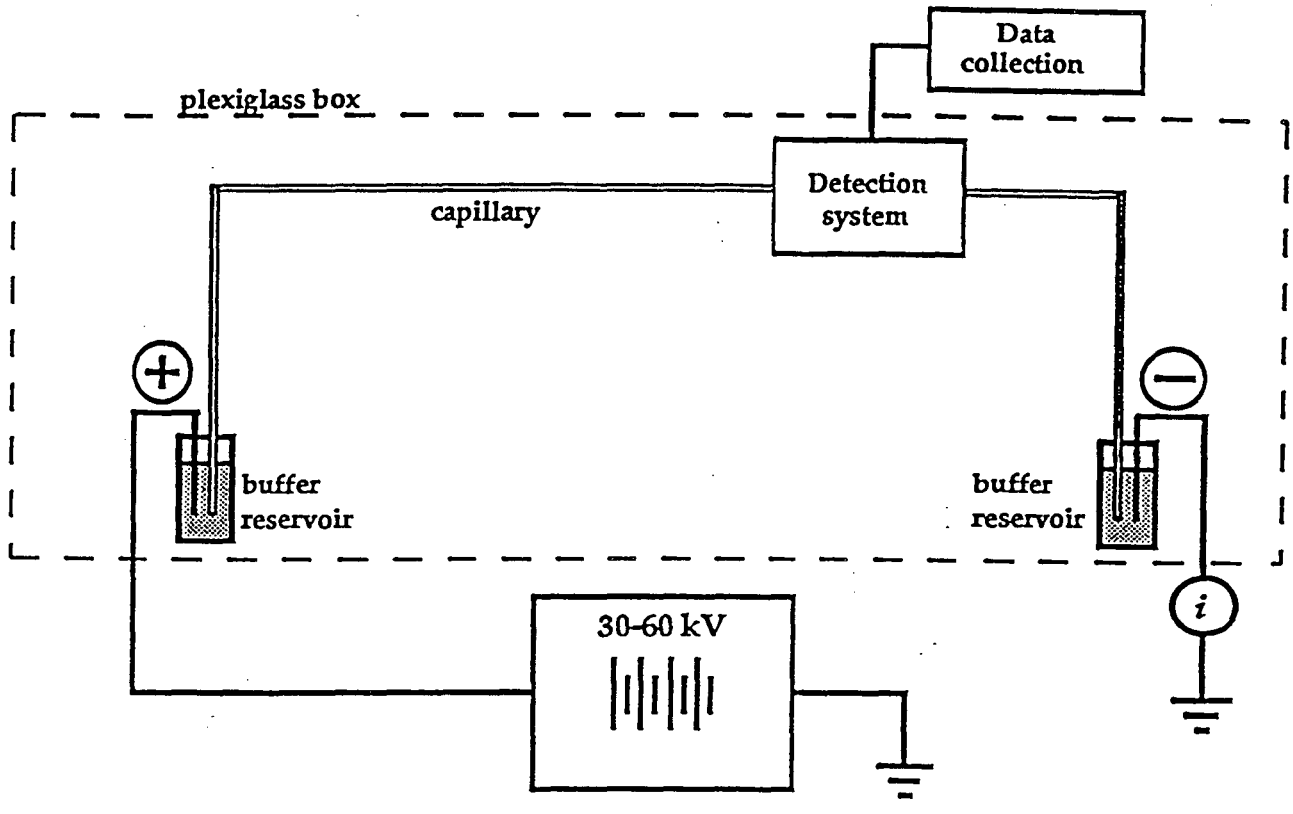
concentrations.

Figure 7. 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.

Table 1: Experimental viscosity for mixed HEC and HEC 105,000 solutions

Concentration HEC 105,000 [% (w/w)]	**Viscosity η of 27,000 (0.3 %)/105,000 Solutions at 30° C (cP)**	**Viscosity η of 105,000 Solutions at 30° C (cP)**
0.00156	1.1553	0.8984
0.00313	1.1737	0.9070
0.00625	1.1909	0.9198
0.0125	1.2450	0.9456
0.025	1.3476	1.0116
0.05	1.5569	1.1735
0.1	2.1567	1.6612
0.2	4.0058	3.3455
0.4	12.7966	12.1849

viscosity of polymer-free aqueous solution $\eta_o = 0.8894$ cp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plexiglass box
Detection
system
capillary
buffer
reservoir
buffer
reservoir
30-60 kV
i

Figure 1

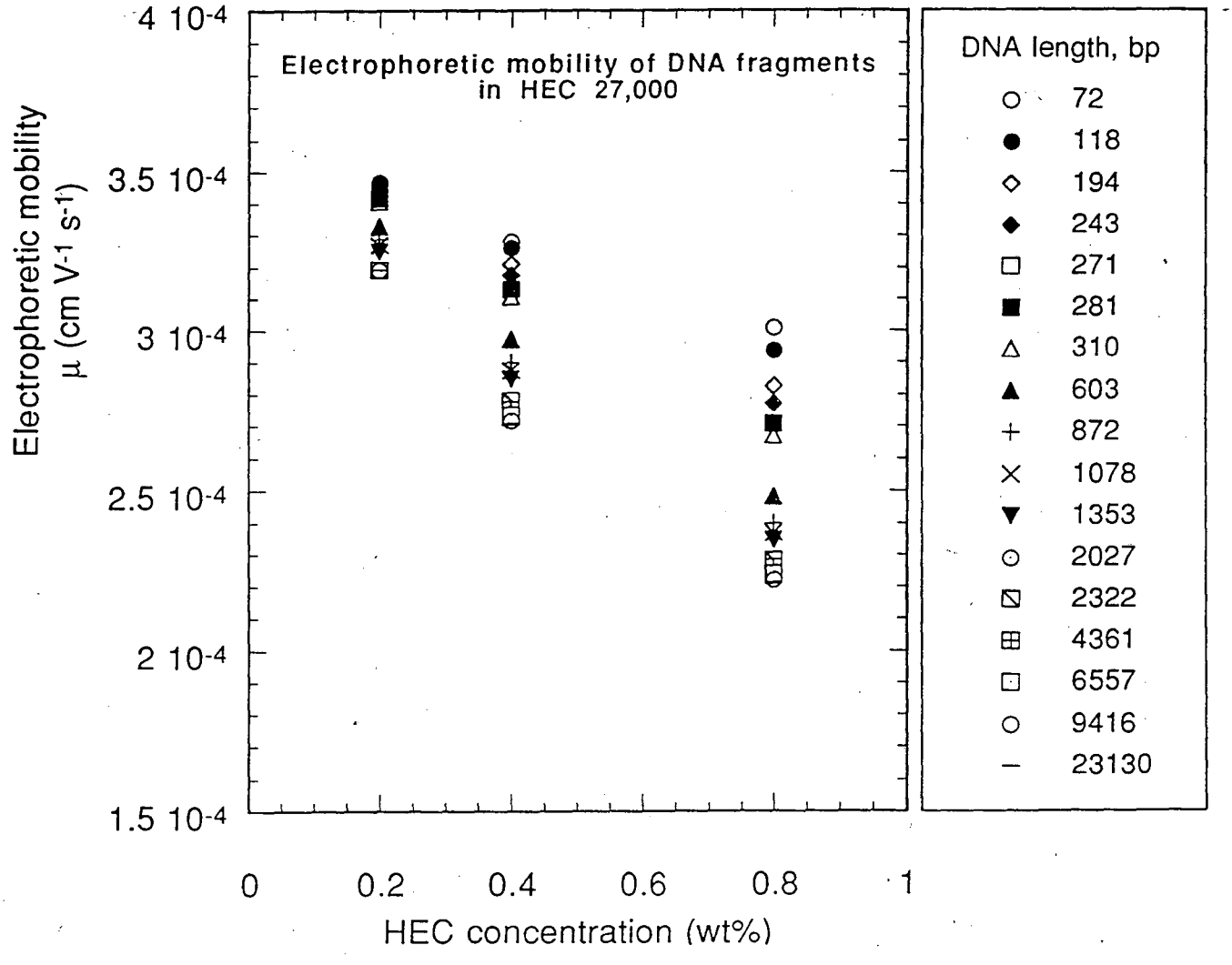
Electrophoretic mobility of DNA fragments
in HEC 27,000
Electrophoretic mobility
μ (cm V-1 s-1)
4 10-4
3.5 10-4
3 10-4
2.5 10-4
2 10-4
1.5 10-4
0
0.2
0.4
0.6
0.8
1
HEC concentration (wt%)
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130

Figure 2

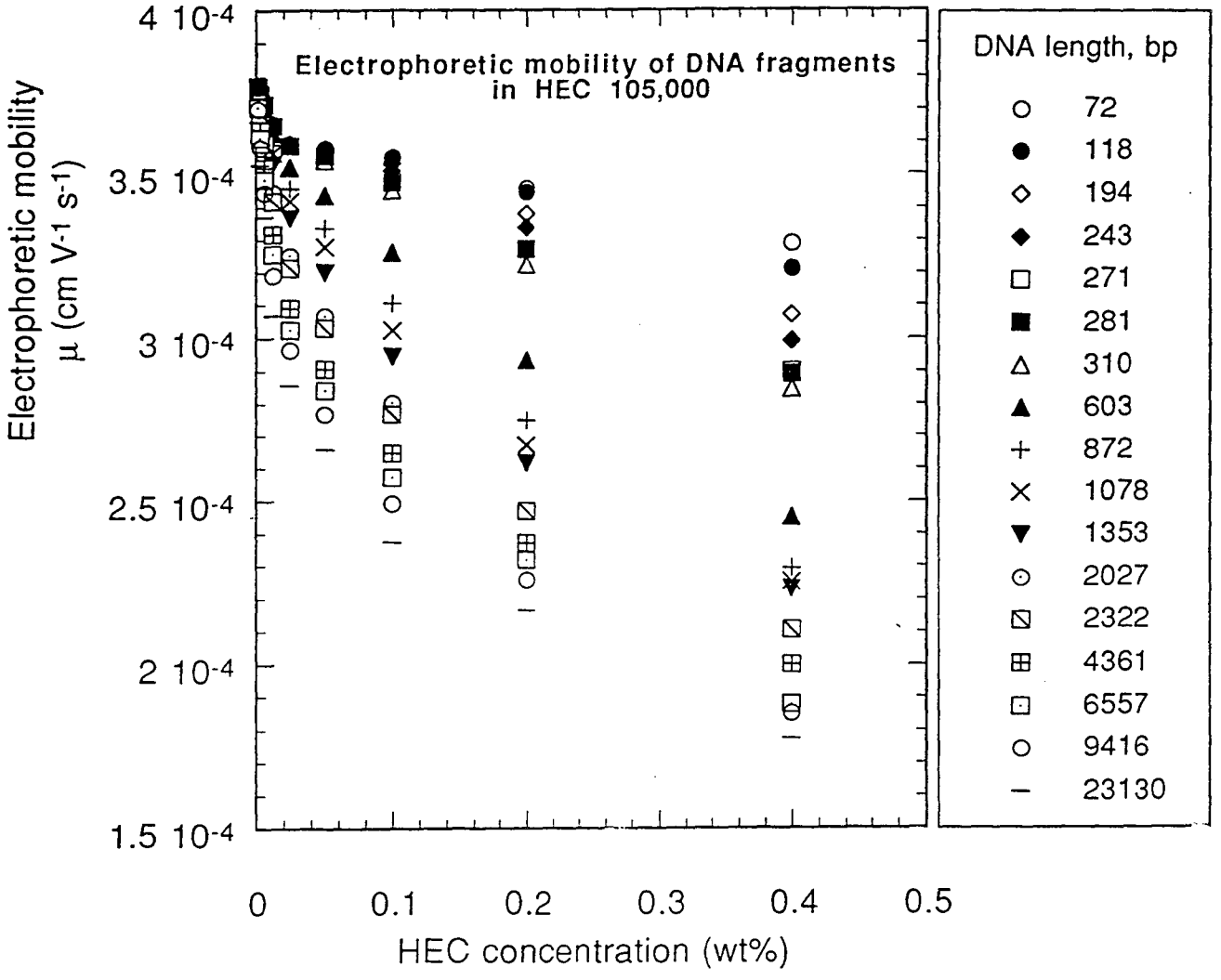
Electrophoretic mobility of DNA fragments
in HEC 105,000
Electrophoretic mobility
μ (cm V-1 s-1)
4 10-4
3.5 10-4
3 10-4
2.5 10-4
2 10-4
1.5 10-4
0
0.1
0.2
0.3
0.4
0.5
HEC concentration (wt%)
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130

Figure 3a

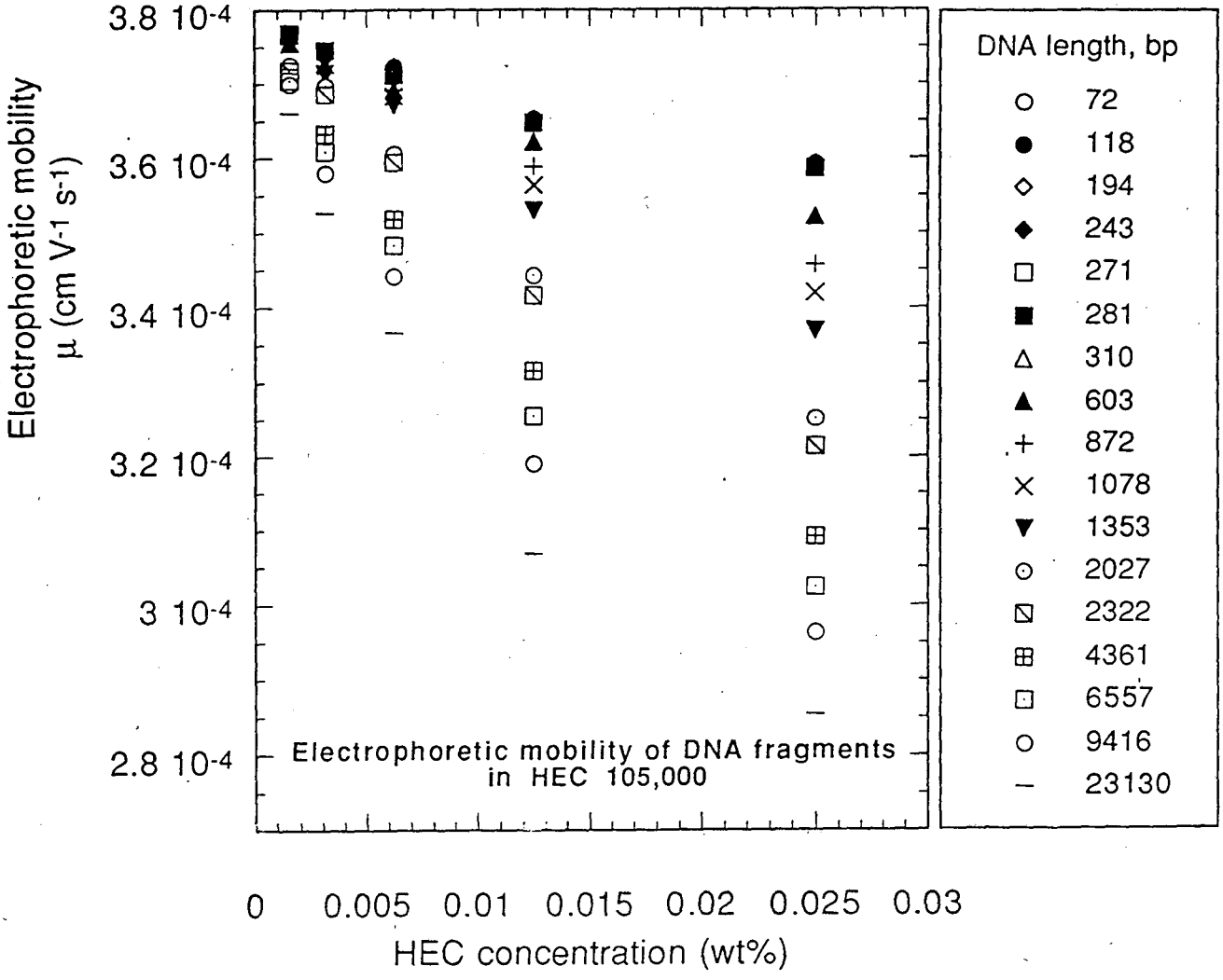
Electrophoretic mobility
μ (cm V-1 s-1)
3.8 10-4
3.6 10-4
3.4 10-4
3.2 10-4
3 10-4
2.8 10-4
Electrophoretic mobility of DNA fragments
in HEC 105,000
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130
0
0.005
0.01
0.015
0.02
0.025
0.03
HEC concentration (wt%)

Figure 3b

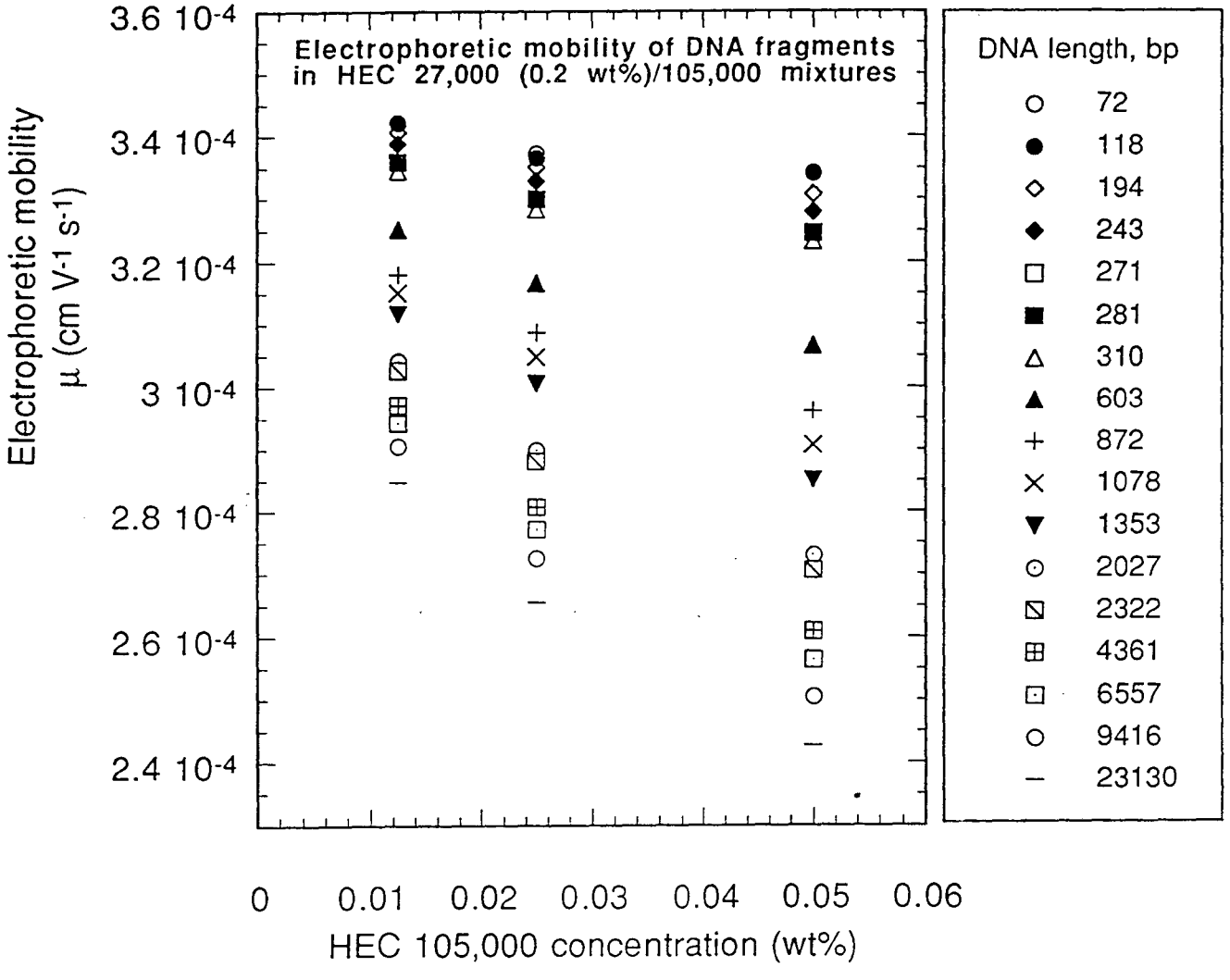
Electrophoretic mobility of DNA fragments in HEC 27,000 (0.2 wt%)/105,000 mixtures
Electrophoretic mobility μ (cm V-1 s-1)
3.6 10-4
3.4 10-4
3.2 10-4
3 10-4
2.8 10-4
2.6 10-4
2.4 10-4
0
0.01
0.02
0.03
0.04
0.05
0.06
HEC 105,000 concentration (wt%)
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130

Figure 4a

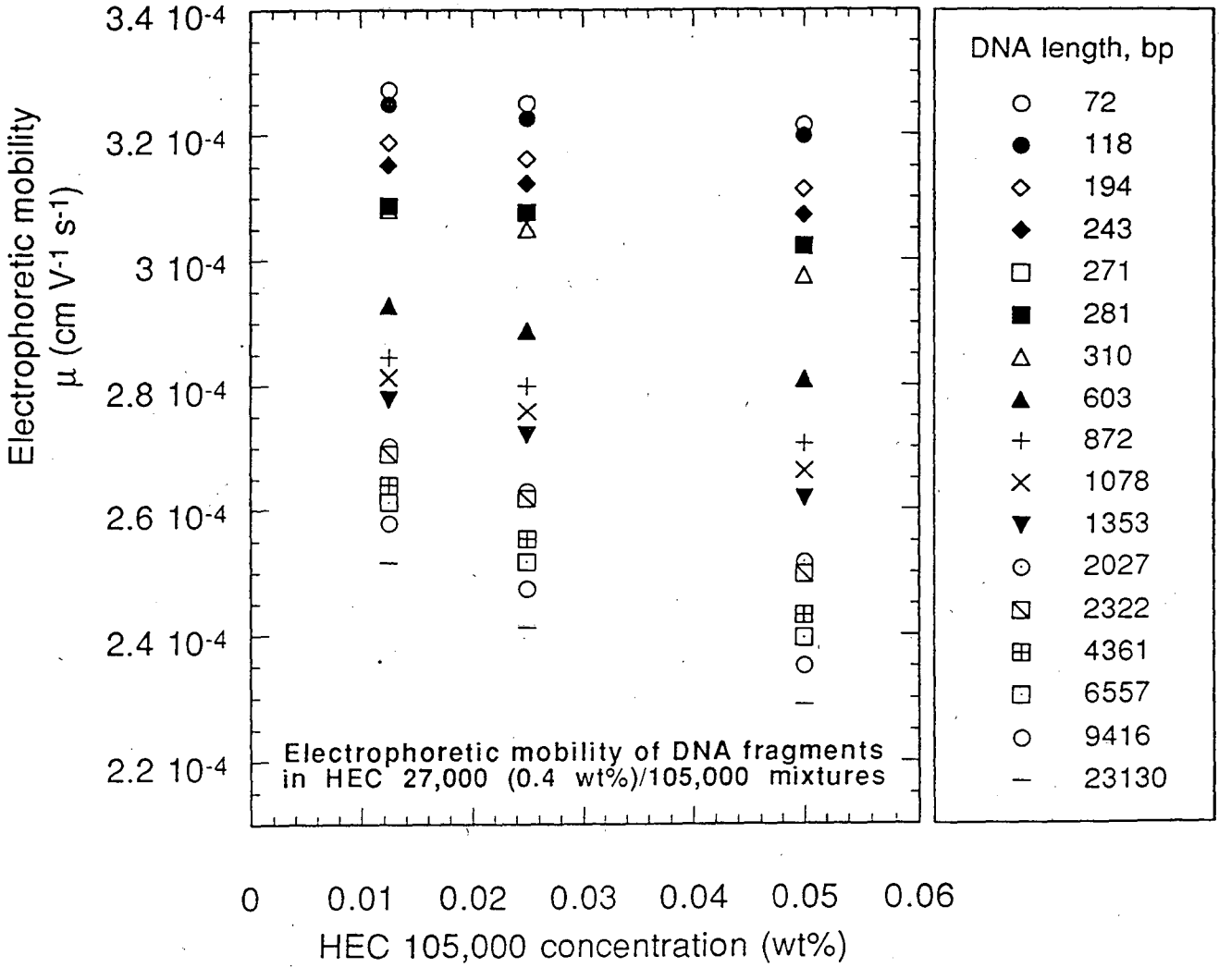
Electrophoretic mobility
μ (cm V-1 s-1)
3.4 10-4
3.2 10-4
3 10-4
2.8 10-4
2.6 10-4
2.4 10-4
2.2 10-4
Electrophoretic mobility of DNA fragments
in HEC 27,000 (0.4 wt%)/105,000 mixtures
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130
0
0.01
0.02
0.03
0.04
0.05
0.06
HEC 105,000 concentration (wt%)

Figure 4b

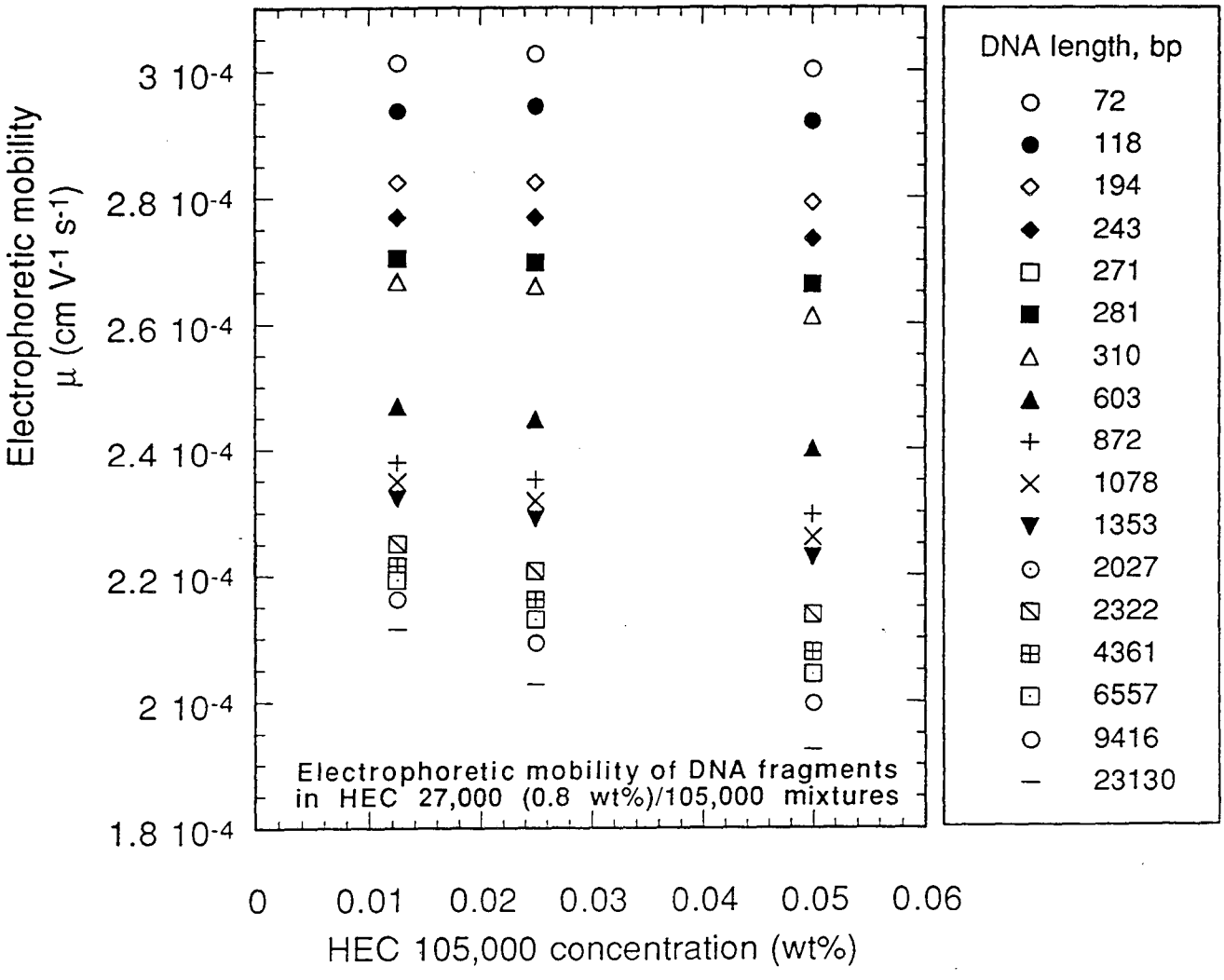
Electrophoretic mobility
μ (cm V-1 s-1)
3 10-4
2.8 10-4
2.6 10-4
2.4 10-4
2.2 10-4
2 10-4
1.8 10-4
0
0.01
0.02
0.03
0.04
0.05
0.06
HEC 105,000 concentration (wt%)
Electrophoretic mobility of DNA fragments
in HEC 27,000 (0.8 wt%)/105,000 mixtures
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130

Figure 4c

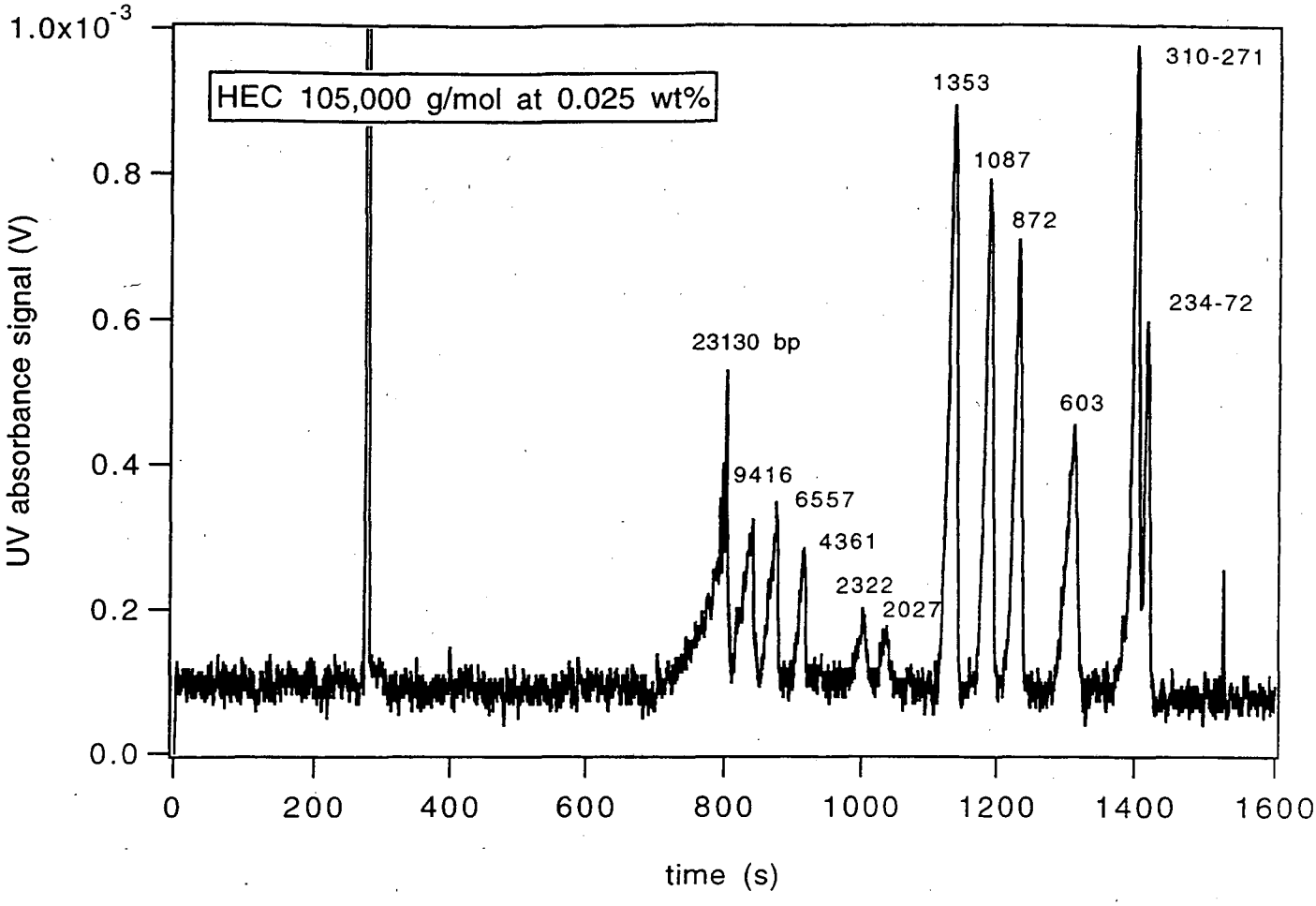
1.0x10^-3
0.8
0.6
0.4
0.2
0.0
UV absorbance signal (V)
HEC 105,000 g/mol at 0.025 wt%
23130 bp
9416
6557
4361
2322
2027
1353
1087
872
603
310-271
234-72
0
200
400
600
800
1000
1200
1400
1600
time (s)

Figure 5a

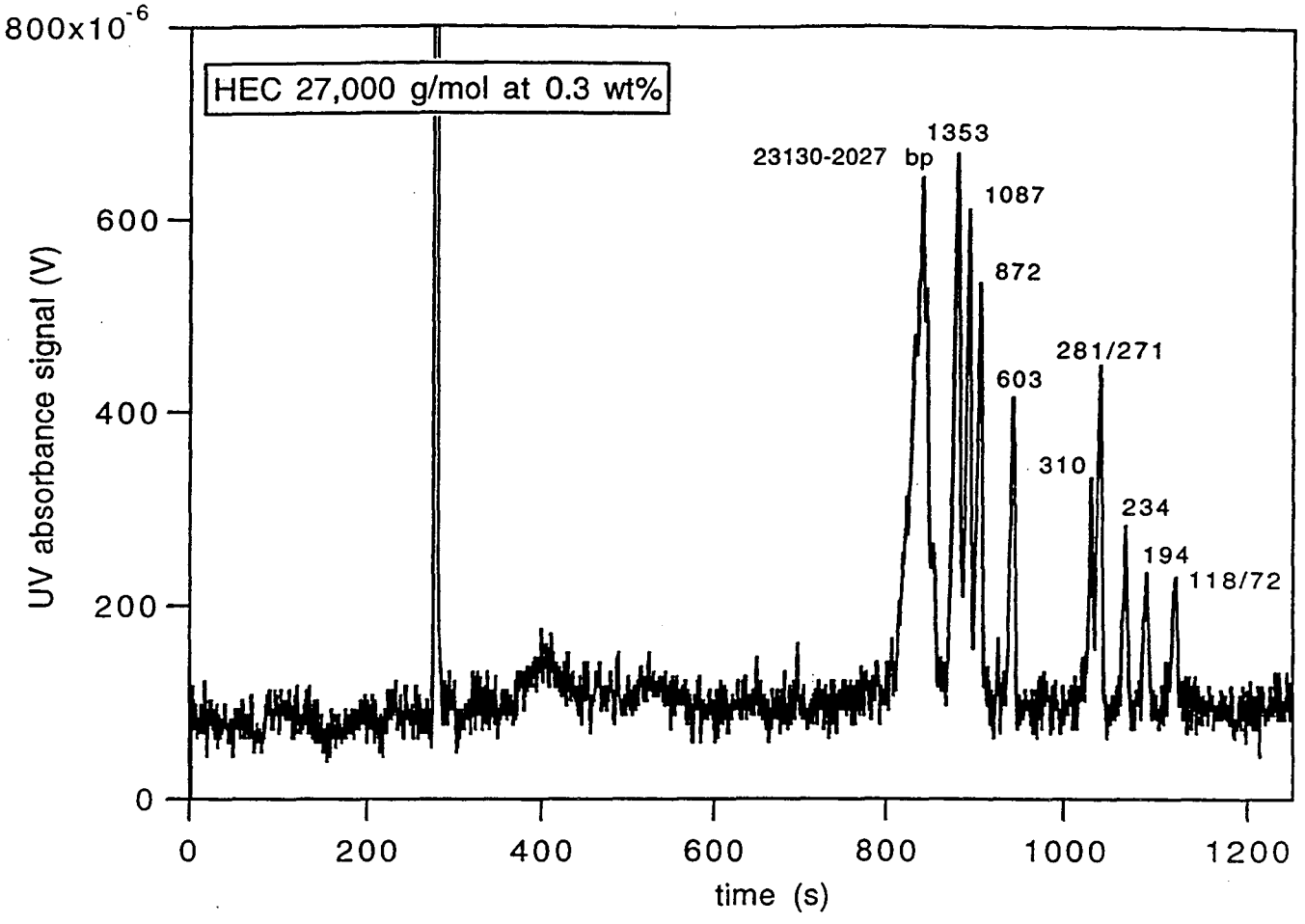
800x10^-6
HEC 27,000 g/mol at 0.3 wt%
23130-2027 bp
1353
1087
872
603
281/271
310
234
194
118/72
UV absorbance signal (V)
0
200
400
600
0
200
400
600
800
1000
1200
time (s)

Figure 5b

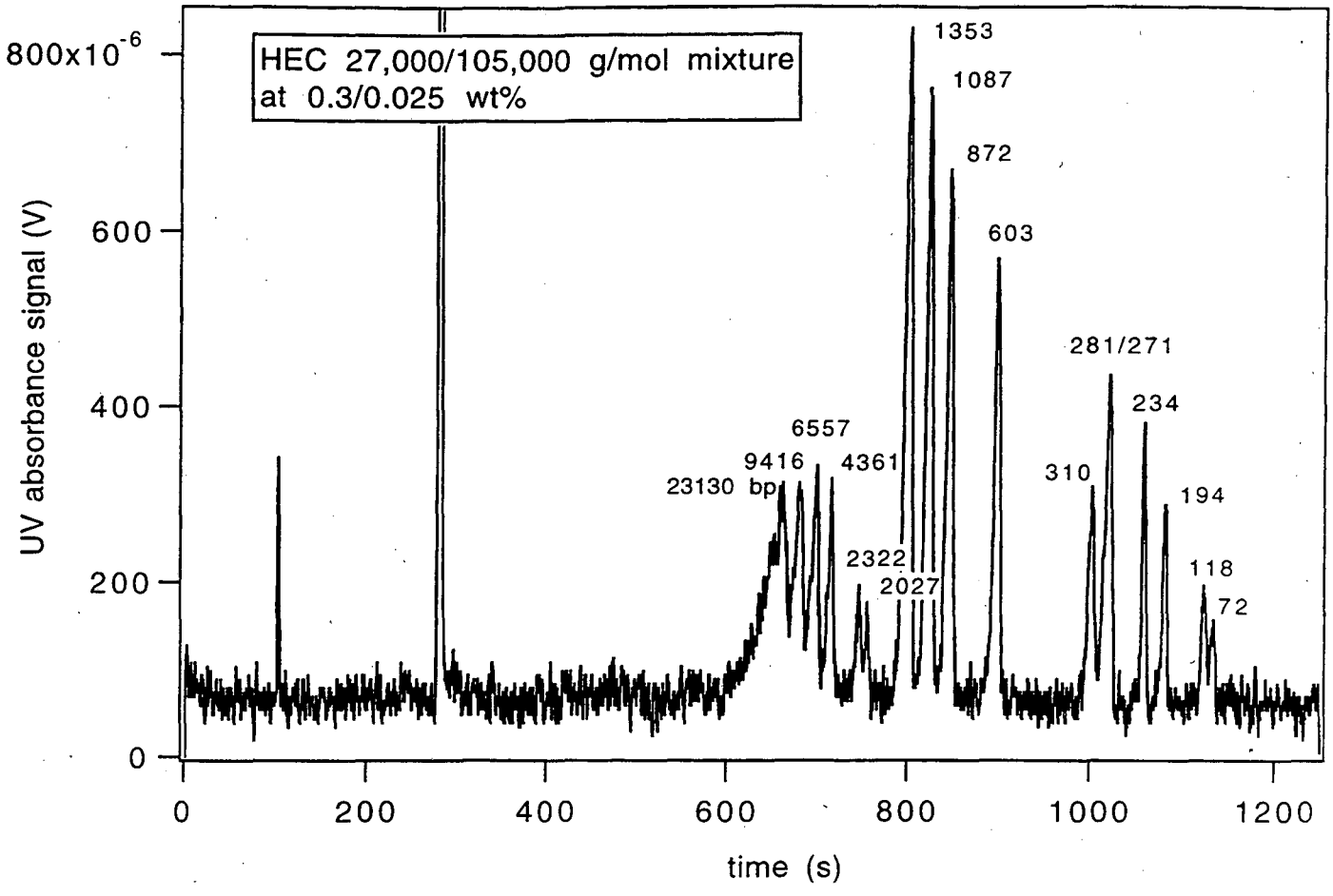
HEC 27,000/105,000 g/mol mixture
at 0.3/0.025 wt%
800x10-6
600
400
200
0
UV absorbance signal (V)
0
200
400
600
800
1000
1200
time (s)
23130 bp
9416
6557
4361
2322
2027
1353
1087
872
603
310
281/271
234
194
118
72

Figure 5c

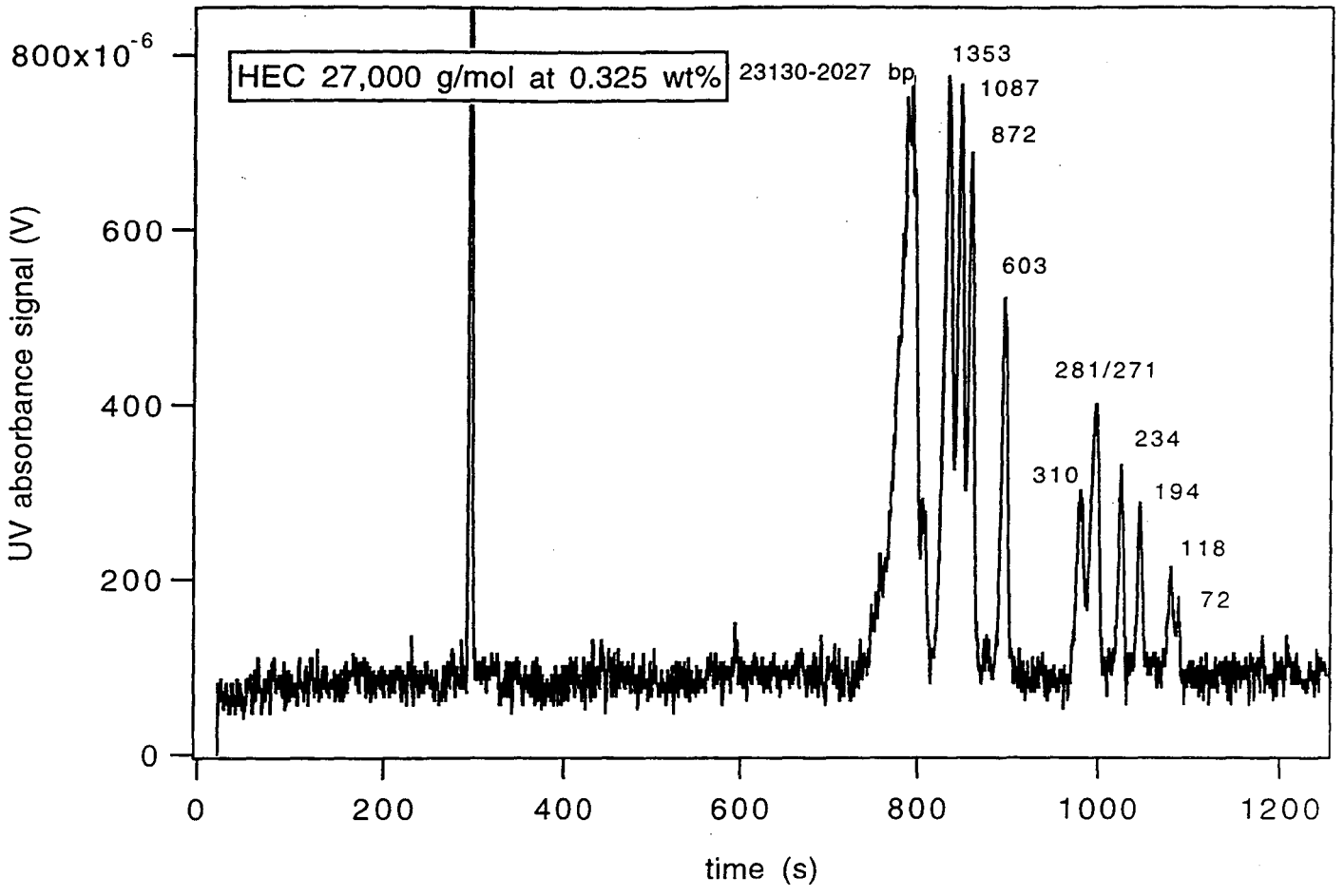
HEC 27,000 g/mol at 0.325 wt%
23130-2027 bp
1353
1087
872
603
281/271
234
310
194
118
72
800x10^-6
600
400
200
0
UV absorbance signal (V)
0
200
400
600
800
1000
1200
time (s)

Figure 5d

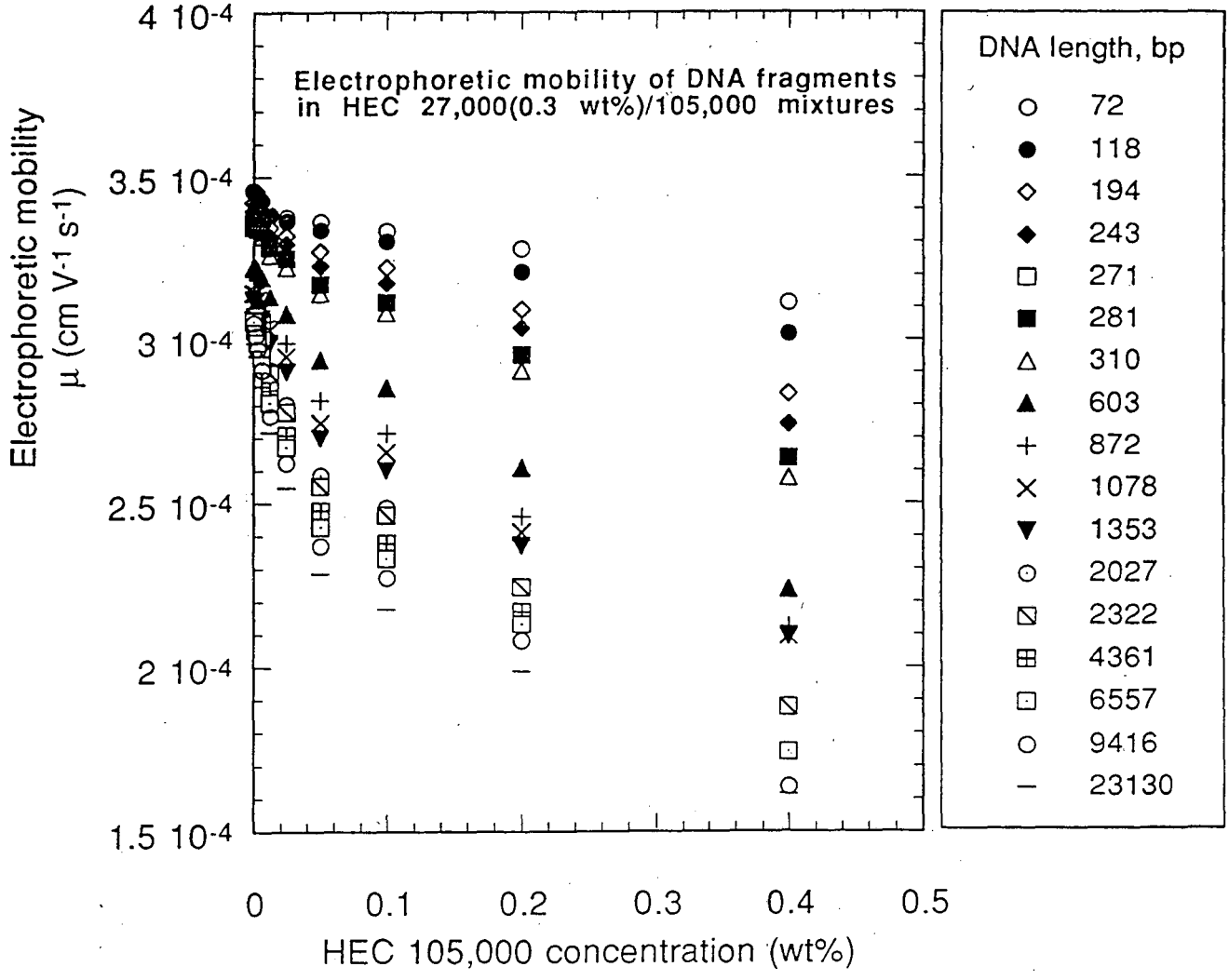
Electrophoretic mobility of DNA fragments
in HEC 27,000(0.3 wt%)/105,000 mixtures
Electrophoretic mobility
μ (cm V-1 s-1)
4 10-4
3.5 10-4
3 10-4
2.5 10-4
2 10-4
1.5 10-4
0
0.1
0.2
0.3
0.4
0.5
HEC 105,000 concentration (wt%)
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130

Figure 6a

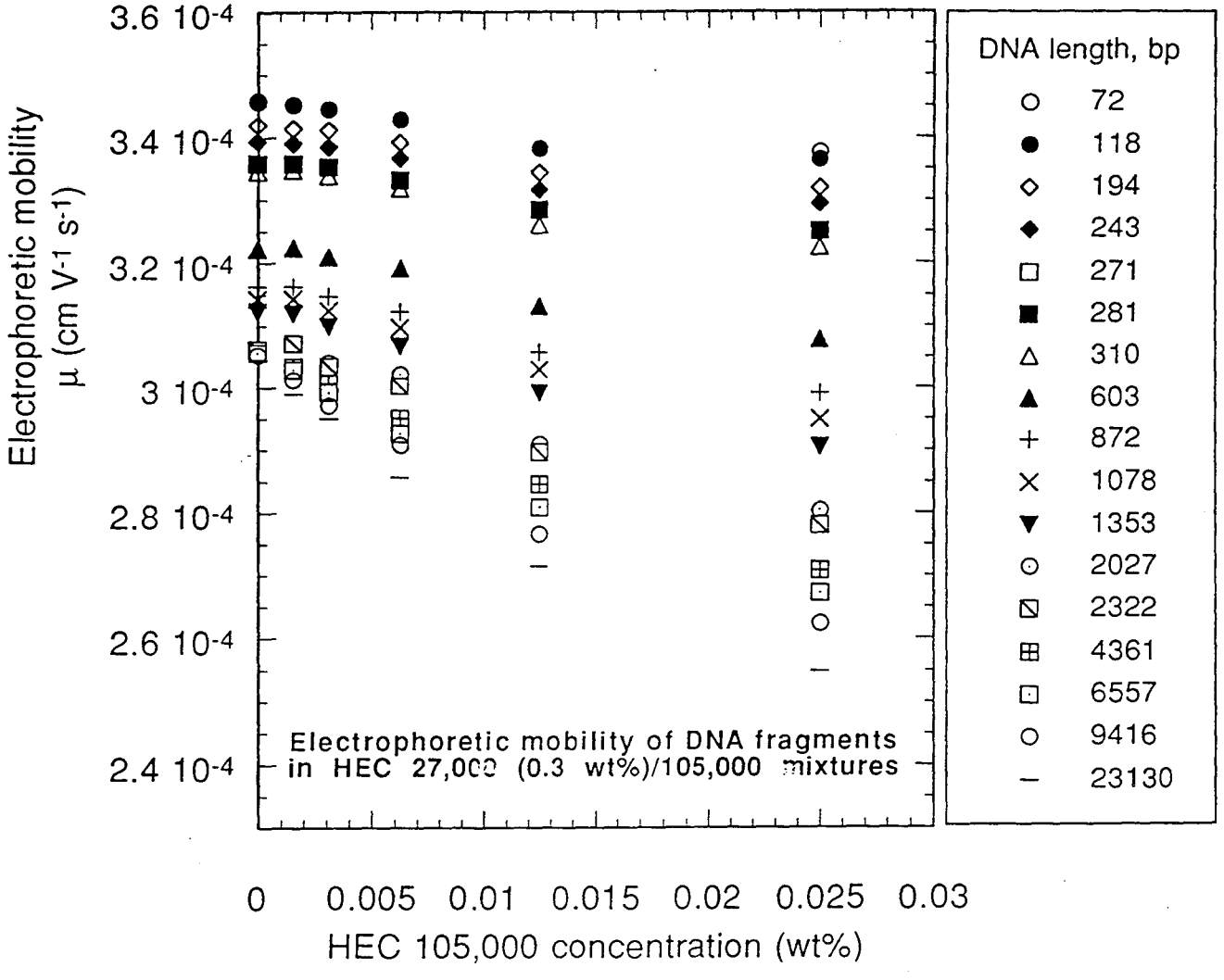
Electrophoretic mobility
μ (cm V-1 s-1)
3.6 10-4
3.4 10-4
3.2 10-4
3 10-4
2.8 10-4
2.6 10-4
2.4 10-4
Electrophoretic mobility of DNA fragments
in HEC 27,000 (0.3 wt%)/105,000 mixtures
DNA length, bp
72
118
194
243
271
281
310
603
872
1078
1353
2027
2322
4361
6557
9416
23130
0
0.005
0.01
0.015
0.02
0.025
0.03
HEC 105,000 concentration (wt%)

Figure 6b

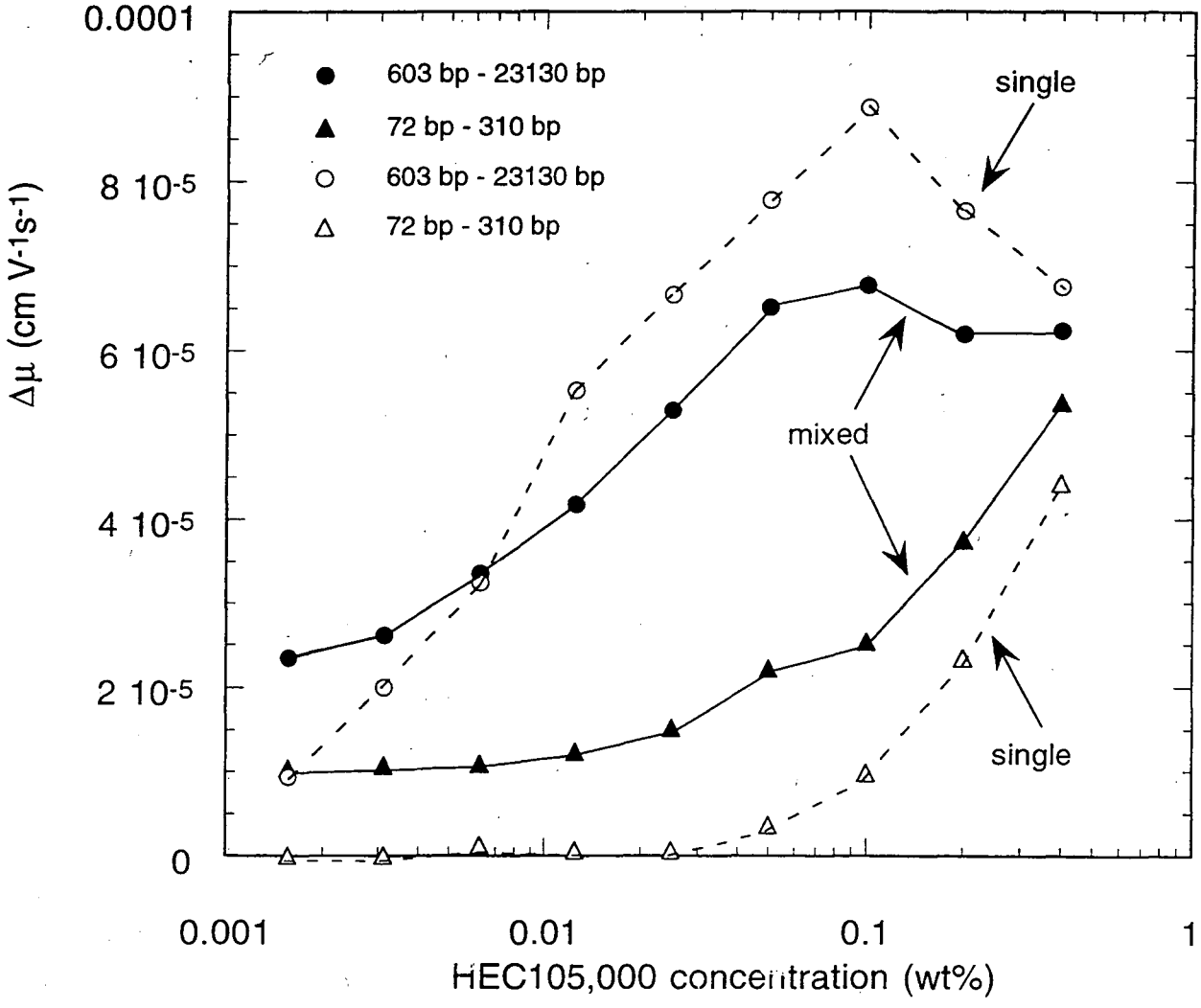
0.0001
8 10-5
6 10-5
4 10-5
2 10-5
0
Δμ (cm V-1s-1)
603 bp - 23130 bp
72 bp - 310 bp
603 bp - 23130 bp
72 bp - 310 bp
single
mixed
single
0.001
0.01
0.1
1
HEC105,000 concentration (wt%)

Figure 7

LAWRENCE BERKELEY NATIONAL LABORATORY
UNIVERSITY OF CALIFORNIA
TECHNICAL & ELECTRONIC INFORMATION DEPARTMENT
BERKELEY, CALIFORNIA 94720