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Modification of the Electrokinetic Properties of Reversible Electrophoresis Gels for the Separation and Preparation of DNA*

Addition of Linear Polymers

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Abstract

The effect of adding linear polymers to a novel reversible electrophoretic media was measured. Reversible gels are formed using the polyanionic carbohydrate polymer, gellan gum. Gellan gum forms strong stable gels in the presence of divalent cations or diamines. The gels are reversible (return to solution) by changing the ionic environment or pH. Gellan gum is an anionic polymer, and the electrophoresis gels have considerable electroosmotic flow (EOF) toward the negative electrode. We measured the EOF in gellan gum electrophoresis gels as a function of gel concentration, buffer composition, and linear polymer additive. The linear polymers used in this study were polyethylene oxide and hydroxyethyl cellulose. Both polymers reduced EOF in the gels, in a manner dependent on molecular weight. Polymers with high molecular weight were more effective at reducing EOF. The addition of polymers increased the resolution of low molecular weight DNA. Native gellan gum resolved DNA from approx 50,000 to 1000 bp. Addition of the polymers resolved DNA down to approx 50 bp, in some instances. The influence of the polymers on circular plasmid DNA was also investigated. Addition

*Certain commercial equipment, instruments, and materials are identified in this article to specify adequately the experimental procedures. Such identification does not imply recommendation by the National Institute of Standards and Technology nor does it imply that the materials or equipment are necessarily the best available for the purpose.

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of high molecular weight polyethylene oxide reduced the electrophoretic mobility of the nicked circular form compared to the supercoiled form.

Index Entries: Electrophoresis gel; DNA; electroosmosis; separation; reversible; preparative; gellan gum.

Introduction

In a recent publication, we introduced new reversible gels for the separation and isolation of DNA *(1)*. These gels are based on the use of a polyanionic carbohydrate polymer, gellan gum (referred to as gellan). In the original report *(1)*, we explored the use of adding a polymer (hydroxyethyl cellulose [HEC]) to a gellan gel, and it was found that the resolution of low molecular weight DNA was increased. Adding additional polymers to the basic gellan electrophoresis matrix is a powerful tool to modify the characteristics of the gel, but there were no guidelines to help us select the conditions for the separations. Adding polymers to gellan gels both influences the electrokinetic properties of the gel and affects DNA electrophoretic mobilities. The present study was motivated by the need for analytical data necessary to guide the selection of conditions for selective preparative separations of DNA.

The inspiration for adding polymers to gellan gels came from a few important references on the addition of polymers to agarose gels *(2)*, and from a large base of literature on the use of polymer solutions in capillary electrophoresis *(3–7)*. These systems are quite different from gellan. Agarose is a carbohydrate polymer that is neutral except for varying amounts of pyruvate and sulfate groups covalently attached to the polymer. Agarose gels are thermally set by formation of the polymer strands that form double helixes and higher-order formation into bundles with large spaces or pores between the bundles of double helixes (reviewed in ref. *8*). Capillary electrophoresis is done in the lumen of a fused silica tube. The walls of the capillary have a uniform charge owing to charged silanol groups. The chemical and physical properties of these systems are quite different from those of gellan gels held together by divalent cations or diamines. It was discovered that it was essential to add a low concentration of divalent cation or diamine to the electrophoresis buffer and rapidly circulate the buffer. If this was not done, the gel dissolved, starting from the end at the positive electrode. This indicates that the gel crosslinking is probably dynamic and also illustrates another way to reverse the gel (using an electric field).

We feel that gellan is a very promising preparative technique for the isolation of DNA and potentially other biological compounds. We regard gellan as a platform that functions as a reversible support in which to do electrophoretic separations. This base matrix is extremely flexible, forming gels at concentrations as low as 0.03%, and additional polymers can be added at concentrations greater than 10% without destroying the gel-forming capabilities of the gellan. More data are needed to guide the use of gellan for electrophoretic separations.

Gellan is made up of linear repeats of a tetrasaccharide unit. The repeating units consist of β-D-glucose, β-D-glucuronic acid, β-D-glucose, and α-L-rhamnose *(9,10)*. The glucuronic acid imparts a negative charge along the gellan gum chain at regular intervals. The average molecular weight of gellan was measured at approx 50,000 *(11)*. Gellan undergoes a transition from an unordered state at temperatures above approx 36°C to an ordered state corresponding to a double helix formation at lower temperatures *(12)*. The nature and concentration of anions in the solution determine the degree of aggregation and gel formation. Recent studies of gellan gum crystals indicate the structure to be a left-handed, threefold parallel helix *(13,14)*. When gellan gum is produced by fermentation, it is partially esterified by acetate and glycerate groups. The commercially available products are processed to remove most of these groups *(15)*.

We chose to concentrate mainly on two polymers, HEC and polyethylene oxide (PEO). These polymers have the advantages of high solubility in aqueous solutions, and they are available in a range of molecular weights. They have different chemical and physical properties. HEC is derived from a naturally occurring carbohydrate and PEO is made by a polymerization reaction. We examined gellan gum gel concentration, buffer used, molecular weight of the polymer, and polymer concentration as variables that could influence electroosmotic flow (EOF). We used mixtures of linear DNAs and circular plasmid DNAs with different molecular weights to determine the separation in the gels. The circular plasmid DNA preparations contained both supercoiled and nicked circular (relaxed) forms.

Materials and Methods

Materials

PEO was obtained from Aldrich (Milwaukee, WI) with approximate molecular weights of 5,000,000 (5M, product no. 18,947-2); 1,000,000 (1M, product no. 37,278-1); and 200,000 (200K, product no. 18,199-4). HEC with approximate molecular weights of 1,300,000 (1.3M, product no. 43,498-1), 250,000 (250K, product no. 30,863-3), and 90,000 (90K, product no. 43,496-5) were obtained from Aldrich. These average molecular weights were determined by viscosity. Dextran with an average mol wt of 485,000 (#T500) was obtained from Pharmacia (Piscataway, NJ). Stock solutions of polymers were made in buffer by shaking a flask on a rotary mixer until dissolved. The high molecular weight polymer solutions took periods up to several days to dissolve fully. One-kilobase DNA ladder, lambda DNA, restriction enzymes, agarose (low EO), and boric acid were obtained from Life Technologies (Rockville, MD). Phytagel (gellan gum), Dowex-50WX8-40, Ficoll 400 (mol wt 400,000), tris(hydroxymethyl)aminomethane (Tris), bis(2-hydroxyethyl)iminotris-(hydroxymethyl)methane (Bis-Tris), 1,3-diamino-2-hydroxypropane (DAHP), myoglobin (horse heart), and cyanocobalamin were obtained from Sigma (St. Louis, MO).

Table 1
Compositions and Properties of the Buffers[a]

Buffer	Composition	pH	Cond. (μS/cm)	EOF	BPB	Corrected BPB
TG (1 mmol/L $CaCl_2$)	3.9 mmol/L Tris, 47 mmol/L glycine, 1 mmol/L $CaCl_2$	8.4	350	15.0 ± 1.0 (n = 3)	13.2 ± 0.8 (n = 3)	28.3
BBE (5 mmol/L DHAP)	22 mmol/L Bis-Tris, 75 mmol/L boric acid, 1 mmol/L EDTA, 5 mmol/L DAHP	7.5	654	12.9 ± 0.3 (n = 3)	11.7 ± 1.3 (n = 3)	24.6
BBE (1 mmol/L DHAP)	22 mmol/L Bis-Tris, 75 mmol/L boric acid, 1 mmol/L EDTA, 1 mmol/L DAHP	6.8	310	—	—	—

[a]Cond., conductivity. BPB, bromophenol blue. Mobilities and EOF are in units of $\times 10^{-5}$ cm^2 V^{-1} s^{-1}.

Buffers and Solutions

Table 1 gives the buffers used in this study. DNA samples were diluted with buffer containing Ficoll at a mol wt of 400,000 (2% by weight, final concentration) or sucrose (5% by weight, final concentration), and bromophenol blue (0.1 mg/mL).

Gel Formation

Gellan gum (potassium salt) was prepared starting from Phytagel using a deionization and precipitation procedure *(16)*. Gellan gum solutions were prepared by a boiling procedure on a hot plate. The powder was placed in a flask along with water (80 or 70% of the final volume, depending on final additions; *see below*) and a stir bar. The solution was heated to boiling and stirred for 10 min to ensure that all particles of the polymer were dissolved. A concentrated solution of either $CaCl_2$ (50 mmol/L) or DAHP (50 mmol/L) was added so that the final concentration was 5 mmol/L. A solution of buffer (10-fold concentrated) was also added at this time. In some cases, a solution of polymer or the dry powder was added by weight. The solution was stirred for an additional 10 min. The temperature of the solution was reduced to approx 60°C, and the solution was poured into a gel tray and allowed to solidify for 45 min at ambient temperature and 20 min at 4°C. The solidified gel was flooded with buffer (950 mL) and the comb removed.

Electrophoresis Apparatus

A flat-bed submarine gel electrophoresis apparatus (Econo-Submarine, CBS Scientific, 14 cm wide × 20 cm long tray; VWR Scientific, Boston, MA) was used. Water was circulated through the cooling chamber and maintained at 20°C by the use of a circulating water bath. Buffer was removed

from one electrode chamber through a coil of tubing in the water bath and returned to the opposite electrode chamber by means of a peristaltic pump. The gel slab anchors at the ends of the gel tray (14 × 20 cm) were enlarged, using a small rotary grinder. For some gels, strips of porous polyethylene (1.5 mm thick × 5 mm wide) were taped along the edge of the open ends (facing the electrode chamber) of the gel tray. These strips stabilize the gel into position, while allowing the free passage of buffer and the electric field. A comb with 16 teeth (2 mm thick) was suspended in the gel to form the sample wells. The strips are necessary for gels below 0.1% in concentration, but with care the gels can be handled without them. The gels are brittle and must remain on the gel tray or they will break.

Staining and Imaging Gels

Gels were always supported by the use of a gel tray during staining and imaging. Gels were stained in custom-made acrylic boxes that closely fit the gel trays. Solutions were removed from the gels with a turkey baster. DNA gels were stained using ethidium bromide (1 µg/mL) in 0.1 mol/L of KCl with gentle shaking for 30–60 min. The gels were destained for 20 min in a solution of 0.1 mol/L of KCl, followed by 20 min in 0.5 mmol/L of EDTA, pH 6.5, for 20 min. Gels were placed on either a light box or ultraviolet transilluminator and imaged.

An alternative way to stain the DNA in gellan gels with much higher sensitivity (5- to 10-fold) is to use SYBR Green I stain (Molecular Probes, Eugene, OR). Gels are stained using a 1:10,000- or 1:5000-fold dilution of the concentrated stock solution in a buffer (0.1 mol/L of KCl, 0.01 mol/L of Tris, and 1 mmol/L of EDTA, pH 7.5). The gels were destained using the same solution and photographed using the recommended filters.

Measurements of EOF and Electrophoretic Mobility

A solution of cyanocobalamin was prepared by dissolving 7 mg in 0.25 mL of ethanol and then diluting to 5 mL of buffer containing 20% sucrose (by weight). After electrophoresis, the lanes of the gel were cut into strips (10 mm wide). A 10% solution (by volume) of mesityl oxide was prepared in TG buffer containing 20% sucrose (by weight). The lane strips were cut every 5 mm from the well (the origin), forming rectangular cubes of gel (each representing 5 mm of migration from the origin). The cubes were placed into tubes and 0.1 mL of 45 mmol/L of EDTA added along with 0.7 mL of water. The tubes were mixed to dissolve the gel, and the solute was placed into a cuvet for absorbance measurements.

Before measuring mobility, the gels were preelectrophoresed for at least 2 h at 4 V/cm. EOF and the electrophoretic mobility of visible markers were calculated by measuring migration every 30 min for a total of 2 h. The electric field was halted and the mobility of the visible markers was measured from the wells. The electric field was then turned back on for the next period. The data points (distance migrated vs time) were entered on a

spreadsheet, and a linear regression program (data forced through origin) was used to calculate the slope of the line. The fit of the data to the line gave an R^2 value >0.95.

Plasmid DNA and DNase I Treatment

Plasmid DNA preparations of pBR322 (4.4 kb), pDELTA2 (8.0 kb), and pYA101 (13.1 kb) were from Life Technologies. The entire bottle of lyophilized DNase I (20,000 U) (Boehringer Mannheim, Indianapolis, IN) was gently suspended in 1 mL of 20 mmol/L of Tris and 1 mmol/L of $MgCl_2$, pH 7.5, and stored as aliquots at –80°C. The reaction buffer was 50 mmol/L of Tris and 10 mmol/L of $MgCl_2$, pH 7.5. Dilutions (1:100,000 and 1:1,000,000) of DNase I were freshly prepared in reaction buffer. The diluted DNase I solutions (10, 5, 2, and 1 µL) were added to tubes containing 0.5 µg of plasmid DNA in a total of 50 µL of reaction buffer. The tubes were incubated for 1 h at ambient temperature (21°C). A solution (2 µL) of 0.5 mol/L of EDTA, pH 8.0, was added to the tubes to stop the reaction.

Results

Measurement of EOF in Gellan Gum Gels

Measurement of EOF in gellan gum gels was facilitated by the use of the red marker, cyanocobalamin. Cyanocobalamin has a low mol wt of 1355, and has been used for the measurement of electroosmosis flow in agarose *(17)*. The mobility of cyanocobalamin was compared with the mobility of another low molecular weight neutral marker, mesityl oxide (4-methyl-3-penten-2-one; mol wt 98). The results in Fig. 1 show that cyanocobalamin and mesityl oxide had the same migration at this level of resolution (16.8 ± 0.5 cm migration distance). Because cyanocobalamin can be easily detected compared to the colorless marker, mesityl oxide, cyanocobalamin was used in all subsequent experiments as a marker for EOF.

Figure 2 shows the influence of the concentration of gellan gum on the mobility of bromophenol blue, a colored protein (myoglobin), and cyanocobalamin. The measurements of cyanocobalamin indicate that increasing the gel concentration only slightly increased the EOF at the highest gel concentration tested. The anionic dye bromophenol blue traveled toward the positive electrode with an apparent electrophoretic mobility. Adding the EOF to the apparent electrophoretic mobility of bromophenol blue yields a corrected electrophoretic mobility that should be the same as the free solution mobility, assuming that there are no interactions between the gel matrix and bromophenol blue. Table 1 shows the effect of buffer on the EOF. The buffer BBE5 has lower EOF and mobility of bromophenol blue. This is expected because of the lower pH of BBE, resulting in lower charges on the gel and dye.

The apparent mobility of the protein myoglobin is also shown in Fig. 2. Myoglobin is a protein with a p*I* near neutrality and has a low electro-

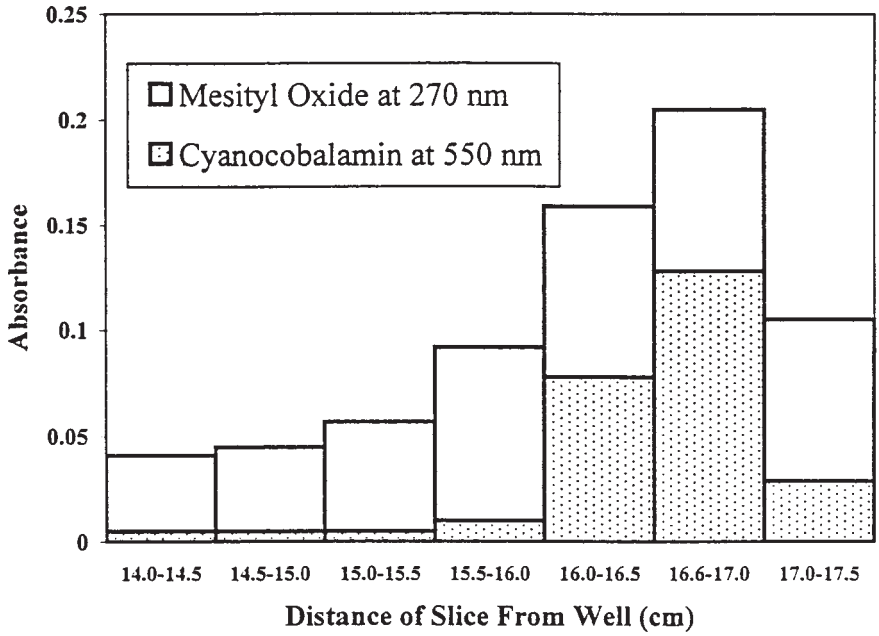


Fig. 1. Comparison of the migration of cyanocobalamin and mesityl oxide as markers for electroosmotic flow. A 0.2% gellan gum gel was cast using the buffer TG (5 mmol/L of $CaCl_2$). The running buffer was TG (1 mmol/L of $CaCl_2$). Gels were run at 2 V/cm for 20 min and then followed by 6.3 V/cm for 261 min. The gel was sliced and dissolved, and the absorbance was measured as described in the text.

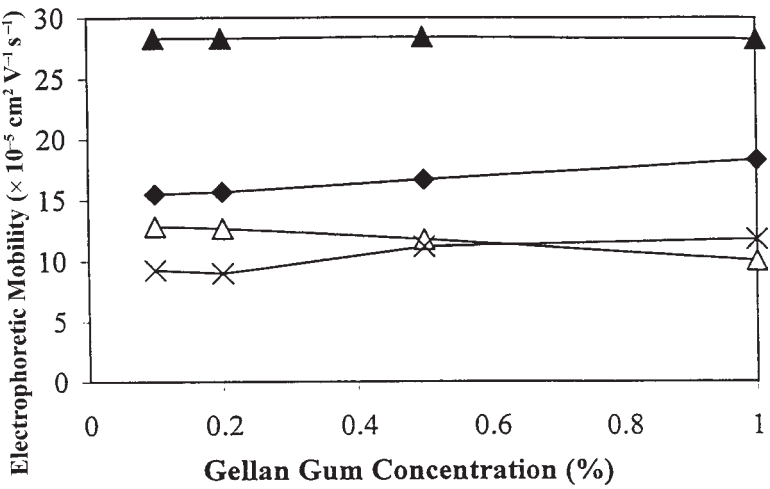


Fig. 2. Measurement of electroosmotic flow in native gellan gels as a function of gel concentration. The indicated concentration gels were cast using TG (5 mmol/L of $CaCl_2$). The running buffer was TG (1 mmol/L of $CaCl_2$). Wells were loaded with 40 µL of cyanocobalamin, bromophenol blue, or myoglobin. Gels were run at 2 V/cm for 3 h. Mobility was determined as described in Materials and Methods. (—◆—), Cyanocobalamin; (—△—), bromophenol blue; (—×—), myoglobin; (—▲—), corrected bromophenol blue.

phoretic mobility *(18)* in the TG buffer (pH 8.3). Myoglobin is carried toward the negative electrode by the EOF. The corrected mobility of myoglobin, in this case, will be the difference between the EOF and the apparent (measured) mobility. This average value, $5.8 \pm 0.2 \times 10^{-5}$ cm^2 V^{-1} s^{-1} (n = 4), is within the experimental error of the free solution value of the electrophoretic mobility previously measured *(18)*. The measured mobility of myoglobin did not decrease with increasing gel concentration, indicating that the gel does not exert a significant sieving effect on this relatively low molecular weight protein. The measurements of EOF (Table 1) indicate that the EOF in native gellan gels was measured at 15×10^{-5} cm^2 V^{-1} s^{-1}. The measurements of EOF in agarose gels indicate that native gellan gels have approx 5- to 10-fold higher values compared to agarose *(17,19–21)*. The addition of high molecular weight PEO or HEC to gellan gum gels brings the EOF down to levels comparable to the values in agarose gels.

Influence of Polymers on EOF in Gellan Gum Gels

Figure 3A shows the influence of PEO, with a mol wt of 5,000,000 (PEO 5M), on the EOF in gellan gum electrophoresis gels. The high molecular weight PEO is extremely effective at reducing EOF. Figure 3B shows the effect of PEO molecular weight on the reduction of EOF. The lower molecular weight PEO requires significantly higher concentrations to achieve the same reduction of EOF compared to the high molecular weight PEO.

High molecular weight HEC with a mol wt of 1,300,000 (HEC 1.3M) was also effective at reducing EOF (Fig. 4). The lower molecular weight HEC also required higher concentrations to reduce EOF compared to the high molecular weight polymer. Comparison of approximately equivalent average molecular weights of PEO and HEC (1M vs 1.3M and 200K vs 250K in Figs. 3B and 4) indicates that HEC is more effective at reducing EOF.

Influence of Polymers on Mobility and Resolution of Linear DNA

A DNA ladder was used to investigate the effect of polymers on the electrophoretic mobility and resolution of linear DNA. This ladder has DNA ranging in size from 75 to 12,216 bp. Lambda DNA (48,502 bp) was used as a source of high molecular weight DNA. DNA mobility experiments were conducted at least twice, and the measurements were within 5% of each other.

Figure 5A shows the apparent electrophoretic mobility of linear DNA in native gellan gum gels. The horizontal dashed line indicates that the DNA below about 1000 bp is traveling at the same rate. The effect of adding high molecular weight PEO on the apparent mobility of DNA is also shown. The addition of the polymer appears to extend the limits of resolution of the DNA to lower molecular weights. As shown in Fig. 5A, adding low concentrations of PEO 5M increases the apparent mobility of the midrange and low molecular weight DNA, and adding higher concentrations has little effect on the apparent mobility of the mid and high molecular weight DNA.

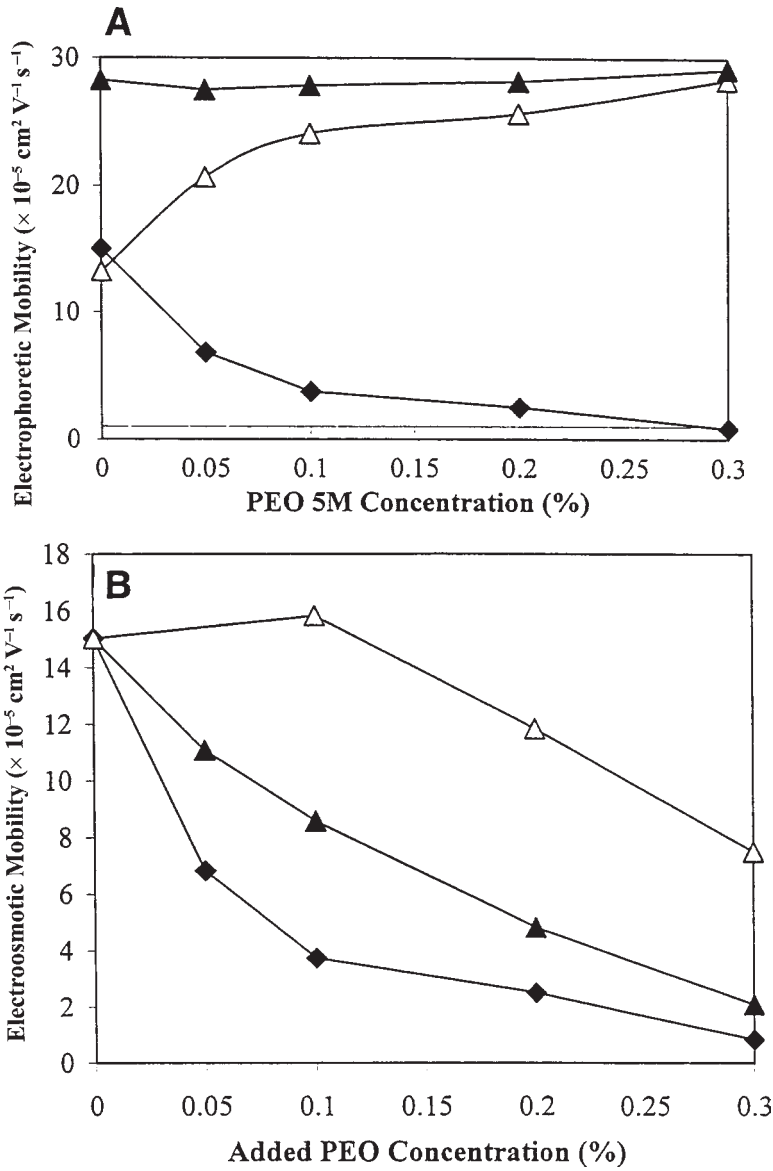


Fig. 3. **(A)** Measurement of electroosmotic flow in gellan gels as a function of added PEO concentration. Gellan gum gels (0.2%) were cast using the buffer TG (5 mmol/L of $CaCl_2$) and with the indicated PEO 5M added. The running buffer was TG (1 mmol/L of $CaCl_2$) and with the indicated PEO 5M added. Wells were loaded with 40 µL of cyanocobalamin or bromophenol blue. Gels were run at 2 V/cm for 3 h. Mobility was determined as described in Materials and Methods. (—◆—), Cyanocobalamin; (—△—), bromophenol blue; (—▲—), corrected bromophenol blue. **(B)** Measurement of electroosmotic flow in gellan gels as a function of added PEO concentration and molecular weight. Gellan gum gels (0.2%) were cast using the buffer TG (5 mmol/L of $CaCl_2$) and with the indicated PEO molecular weight added. The running buffer was TG (1 mmol/L of $CaCl_2$) and with the indicated PEO molecular weight added. Gels were run at 2 V/cm for 3 h. Mobility was determined as described in Materials and Methods. (—◆—), 5M PEO; (—▲—), 1M PEO; (—△—), 200K PEO.

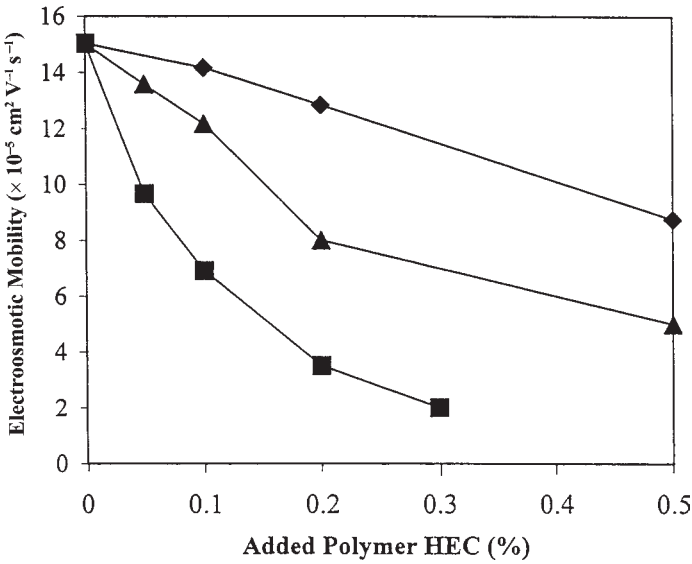
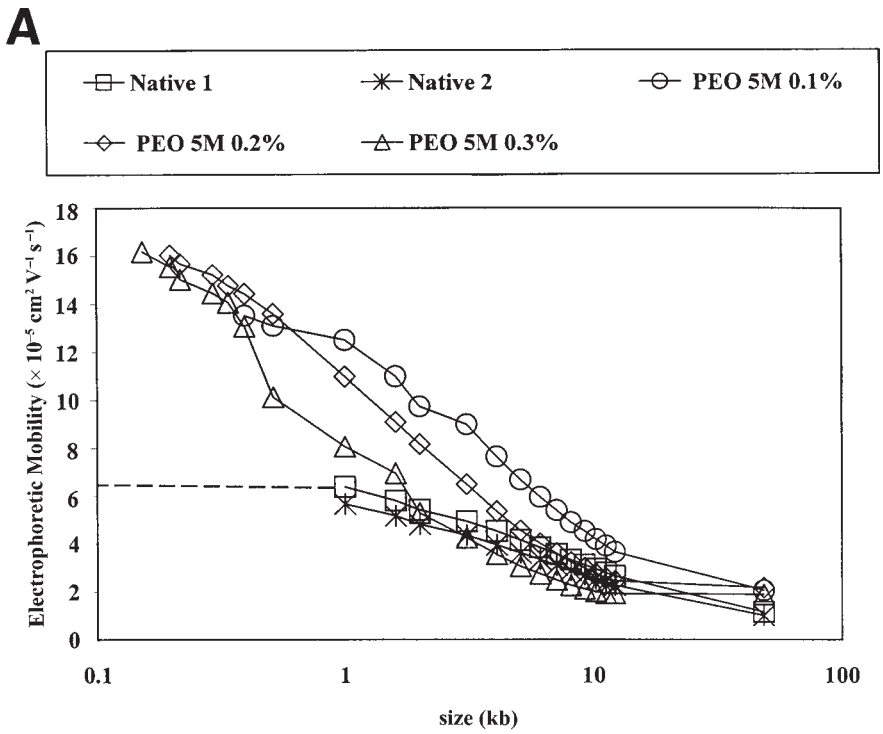


Fig. 4. Measurement of electroosmotic flow in gellan gels as a function of added HEC concentration and molecular weight. Gellan gum gels (0.2%) were cast using the buffer TG (5 mmol/L of $CaCl_2$) and with the indicated HEC molecular weight added. The running buffer was TG (1 mmol/L of $CaCl_2$) and with the indicated HEC molecular weight added. Gels were run at 2 V/cm for 3 h. Mobility was determined as described in Materials and Methods. (—■—), 1.3M HEC; (—▲—), 250K HEC; (—◆—), 90K HEC.

The reduction of EOF by the addition of the polymer would be expected to increase the apparent mobility of DNA in the gel, but the polymer in the gel can also interact with the DNA to affect the mobility. The interactions of DNA with the polymer are dependent on the sizes of the DNA and the polymer, with larger DNA being more likely to interact with high molecular weight polymer.

The measured EOF in the gel (cyanocobalamin mobility) was added to the apparent mobility to obtain a corrected mobility of the DNA (Fig. 5B). The effect of the polymer in the gel on the mobility of DNA now becomes apparent. The greatest effect of the polymer is on the retardation of the highest molecular weight DNA. In the native gel, DNA below about

Fig. 5. *(opposite page)* **(A)** Measurement of the apparent electrophoretic mobility of linear DNA in gellan gels as a function of PEO 5M concentration. Gellan gum gels (0.1%) were cast using the buffer BBE (5 mmol/L of DAHP) and with the indicated PEO 5M concentrations. The running buffer was BBE (5 mmol/L of DAHP) and with the indicated PEO 5M concentrations. Gels were run at 4 V/cm for 4 h. The dashed line indicates that all DNA below this size were traveling at the same rate. **(B)** Electrophoretic mobility data in (A) corrected by adding electroosmotic flow in each gel. The mobility of cyanocobalamin was added to the apparent mobility of the data shown in (A). The dashed line indicates that all DNA below this size were traveling at the same rate.


A
Native 1
Native 2
PEO 5M 0.1%
PEO 5M 0.2%
PEO 5M 0.3%
Electrophoretic Mobility (× 10⁻⁵ cm² V⁻¹ s⁻¹)
18
16
14
12
10
8
6
4
2
0
0.1
1
10
100
size (kb)

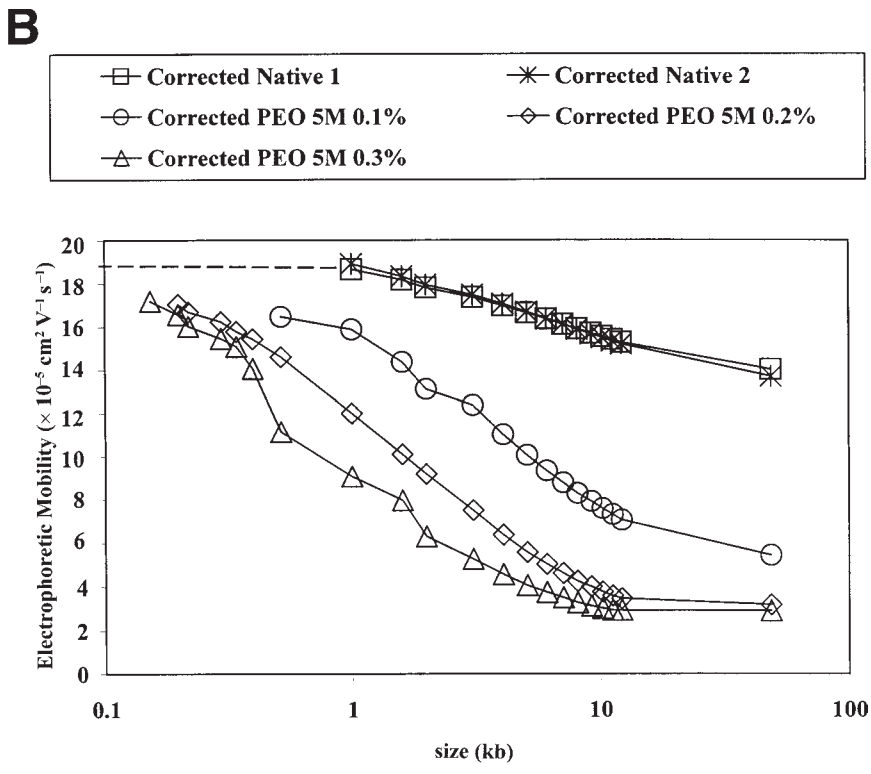

B
Corrected Native 1
Corrected Native 2
Corrected PEO 5M 0.1%
Corrected PEO 5M 0.2%
Corrected PEO 5M 0.3%
Electrophoretic Mobility (× 10⁻⁵ cm² V⁻¹ s⁻¹)
20
18
16
14
12
10
8
6
4
2
0
0.1
1
10
100
size (kb)

Fig. 5.

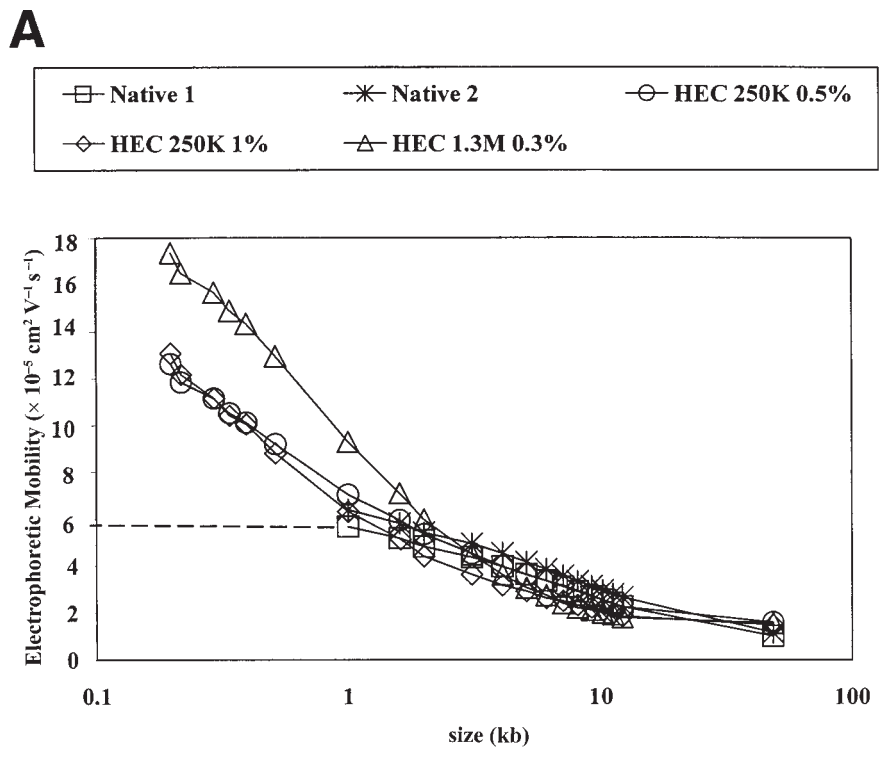
1000 bp is traveling through the gel at approximately the same high mobility (indicated by the dashed horizontal line in Fig. 5A,B). Increasing concentrations of high molecular weight PEO decreases the mobility of the high molecular weight DNA, allowing the lower molecular weight DNA to be resolved. This effect is obscured in the uncorrected mobility data (Fig. 5A) because of the effect of the polymer on reducing the EOF in the gel.

Figure 6A shows the apparent mobility of linear DNA in the presence of HEC. Two different molecular weights of HEC were used. In preliminary experiments, HEC 250,000 (HEC 250K) gave better results with resolution of low molecular weight DNA; therefore, we used HEC 250K and compared it to the HEC with the highest molecular weight HEC (1,300,000). Again, the effect of the polymer on EOF makes the apparent mobilities difficult to compare (Fig. 6A). The mobility of cyanocobalamin on each gel (the EOF) was added to the apparent mobility of the DNA to yield a corrected mobility. The corrected mobilities allow the effect of the added polymers on the mobilities of the DNA to be compared (Fig. 6B). The greatest effect, again, is seen on high molecular weight DNA. The effect of the two molecular weight HECs on the slope of the mobilities is quite different. HEC 1.3M reduces the mobility of the high molecular weight DNA to a greater extent compared to the low and midrange molecular weight DNA. HEC 250K reduces the mobility of the lower and midrange molecular weight DNA to a greater extent compared to HEC 1.3M.

Influence of PEO and HEC on the Mobility and Resolution of Circular DNA

Three plasmid preparations with different molecular weights were used to determine the separation of circular DNA in gellan gum gels. All these plasmid preparations have significant amounts of nicked circular along with the supercoiled circular DNA forms. The identity of the bands was determined by running samples of the plasmid preparations that had been treated with DNase I. Limited digestion of the DNA with DNase I readily converts the supercoiled DNA to the nicked form. With limited digestion, the supercoiled bands decreased in intensity whereas the nicked circular bands increased. More extensive digestion (more enzyme or a longer time) resulted in formation of the linear form and increased amounts of truncated forms of the DNA. Supercoiled and nicked circular bands were

Fig. 6. *(opposite page)* **(A)** Measurement of the apparent electrophoretic mobility of linear DNA in gellan gels as a function of HEC concentration. Gellan gum gels (0.1%) were cast using the buffer BBE (5 mmol/L of DAHP) and with the indicated HEC 250K or 1.3M concentrations. The running buffer was BBE (5 mmol/L of DAHP) and with the indicated HEC 250K or 1.3M concentrations. Gels were run at 4 V/cm for 4 h. The dashed line indicates that all DNA below this size were traveling at the same rate. **(B)** Electrophoretic mobility data in (A) corrected by adding electroosmotic flow in each gel. The mobility of cyanocobalamin was added to the apparent mobility of the data shown in (A). The dashed line indicates that all DNA below this size were traveling at the same rate.


A
Native 1
Native 2
HEC 250K 0.5%
HEC 250K 1%
HEC 1.3M 0.3%
Electrophoretic Mobility (× 10⁻⁵ cm² V⁻¹ s⁻¹)
18
16
14
12
10
8
6
4
2
0
0.1
1
10
100
size (kb)

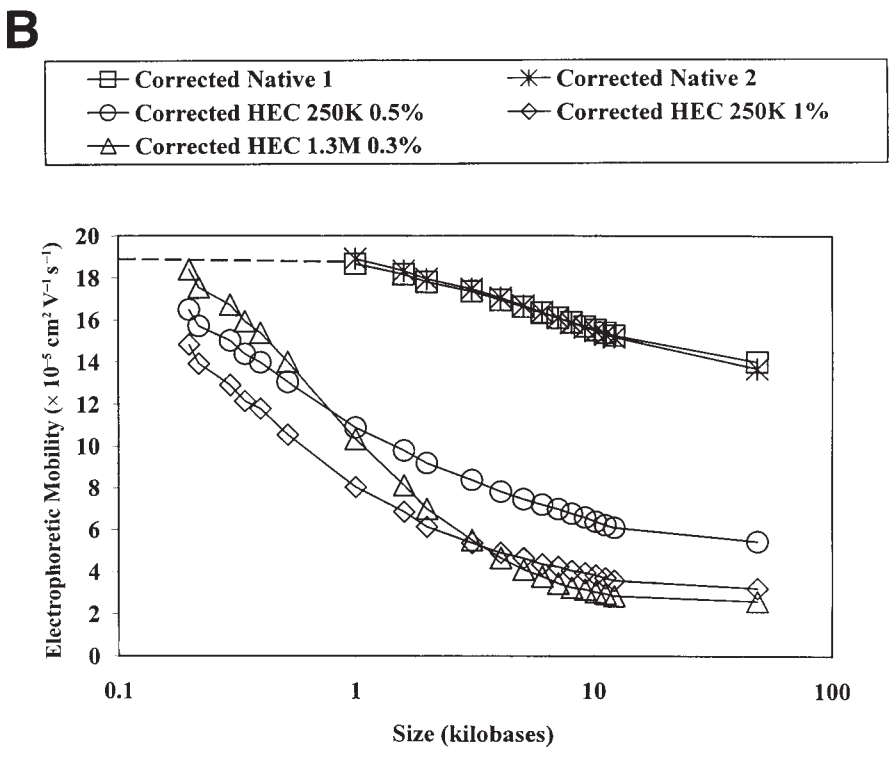

B
Corrected Native 1
Corrected Native 2
Corrected HEC 250K 0.5%
Corrected HEC 250K 1%
Corrected HEC 1.3M 0.3%
Electrophoretic Mobility (× 10⁻⁵ cm² V⁻¹ s⁻¹)
20
18
16
14
12
10
8
6
4
2
0
0.1
1
10
100
Size (kilobases)

Fig. 6.

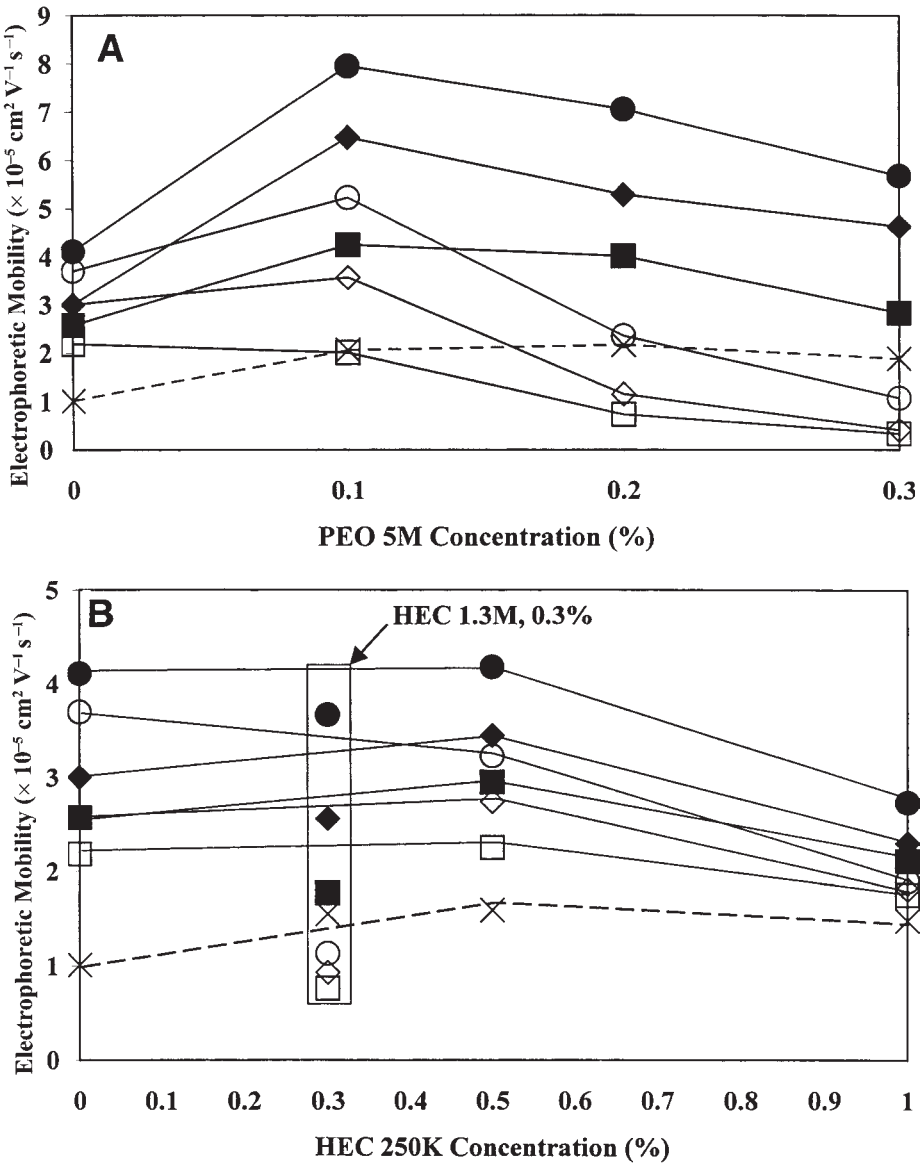


Fig. 7. **(A)** Measurement of the apparent electrophoretic mobility of circular DNA electroosmotic flow in gellan gels as a function of added PEO concentration. Gellan gum gels (0.1%) were cast using the buffer BBE (5 mmol/L of DAHP) and with the indicated PEO 5M concentrations. The running buffer was BBE (5 mmol/L of DAHP) and with the indicated PEO 5M concentrations. Gels were run at 4 V/cm for 4 h. **(B)** Measurement of the electrophoretic mobility of circular DNA as a function of added HEC concentration. Gellan gum gels (0.1%) were cast using the buffer BBE (5 mmol/L of DAHP) and with the indicated HEC 250K or 1.3M concentrations. The running buffer was BBE (5 mmol/L of DAHP) and with the indicated HEC 250K or 1.3M concentrations. Gels were run at 4 V/cm for 4 h. (—●—), 4.4-kb supercoiled; (—◆—), 8.0-kb supercoiled; (—■—), 13.1-kb supercoiled; (—×—), lambda DNA (48.5 kb); (—○—), 4.4-kb nicked circular; (—◇—), 8.0-kb nicked circular; (—□—), 13.1-kb nicked circular.

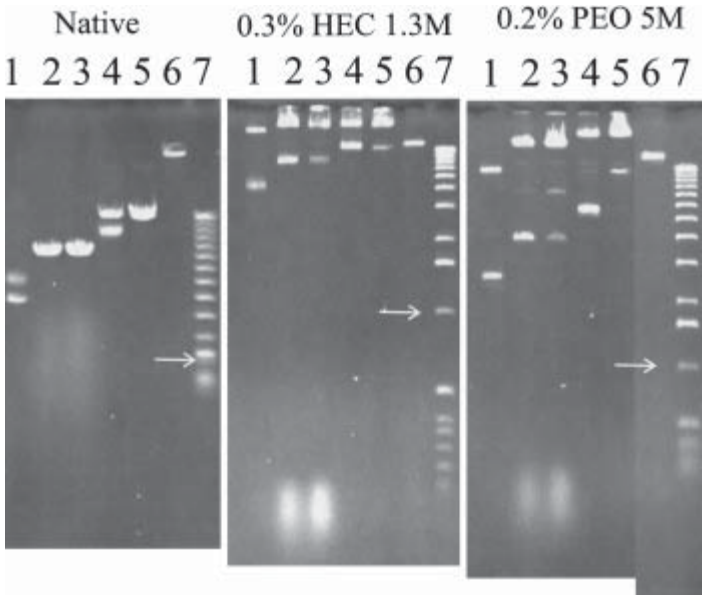


Fig. 8. Comparison of native gellan, and gels with HEC 1.3M **(middle)** and PEO 5M **(left)**. Gellan gels (0.1%) were cast using the buffer BBE (5 mmol/L of DAHP) and with the indicated polymer added. The running buffer was BBE (1 mmol/L of DAHP) with the indicated polymer. The gels were run at 2 V/cm for 31 min and 4 V/cm for 396 min (native); 2 V/cm for 21 min and 4 V/cm for 208 min (HEC gel); and 2 V/cm for 37 min and 4 V/cm for 188 min (PEO gel). Lane 1, pBR322 plasmid DNA; lane 2, pDELTA2 (8.0 kb); lane 3, pDELTA2 plasmid DNA-treated with DNase I; lane 4, pYA101 (13.1 kb) plasmid DNA; lane 5, pYA101 (13.1 kb) plasmid DNA-treated with DNase I; lane 6, lambda DNA; lane 7, kilobase DNA ladder. The arrow indicates the position of the 1-kb DNA band.

also isolated from gellan gum gels and run on agarose gel electrophoresis to confirm their identity.

In native gellan gum gels, the supercoiled and nicked circular forms from each of the plasmids had very similar apparent electrophoretic mobilities (Fig. 7A). On the native gels, the circular forms migrated close to the linear forms of the plasmid, indicating that native gellan gum gel electrophoresis is not very sensitive to shape of the DNA (results not shown). Addition of PEO 5M enhanced the separation between the circular forms of the three plasmids (Fig. 7A). Increasing concentrations of PEO 5M resulted in decreases in mobility of both forms of DNA, but the migration of the nicked circular form was decreased to a greater extent compared to the supercoiled form. Increasing the concentration of PEO to 0.3% resulted in very low apparent mobilities of the nicked circular forms. The dashed line in Fig. 7A indicates the apparent mobility of high molecular weight DNA (lambda DNA). The higher concentration of PEO also separates the circular forms from high molecular weight DNA, a likely contaminant in plasmid preparations.

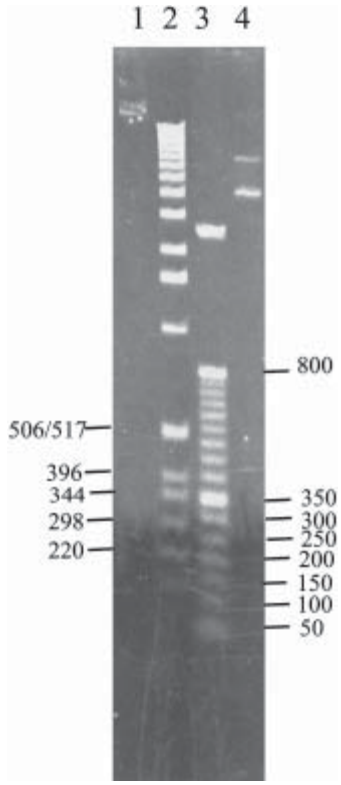


Fig. 9. Separation of low molecular weight DNA on a gellan gel with added HEC 250K. A gellan gel (0.1%) was cast using the buffer BBE (5 mmol/L of DAHP) and with 0.5% HEC 250K. The running buffer was BBE (1 mmol/L of DAHP). The gel was run at 8.6 V/cm for 2.5 h. Lane 1, lambda DNA; lane 2, 1-kb DNA ladder; lane 3, 50-bp ladder; lane 4, pBR322 plasmid DNA.

Figure 7B shows the effect of two different molecular weights of HEC on the mobilities of the circular DNA. This indicates that the HEC 250K is not as efficient at separating the supercoiled and nicked circular forms of DNA compared to HEC 1.3M. PEO 5M was more efficient at reducing the mobility of the nicked circular DNA compared to HEC 1.3M (cf. Fig. 7A and B, noting that the scales are different).

Figure 8 is an example of some of the gels the data were obtained from. It illustrates the resolution of gellan gum and the effect of the polymers on the resolution and separation of the different forms of DNA. The native gel was run approximately twice as long as the other gels. Figure 9 is an image of a gellan gum gel, illustrating the separation of low molecular weight DNA. This gel was run under conditions that decrease the electrophoresis time and make the separation more convenient. The gel was run at a higher voltage using a lower conductivity buffer (BBE1), and polymer was added to the gel but not to the electrophoresis buffer. This gel shows that under these conditions, DNA can be resolved down to approx 50 bp.

Discussion

Gellan is a very flexible electrophoresis matrix. Gellan gels do not scatter light and appear like glass. Like glass, the gels are brittle and must be supported by a gel tray. The brittleness is a potential advantage because it is quite easy to make a slurry out of a gellan gel by mixing, facilitating recovery. In the original report *(1)*, it was noted that the gels did not stain well with ethidium bromide. This is probably due to the negative charge on the gellan binding the positively charged ethidium bromide. If increased sensitivity of DNA staining (similar to results obtained with DNA in agarose; unpublished data) is desired, the gels can be stained with SYBR green as described in Materials and Methods. The viscosity of the solution resulting from dissolving gellan gum is not significantly higher than buffer because of the low gellan concentration. The additional linear polymers will increase the viscosity, but this does represent a significant obstacle to their use, because of the low concentrations that can be used. We have been able to recover DNA from these gels with high purity and high yield (unpublished data).

Carbohydrate polymers *(22)* and linear polyacrylamide *(23)* have been added to agarose gels to reduce the electroosmosis in order to improve the electrophoretic and isoelectric focusing performance. Bode *(24)* added polyethylene glycol to cellulose acetate membranes and demonstrated that a linear polymer could substitute for a crosslinked gel to separate proteins by size. He utilized linear polyacrylamide stabilized in cellulose acetate membranes to separate proteins and RNA *(25)*. Perlman et al. *(2)* measured the effect of adding polysaccharides to agarose gels on DNA electrophoresis. They found that HEC and galactomannan increased the resolution of DNA below approx 1000 bp. These polymers had the greatest effect on decreasing the mobility of the larger molecular weight DNA. The effect of HEC was dependent on polymer concentration, and high molecular weight preparations (based on higher viscosity preparations) were more effective at enhancing the separation. Their results in agarose are similar to the results presented here but were not complicated by high EOF in native gellan gum. Perlman et al. *(2)* also found that dextran and Ficoll (400,000 mol wt) when incorporated into agarose did not significantly alter the migration rate of the DNA. In preliminary experiments, screening polymers for reduction of EOF, we found that PEO and HEC were superior at reducing EOF compared to polymers such as Ficoll (400,000 average mol wt) and dextran (485,000 average mol wt).

The negatively charged walls of silica capillaries gives rise to high EOF when used for electrophoresis. High-resolution separations of DNA are commonly done in the presence of high EOF with a polymer added to the buffer to separate the DNA by size (reviewed in refs. *4* and *26*). In the case of uncoated capillaries, the DNA migrates toward the negative electrode because of the high EOF. A similar process occurs in the case of gellan gum. The high EOF in the native gels results in a significant decrease in the migration of the DNA toward the positive electrode.

Noncrosslinked polymer solutions have proved to be capable of replacing gels for separation of DNA in capillary electrophoresis. A variety of polymers have been shown to be effective for electrophoresis of DNA (reviewed in refs. *4* and *27*). Heller *(28)* has made a systematic study of the selection of a polymer that can be pumped into a capillary tube for the electrophoretic separation of DNA. He calculated the theoretical value and experimentally measured the concentration at which the various polymers become entangled (entanglement threshold) determined by the point at which the dependence of viscosity on concentration departs linearity.

Significant efforts have been expended in reducing EOF in capillary electrophoresis. One successful method of reducing EOF and adsorption to silica capillaries has been to coat the walls with a thin layer of polymer *(29)*. Hjerten *(29)* points out that increasing the viscosity of the solvent will reduce EOF and also electrophoresis of solutes and will not accomplish anything. Coating the surface of the capillary with a thin layer of a polymer of high viscosity will have the effect of decreasing EOF and allowing electrophoresis to occur in the lumen of the capillary.

Our results indicate that PEO is an effective additive for increasing the separation of supercoiled from nicked circular plasmid DNA. Mickel et al. *(30)* studied the separation of a series of plasmids (from 5.4 to 83 kb pairs) in agarose gel electrophoresis. They measured the arrest of nicked circular plasmid DNA in agarose gels that was dependent on the size of DNA, the electric field strength, and buffer ionic strength. They postulated a "hoop and stick" (impalement) mechanism to explain the arrested migration of nicked circular DNA (starting with plasmids about 23 kb pairs in size). We did not see any significant differences in the migration of these plasmids when we increased the voltage from 4 to 8.6 V/cm in 0.1% gels containing 0.3% PEO 5M at 4°C (results not shown). These results indicate that an impalement mechanism is not a likely explanation for the enhanced separation. Experiments done in free-solution electrophoresis (stabilized by a density gradient) showed that PEO 5M could be used to separate supercoiled plasmid DNA because of its greater reduction in the mobility of the nicked circular DNA over supercoiled DNA *(31)*. In free-solution electrophoresis experiments, PEO 5M was effective at enhancing the separation whereas lower molecular weight PEO was not. In the previous study, the DNA was readily recovered from solutions containing PEO 5M by precipitation with ethanol.

Gellan gum offers a unique opportunity to study the mobility of DNA with different shapes ranging from conditions in native gel that has high EOF and little shape discrimination, to polymer-containing gels that have low EOF and a much higher shape discrimination.

Our study has shown the utility of adding linear polymers to gellan gum gels. The results indicate that higher molecular weight polymers are more efficient at reducing EOF and that the carbohydrate polymer HEC is more efficient at reducing EOF compared to PEO. The high molecular weight polymers were more efficient at reducing the mobility of high

molecular weight DNA in the gels. The lower viscosity of the low molecular weight polymers makes them easier to use in higher concentrations. High concentrations of low molecular weight polymers were efficient at increasing the resolution to low molecular weight DNA. PEO 5M at relatively low concentrations was effective at increasing the shape discrimination of gellan gum gels. We are interested in exploiting the enhanced shape separation for the preparation of supercoiled plasmid DNA. The use of linear polymers extends the range of separation possible using gellan gum electrophoresis and will increase the preparative applications of this reversible gel.

The data we have presented on the analytical separations of DNA will serve to guide the choosing of conditions for selective preparative separations. We believe that gellan is a remarkable matrix for DNA separations. Gels as low as 0.03% can be used to separate DNA, and polymers can be added at high concentrations without disrupting gellan's gel-forming capabilities. The DNA separations obtained with gellan in the presence of linear polymers are similar to those obtained with capillary electrophoresis, but with the advantage that the separations can be scaled up easily. We intend to utilize these high-resolution DNA separations on preparative scales.

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