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ELECTROPHORESIS

**Separation of DNA by Capillary
Electrophoresis in Uncoated Silica Columns
Using Hydroxypropylmethyl Cellulose
as the Sieving Matrix**

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ABSTRACT

The use of uncoated fused silica capillary columns and hydroxypropylmethyl cellulose (HPMC) in capillary electrophoresis (CE) to separate DNA ladders and plasmids is described. In these studies HPMC is used as a sieving matrix to separate DNA. Many previous studies have focused on using coated and gel-filled capillaries to

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separate DNA fragments. These techniques suffer from several disadvantages including expense and column degradation over time. Few studies have focused on using uncoated capillary columns to separate and analyze DNA. In this paper we describe the use of HPMC and uncoated fused silica capillary columns to separate DNA fragments and plasmids isolated from the *Escherichia coli* strain RRI. We believe that the use of dynamically coated capillaries with polymer solutions provides for a stable, reproducible, less toxic method for the analysis of biopolymers by CE.

INTRODUCTION

Capillary electrophoresis (CE) is a versatile technique for separating biomolecules that has gained wide acceptance as a complimentary analytical tool to high performance liquid chromatography (HPLC).^[1-3] In CE molecules are separated based on their charge to mass ratio. Capillary electrophoresis has been used to separate a wide range of molecules including proteins, carbohydrates, oligonucleotides, anions, cations, and neutral compounds. During the past decade a number of different CE applications have been developed including micellar electrokinetic chromatography (MEKC), capillary isoelectric focussing, capillary electrochromatography (CEC), capillary isotachopheresis, and capillary gel electrophoresis (CGE).

In CGE the capillary is filled with a gel (polymer) that is either a viscous fluid or solid. This polymer functions as a sieving matrix and separates molecules based on their size. The polymer concentration and respective pore size retards species during electrophoresis. Two types of gels have been employed to separate DNA fragments; one, chemically cross-linked gels with high viscosity, and; two, non-cross-linked gels of low viscosity.

Cross-linked gels, or chemical gels, have well-defined pore structure and are usually covalently bound to the capillary wall to prevent electroosmotic flow (EOF) during electrophoresis thereby reducing changes in pore size. They are heat sensitive and are susceptible to bubble formation. Non-cross-linked gels, or physical gels, have a flexible pore structure and are not covalently bound to the capillary wall. Their pore size can be modified easily by changing electrophoresis parameters (e.g., temperature, voltage, buffer ionicity). Unlike chemical gels, physical gels, are not heat sensitive and are not as susceptible to formation of bubbles during electrophoresis.



Separation of DNA by Capillary Electrophoresis

453

Capillary gel electrophoresis is a powerful tool for the analysis of oligonucleotides mainly because of its high efficiency and resolving power.^[4–15] For example, CGE has been used to analyze PCR products and restriction enzyme digest fragments and as a check of purity of primers for DNA sequencing, point mutation analysis, and DNA diagnostics.^[16–18] Perhaps its greatest role has been in the sequencing of the human genome.

Much of this work in CGE has involved cross-linked polymers such as polyacrylamide mainly because of its success in separating DNA and proteins in slab gel electrophoresis. Physical gels have also become very popular in DNA separations including non-crosslinked polyacrylamides,^[4–8] cellulosic derivatives,^[9–13] and poly(ethylene oxide) (PEO).^[14,15] Although a number of advances have been made in the separation of DNA fragments by gels most of this work has utilized coated capillary columns that have been found to be unstable with prolonged use. In addition, the polymerization process is difficult to reproduce from one column to the next. Because of the inherent problems associated with coated columns a more reliable separation technique using uncoated fused silica columns is warranted.

Although HPMC has been utilized in earlier studies to separate DNA, this work employed expensive coated capillary columns and was conducted in a negative polarity setting requiring long experimental runs.^[19–27] In this article, we report our findings on the use of uncoated fused silica capillary columns and HPMC in the separation of DNA base pair ladders and in the digestion of plasmid to linear DNA by CE. This work demonstrates the advantages of using CE and uncoated columns to separate DNA and plasmids.

EXPERIMENTAL

Chemicals and Reagents

All chemicals were analytical grade. pUC18 DNA *MspI* digest, step ladder from lambda DNA, 1 kb ladder were purchased from Sigma Chemical Company (St. Louis, MO, USA) and used without further purification. Mesityl oxide (MO) was purchased from Calbiochem (San Diego, CA, USA). Stock solutions of pUC18 (50/50 v/v buffer) was prepared by dissolving the DNA in buffer (89 mM Tris–89 mM boric acid–2 mM EDTA–1 μ M ethidium bromide; pH 8.3). The running buffer consisted of varying concentrations of HPMC in similar buffer.



Apparatus

The capillary electrophoresis (CE) system used in this study was a Beckman Model P/ACE 5510 (Fullerton, CA, USA). The capillary tubing (Polymicro Technologies, Inc., Phoenix, AZ, USA) used for the ACE experiments was of uncoated fused silica with an internal diameter of 50 μm , length from inlet to detector of 40.5, and a length from detector to outlet of 6.5 cm. The conditions used in CE were as follows: voltage, 12 kV current, 7.6–8.0 μA ; detection, 260 nm; temperature, $25.0 \pm 0.1^\circ\text{C}$. Data were collected and analyzed with Beckman System Gold software.

Procedures

The capillary was initially rinsed with base (0.1 N NaOH), distilled water, and buffer for 2 min. The DNA sample was injected for 6 s at high pressure and electrophoresed in buffer containing HPMC until elution of all species.

RESULTS AND DISCUSSION

In the first experiment DNA fragments from pUC18 *MspI* digest were examined by CE. The pUC18 plasmid is isolated from the *Escherichia coli* strain RRI. It confers ampicillin resistance and complement defects in β -galactosidase in appropriate host strains. *MspI* is an endonuclease from the *Moraxella* species that recognizes the 5'C/CGG-3' sequence in DNA. Twelve fragments ranging from 26–501 base pairs (bp) result from its digestion with *MspI*. The fragments provide a good reference for polymerase chain reaction (PCR) samples because it shows the range of 26–501 bp. High pressure injection was utilized in the experiments. Figure 1(A) shows the separation of the pUC18 *MspI* digest fragment mixture using 1.5% HPMC ($M_n = 86,000$, $n = 4000$ cps) as the sieving matrix. A good separation was achieved of all 12 fragments in less than 14 min. In uncoated capillaries a strong electroosmotic flow (EOF) exists and migration of DNA is toward the negative electrode. Hence, the largest DNA fragments, with the lowest electrophoretic mobilities, elute first from the capillary column. It was not possible to fully separate with baseline resolution the 489 and 501 bp fragments using 1.5% HPMC. The use of 0.5% (Fig. 1(B)) or 0.8% (Fig. 1(C)) HPMC yielded broad peak



Separation of DNA by Capillary Electrophoresis

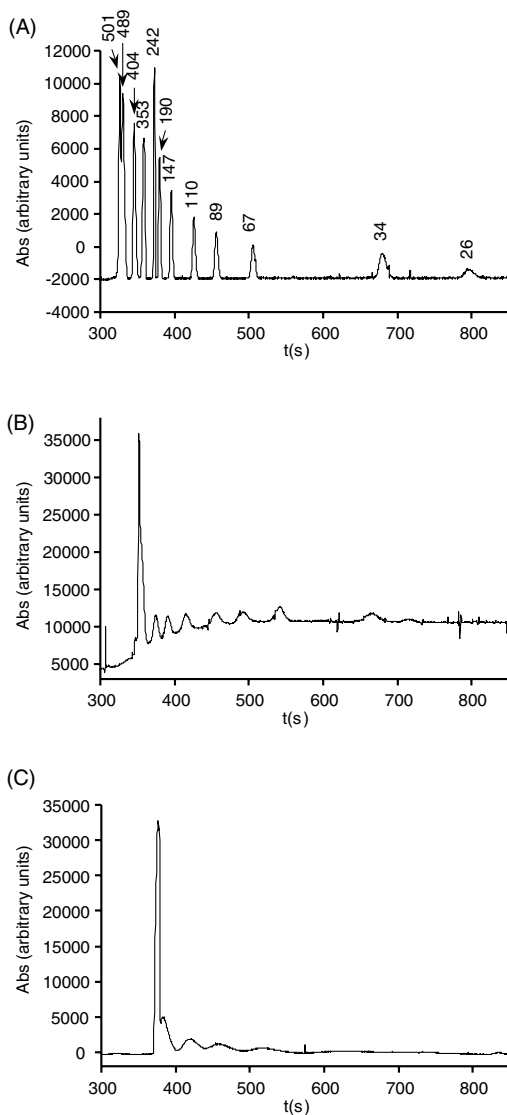


Figure 1. Separations of pUC18 *MspI* digest DNA fragments using (A) 1.5%, (B) 0.8%, and (C) 0.5% HPMC ($M_n = 86,000$, $n = 4000$ cps) (89 mM Tris–89 mM boric acid–2 mM EDTA–1 μ M ethidium bromide; pH 8.3). The total analysis time in each experiment was 15.0 min at 12 kV (current: 7.6–8.0 μ A) using a 40.5-cm (inlet to detector), 50- μ m i.d. open, uncoated quartz capillary.



shapes and reduced baseline resolution than that found using 1.5% HPMC. Of particular interest is the net migration of the initial DNA fragments. In uncoated columns, DNA separation is faster using the higher concentration of HPMC. This is because the DNA fragments elute faster with the running buffer since more HPMC molecules hinder their electrophoretic migration. No peak separation was obtained for the 489 and 501 bp fragments. With 0.8% HPMC only eight peaks were observed in the electropherogram. With 0.5% HPMC only five peaks were observed the majority of which were broad. In addition, the migration times of the DNA fragments increased as the amount of HPMC was decreased. A plot of electrophoretic mobility (μ) vs. log bp of the DNA fragments yielded an S-shaped curve (Fig. 2) for the 1.5% HPMC separation. With cross-linked polyacrylamide gels, a plot of μ vs. log bp (or molecule mass) for nucleic acids should yield a straight line.^[28] It can be seen that an S-shaped curve is obtained. The shallowness of the slope of the curve is a measure of the molecular sieving power of the medium. It has been shown that the steeper the slope of the curve, the less effective is the sieving. In comparison to previous studies the present polymer is quite effective in its sieving ability.^[28] Attempts to separate DNA using a greater percentage of HPMC neither realized improved separation or baseline resolution. In addition, bubbles were more pronounced leading to more noise in the electropherogram.

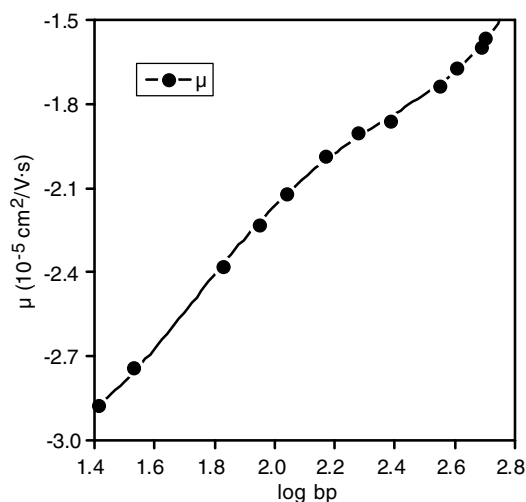


Figure 2. Plot of electrophoretic mobility (μ) vs. log basepair (bp) for Fig. 1(A).



Separation of DNA by Capillary Electrophoresis

457

In a separate experiment 2% HPMC ($M_n=90,000$, $n=15,000$ cps) was used to determine if a change in viscosity could effect peak separation. Using the same *MspI* digest a similar peak pattern was obtained as that seen for 1.5% HPMC (viscosity 4000 cps) (Fig. 3). The only difference was that the largest peak fragments (353, 404, 489, and 501 bp) could not be resolved or with baseline resolution. In addition, the largest two fragments (489 and 501 bp) eluted as one peak like the lower viscosity of HPMC. A similar linear relationship between μ and log bp was obtained (Fig. 2) with this concentration and viscosity of HPMC.

We also examined a step-ladder containing a DNA ladder size ranging from 50 to 3147 bp from lambda DNA. Figure 4(A) shows the separation of this step-ladder using 2% HPMC ($M_n=90,000$, $n=15,000$ cps). Although baseline resolution could be achieved on the majority of fragments appreciable noise in the electropherogram was observed. A similar S-shaped curved was obtained as that found using a 1.5% HPMC solution (Fig. 4B). There is also deviation from linearity with the larger DNA fragments. This result has been observed by others and can be attributed to electric field effects.^[29] It has been shown that field effects on mobility may occur in CE at much smaller fragment sizes than found in conventional gel electrophoresis because the field strength in CE is generally much higher. The separation does not proceed as nicely using the lower viscosity polymer as a number of DNA fragments elute at similar migration times.

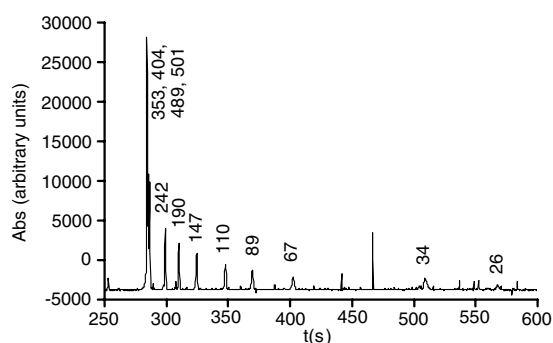


Figure 3. Separation of pUC18 *MspI* digest DNA fragments using 2% HPMC ($M_n=90,000$, $n=15,000$ cps) (89 mM Tris–89 mM boric acid–2 mM EDTA–1 μ M ethidium bromide; pH 8.3). The total analysis time was 10.0 min at 12 kV (current: 7.6–8.0 μ A) using a 40.5-cm (inlet to detector), 50- μ m i.d. open, uncoated quartz capillary.

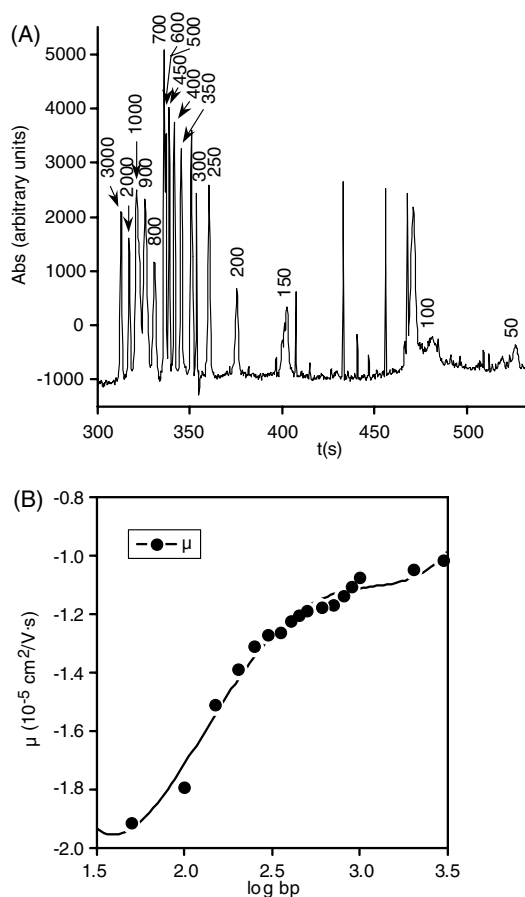


Figure 4. (A) Separation of 50–3147 bp ladder from lambda DNA using 2% HPMC ($M_n=90,000$, $n=15,000$ cps) (89 mM Tris–89 mM boric acid–2 mM EDTA–1 μ M ethidium bromide; pH 8.3). The total analysis time was 10.0 min at 12 kV (current: 7.6–8.0 μ A) using a 40.5-cm (inlet to detector), 50- μ m i.d. open, uncoated quartz capillary. (B) Plot of μ vs. log bp.

Finally, we used HPMC to separate a DNA plasmid and its open-chain linear DNA form. We initially showed that the pUC18 plasmid elutes in a 1% solution of HPMC polymer ($M_n=86,000$, $n=4000$ cps) yielding a single peak for the plasmid (Fig. 5(A)). We then reacted the pUC18 plasmid off-column with the endonuclease *AccI* cutting the



Separation of DNA by Capillary Electrophoresis

459

plasmid at one site, thereby, generating a linear DNA fragment of the same DNA structure as the plasmid (Fig. 5(B)). The hydrodynamic radius of the linear DNA is much smaller than for the plasmid. Hence, it was more greatly affected by the HPMC sieving matrix than the plasmid and eluted at a greater migration time.

Only a few studies have focused on using uncoated capillary columns to analyze DNA.^[21–27] The problems associated with the separation of DNA using uncoated fused silica columns are well known. One, DNA is a sticky molecule and has a high propensity to adsorb onto uncoated capillary columns. Two, DNA molecules show a mobility independent of molecular weight due to their constant linear charge density making separation difficult.

The use of HPMC and uncoated fused silica capillary columns has several advantages as a method to separate biopolymers. First, it does not cause changes in pore size unlike the covalently coated columns which have a propensity to lyse over time. Second, the electrophoresis is

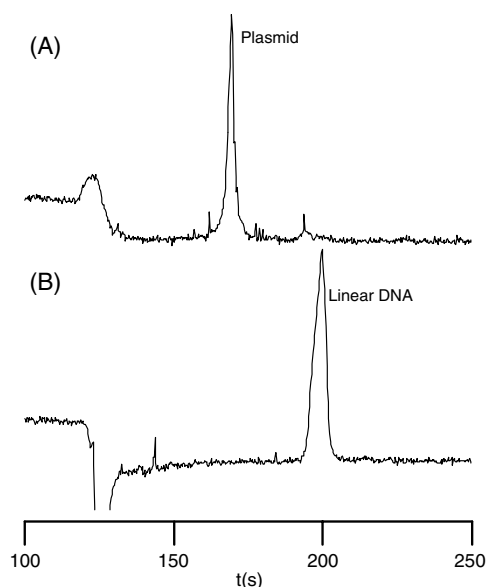


Figure 5. Separation of pUC18 plasmid and linear DNA after *AccI* digest using 1% HPMC ($M_n = 86,000$, $n = 4000$ cps) (89 mM Tris–89 mM boric acid–2 mM EDTA–1 μ M ethidium bromide; pH 8.3). The total analysis time was 6.0 min at 12 kV (current: 7.6–8.0 μ A) using a 40.5-cm (inlet to detector), 50- μ m i.d. open, uncoated quartz capillary.



conducted in a positive polarity setting, thereby decreasing the length of time for the experiment. Third, the column can be conveniently refilled with polymer using pressure injection technique.

CONCLUSION

The work presented here successfully demonstrates the use of hydroxypropylmethyl cellulose (HPMC) and uncoated fused silica capillary columns to separate DNA fragments and plasmids. The use of dynamically coated capillaries with polymer solutions provides for a stable, reproducible, less toxic method for the analysis of biopolymers by CE. The percentage and viscosity of HPMC were varied thereby changing the separation of DNA fragments. The use of 1.5% HPMC ($M_n = 86,000$, $n = 4000$ cps) successfully separated fragments from pUC18 digest. A corresponding experiment with 2% HPMC ($M_n = 90,000$, $n = 15,000$ cps) showed a similar separation. The use of 2% HPMC ($M_n = 90,000$, $n = 15,000$ cps) was successful in separating a 50–3147 bp ladder from lambda DNA. Plots of electrophoretic mobility vs. log basepair yielded linear relationships. A 1% HPMC ($M_n = 86,000$, $n = 4000$ cps) solution separated DNA plasmid from the corresponding linear DNA molecule generated by reaction with the endonuclease *AccI*. Further work to demonstrate the versatility of HPMC is in progress.

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Separation of DNA by Capillary Electrophoresis

461

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Separation of DNA by Capillary Electrophoresis

463

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