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Effect of ionic strength, pH and polymer concentration on the separation of DNA fragments in the presence of electroosmotic flow

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Abstract

DNA separations in the presence of electroosmotic flow (EOF) using poly(ethylene oxide) (PEO) solutions have been demonstrated. During the separations, PEO entered capillaries filled with Tris–borate (TB) free buffers by EOF and acted as sieving matrices. We have found that ionic strength and pH of polymer and free solutions affect the bulk EOF and resolution differently from that in capillary zone electrophoresis. The EOF coefficient increases with increasing ionic strength of the free TB buffers as a result of decreases in the adsorption of PEO molecules. In contrast, the bulk EOF decreases with increasing the ionic strength of polymer solutions using capillaries filled with high concentrations of free TB buffers. Although resolution values are high due to larger differential migration times between any two DNA fragments in a small bulk EOF using 10 m*M* TB buffers, use of a capillary filled with at least 100 m*M* TB free buffers is suggested for high-speed separations. On the side of PEO solutions, 1.5% PEO solutions prepared in 100 to 200 m*M* TB buffers are more proper in terms of resolution and speed. The separation of DNA markers V and VI was accomplished less than 29 min in 1.5% PEO solutions prepared in 100 m*M* TB buffers, pH 7.0 at 500 V/cm using a capillary filled with 10 m*M* free TB buffers, pH 7.0.

Keywords: Electroosmotic flow; Buffer composition; pH effects; Ionic strength; DNA; Poly(ethylene oxide)

1. Introduction

Capillary electrophoresis (CE) has become one of the most attractive DNA separation techniques due to its high resolving power and extremely high speed compared to conventional gel electrophoresis [1–4]. Use of capillaries filled with polymer solutions or cross-linked gel is required for DNA separation because the charge densities of DNA fragments are independent of molecular size. Compared to gel, polymer solutions offer advantages of having a dynamic pore structure, flexibility, low viscosity and easy preparation and are more commonly used for DNA separation.

In recent years, entangled polymer solutions prepared from linear polymers, such as linear polyacrylamide (LPA) and its derivatives [5–7], cellulose and its derivatives [8,9], agarose [10] and poly(ethylene oxide) (PEO) [11,12], were more and more frequently employed as sieving media to achieve high-resolution DNA sizing and sequencing. In addition to the sieving mechanism, the separation of DNA up to 23 kilobase pairs (kbp) based on the transient entanglement between DNA and polymer molecules has been suggested in ultradilute solutions [13–15]. Furthermore, one technique called counter-migration CE in the presence of electroosmotic flow

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(EOF) has been developed for rapid separations of DNA fragments in the size range of 100–7000 kbp [16].

Although resolution is hard to predict due to a lack of complete understanding of electrophoretic migration of DNA fragments in polymer solutions, the sieving ability varies greatly according to the polymer concentration and chemical component [17–20]. For example, resolution of separating DNA fragments ranging from 18 to 2176 bp was optimized by using polydispersed polymer solutions prepared from different sizes of PEO [21]. Effects of pH and salts on resolution have also been demonstrated under alkaline conditions using entangled polymer solutions [22]. With increasing pH, the diffusion and electrophoretic mobility of DNA decrease due to changes in DNA conformation. It has been found that denaturation of DNA is usually complete above pH 11.5 [23]. Compared to the separation under neutral conditions, the high-efficiency DNA separation has been achieved due to sharper bands under strongly alkaline conditions [22]

The separation of DNA in the presence of EOF using entangled polymer solutions has been demonstrated by this group [24,25]. PEO solutions entered capillaries by the high EOF during the separation and acted as sieving matrices for high-resolution DNA separation. Due to the existence of a high EOF after washing capillaries with 0.5 *M* NaOH at 1 kV for 10 min, any highly viscous polymer solutions can be introduced into a very small-size capillary with reproducibility. Furthermore, filling the capillary with a number of neutral chemicals and polymers is possible in the presence of the EOF during the separation. For example, we have demonstrated DNA separations by a stepwise technique of PEO concentration [26].

In the presence of the EOF, resolution depends not only on sieving matrices as well as DNA characteristics, but also on the EOF. Therefore factors such as pH, ionic strength and PEO concentration, affecting the EOF must be carefully controlled [27]. Note that the PEO adsorption on the capillary wall is mainly through hydrogen bonding and hydrophobic interactions [28]. In an attempt to further understand factors affecting resolution and speed of DNA separation in the presence of the EOF using PEO solutions, we performed DNA separations using different concentrations of PEO solutions prepared in a variety of Tris–borate (TB) buffers at various pH values. Effects of polymer concentration, pH, and ionic strength on the bulk EOF, resolution and migration times were carefully elucidated.

2. Materials and methods

2.1. Equipment

The basic design of the separation system has been previously described [21]. Briefly, a high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA) was used to drive electrophoresis. The entire detection system was enclosed in a black box with a high-voltage interlock. The high-voltage end of the separation system was put in a laboratory-made Plexiglass box for safety. A 4.0-mW He–Ne laser with 543.6-nm output from Uniphase (Mantence, CA, USA) was used for excitation. The light was collected with a 20× objective (N.A.=0.25). One RG 610 cut-off filter was used to block scattered light before the emitted light reaches the phototube (Hamamatsu R928). The amplified currents were transferred directly through a 10-kΩ resistor to a 24-bit A/D interface at 10 Hz (Borwin; JMBS Developments, Le Fontanil, France) and stored in a personal computer. Capillaries (BIOTAQ-.COM, Potomac, MD, USA) of 75 μm I.D.×365 μm O.D. were used for DNA separations without any further coating process.

2.2. Chemicals

All chemicals for preparing buffer solutions were from Aldrich (Milwaukee, WI, USA). Ethidium bromide (EB) was obtained from Molecular Probes (Eugene, OR, USA). TB free buffers prepared from Tris [tris(hydroxymethyl)aminomethane] were adjusted with boric acid to pH 7.0, 8.2, 9.0 and 10.0, respectively. In this study, *X* m*M* TB buffers means that the buffers were prepared from *X* m*M* Tris and were adjusted by suitable amounts of boric acid. PEO (M_r 8 000 000) solutions containing 5 μg/ml EB were prepared from the above free buffers, respectively. ϕX174 RF DNA-Hae III digest was purchased from Pharmacia Biotech (Uppsala,

Sweden). DNA marker V and VI was purchased from Boehringer Mannheim (Mannheim, Germany).

2.3. Preparation of PEO solutions

Certain amounts of PEO were gradually added to the TB buffer solutions in a beaker stirring in a water bath at 85 to 90°C. During the addition of PEO, a magnetic stirring rod was used at high speed to produce a homogeneous solution. After addition was complete, the suspension was stirred for at least 1 more hour. Finally, polymer solutions were degassed with a vacuum system in an ultrasonic tank. Polymer solutions stored in a refrigerator at 4°C were usable for at least a week.

2.4. Separation

Prior to analysis, capillaries were treated with 0.5 *M* NaOH overnight. After each run, capillaries were washed with 0.5 *M* NaOH at 1 kV for 10 min to remove polymer solutions and refresh the capillary wall. Twenty-five or 10 μg/ml DNA samples were injected into the capillary filled with free TB buffers at 1 kV for 10 s. During the separation, PEO solutions entered the capillary via the high EOF for sieving DNA fragments.

2.5. Calculation

Resolution (R_s) was calculated using Eq. (1):

$$R_s = 2\,(t_2 - t_1)/(t_{w1} + t_{w2}) \quad (1)$$

where t_1 and t_2 are the migration time, and t_{w1} and t_{w2} are the width of the peak at baseline for fragments 1 and 2, respectively. EOF coefficients (μ_{eof}) were calculated according to Eq. (2):

$$\mu_{eof} = Ll/(Vt_0) \quad (2)$$

where L and l are the total length and effective length, respectively, V is the applied voltage, and t_0 is the time when the baseline shifted due to the detection of the PEO matrices.

3. Results and discussion

3.1. Bulk EOF

The EOF coefficient is proportional to the ζ (zeta) potential of the capillary wall. It has been known that the EOF decreases with increasing ionic strength but with decreasing pH (<7.0) due to decreases in the ζ potential in capillary zone electrophoresis (CZE) [29,30]. Effects of ionic strength and pH on the EOF are different in CE using polymer solutions because of dynamic adsorption of polymer molecules on the capillary wall. The adsorption, leading to decreases in the bulk EOF mobility, should depend on pH, ionic strength and species and concentration of polymer solutions and electrolytes. In this study, PEO solutions entered a capillary filled with free TB buffers by the bulk EOF during the separation. Therefore, the bulk EOF mobility is not constant and depends on the local EOF mobilities and the fractions filled with free buffers and PEO solution in the capillary, respectively [31].

Fig. 1A shows the effect of the concentrations of TB buffers used to prepare PEO solutions and fill the capillary on the bulk EOF when the pH values of these solutions were the same. Fig. 1B demonstrates the pH effect on the bulk EOF in 1.5% PEO solutions prepared in different concentrations of TB buffers using a capillary filled with 100 m*M* TB buffers (the pH values were the same as that of PEO solutions). To make it easier to explain the results, we simply divided the bulk EOF mobility into two local EOF mobilities, μ_f and μ_p, in which μ_f represents the local EOF mobility when the capillary was filled with free buffers and μ_p represents the local EOF mobility when the capillary was filled with polymer solutions. In this study, we ignored temporary changes in μ_f, which were probably due to spatial changes in the pH and ionic strength inside the capillary during separations. It is more difficult to estimate μ_p since it depends not only on the viscosity of polymer solutions but also on adsorption ability of polymer molecules. Fig. 1A shows that the bulk EOF mobility increased with increasing concentrations of free buffers, indicating that the PEO adsorption on the capillary wall should affect the bulk EOF mobility. At high concentrations, the interaction between PEO molecules and the capillary wall decreased,

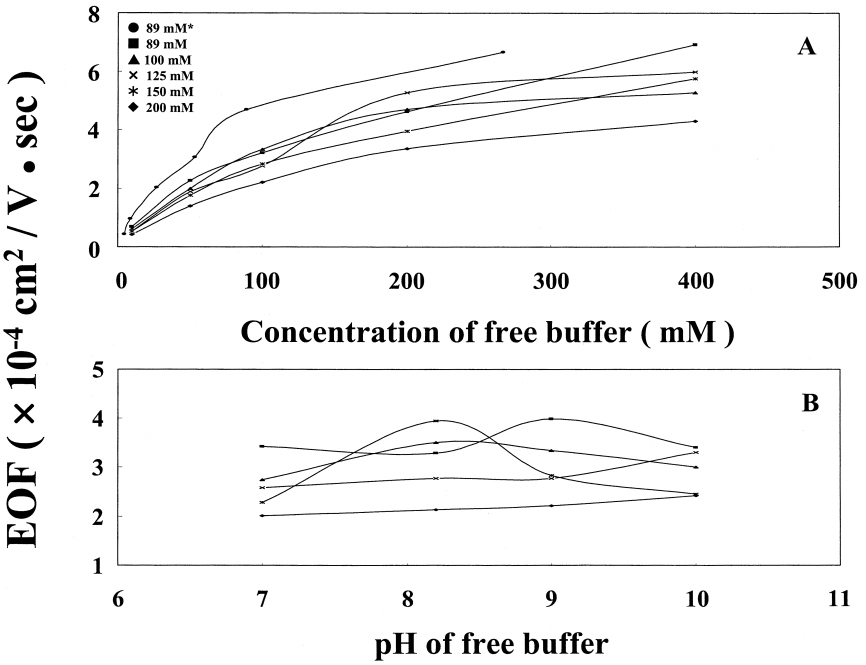

Fig. 1. Effect of concentration (A) and pH (B) of TB buffers used to fill the capillary and prepare PEO solutions on the bulk EOF in DNA separations at 15 kV using 1.5% PEO solutions containing 5 μg/ml EB. Capillary: 40 cm in total length and 30 cm in effective length. The pH values of free buffers and PEO solutions were 8.2 in (A); pH values of free buffers and PEO solutions were the same and the concentration of free TB buffers was 100 m*M* in (B). The symbol '*' represents 1× TBE and symbols in (B) as those in (A).

which is in good agreement with the suggestion that the PEO adsorption on the capillary wall is through hydrogen bonding and hydrophobic interactions [28,32]. Note that the hydrogen bonding is weak under high-salt conditions. On the other hand, the effect of the ionic strength of PEO solutions on the bulk EOF was inconsistent. This is due to the opposite effects of ionic strength on the ζ potential and on the PEO adsorption. The decrease of EOF due to PEO adsorption has been shown in our previous studies and others [25,33]. Since PEO molecules are neutral, its property was not affected as much as that of the capillary wall by changing the ionic strength. Because the PEO adsorption was trivial at very high concentrations, its effect on the ζ potential should be ignored. As a result, the decrease in the ζ potential due to decreases in the thickness of the double layers at higher ionic strengths played a significant role in determining the EOF. In addition, variations of the bulk EOF resulting from Joule heating should be taken into account in some cases shown in Table 1.

The pH effect of free solutions on the bulk EOF was also inconsistent when PEO solutions prepared in different concentrations of TB buffers (pH was the same as that of the free buffers) were used. Because the pH effect on the EOF is small from pH 7.0 to 10.0 using a fused-silica capillary, the variation of the ζ potential due to the dissociation of silanol groups in the capillary wall was ignored in this study. Therefore, changes in the bulk EOF using TB free buffers at different pH resulted mostly from the

Table 1
Current observed in different concentrations and pH of free TB buffers at 15 kV

TB (m*M*)	Current (μA)			
	pH 7.0	pH 8.2	pH 9.0	pH 10.0
10	2.8	1.9	2.3	1.3
50	12.8	5.4	3.2	2.0
100	22.6	13.1	3.8	3.4
200	47.0	21.0	14.8	4.5
400	104.2	48.6	27.2	7.4

variation of interactions between PEO molecules and the capillary wall at different ionic strengths. When 100 m*M* TB free buffers at pH 7.0 (more borate) and pH 10.0 were employed, respectively, the largest bulk EOF mobility was found using PEO solutions prepared in 89 m*M* TB buffers. At pH 8.2, 100 m*M* TB buffers were used to prepare the PEO solution for higher EOF coefficients. To the end, these results suggest that the existence of a high density of the negative charges in the capillary wall is essential to minimize the PEO adsorption.

3.2. Effect of pH and concentration of TB buffers

Fig. 2 shows the effect of the concentration of TB free buffers on the separation of DNA fragments at pH 8.2. The separation of 11 DNA fragments was accomplished within 9 min in 1.5% PEO solution

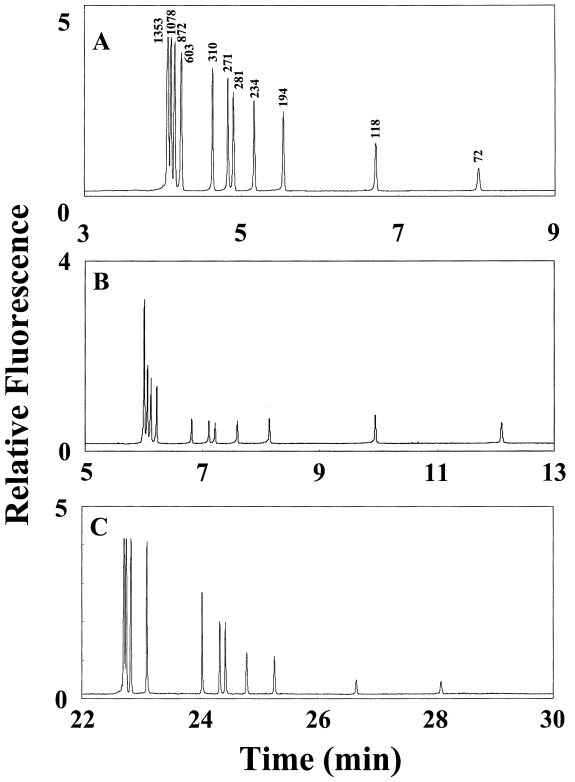

Fig. 2. Separation of 25 μg/ml ΦX174 RF DNA-Hae III digest in a capillary filled with different concentrations of TB buffers, pH 8.2, using 1.5% PEO solutions prepared in 100 m*M* TB buffers, pH 8.2. The concentration of TB buffers: (A) 400 m*M*, (B) 100 m*M*, (C) 10 m*M*. Other conditions as in Fig. 1.

using a capillary filled with a 400 m*M* TB buffer. The separation time was much longer using the 10 m*M* TB buffer, which most likely resulted from the PEO adsorption. Fig. 3 shows the pH effect on DNA separation using a capillary filled with 100 m*M* TB buffers. In comparison with the result of Fig. 2A, the shortest separation time was found at pH 7.0. This is due to a relatively large amount of boric acid used at pH 7.0, which further minimized the PEO adsorption. The longest separation time at pH 9 (a relatively small amount of boric acid used) further implies that borate plays a significant role in determining the PEO adsorption. To further elucidate the pH effect, resolution obtained at different pH values is summarized in Table 2. When the pH of polymer and free solutions were the same, resolution decreased with increasing concentration of TB buffers. At high concentrations, the separation time was short and the differential mobility between any two adjacent peaks became smaller as shown in Table 3. In addition, the effect of pH and borate and ionic strength on the conformation and charge density of DNA fragments, and the stability of the complexes between EB and DNA, leading to changes in the electrophoretic mobility (EPM) and band shapes, may be responsible for changes in resolution at high concentrations [34–36]. On the other hand, resolution was optimized due to increases in the differential migration times at low concentrations. When the pH values of PEO solutions and 400 m*M* free TB buffers were the same, resolution also decreased with increasing pH. However, when the pH values of PEO solutions and TB free buffers were different, the change in resolution was inconsistent with increasing pH as a result of the variation of the bulk EOF. The bulk EOF values were generally smaller using the capillary filled with free TB buffers, pH 10.0 (Table 3), unlike the result shown in Fig. 1B. The results suggested that borate and pH played significant roles in determining the PEO adsorption. In comparison of the data shown in Tables 2 and 3, the loss of resolution at pH 10.0 should be mainly due to diffusion as a result of small EOF coefficients.

Table 4 summarizes the effect of the concentration of TB buffers used to prepare PEO solutions and fill the capillary. The bulk EOF mobility was much smaller when a 10 m*M* free TB buffer was used even in the presence of higher ionic strengths of PEO solutions, presumably because of the PEO adsorp-

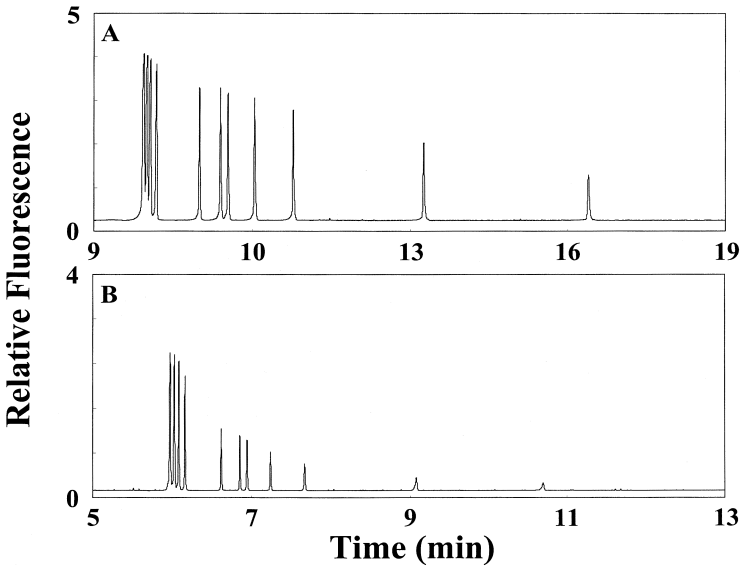

Fig. 3. Separation of 25 μg/ml ΦX174 RF DNA-Hae III digest at different pH. 1.5% PEO solutions were prepared in 100 m*M* TB buffers; free buffers were 100 m*M* TB buffers; pH values of the free and polymer solutions were the same: (A) pH 9.0, (B) pH 7.0. Other conditions as in Fig. 1.

tion. This suggestion was further supported by the result of high bulk EOF coefficients obtained using high concentrations of TB buffers to prepare PEO solutions and fill the capillary. Although the variation of the bulk EOF due to the Joule heating and the effect of ionic strength on the ζ potential must be considered as well at high ionic strengths, a marked decrease in the EOF coefficient implied that their effects were not profound. The fact that the bulk EOF mobility decreased with increasing the ionic strength of polymer solutions at pH 7.0 using a capillary filled with high concentrations of TB free buffers, indicating that the PEO adsorption did not play a crucial role in determining the bulk EOF in this case. However, the changes in the bulk EOF at different ionic strengths were neither consistent at pH 8.2 nor 9.0 because the effect of ionic strength on the EOF and PEO adsorption was competitive. Note that the separation windows were much wider using PEO solutions prepared in 200 m*M* buffer, especially at pH 7.0, presumably because of the decrease of the bulk EOF due to the adsorption of electrolytes, changes in the morphology of the PEO matrices (more compact) and DNA conformations, and increase in the EPM of DNA fragments [37]. Thus, if speed is not an important issue, resolutions can be further optimized using PEO solutions prepared in 200 m*M* TB buffers, pH 7.0. Since the DNA fragments migrated against the bulk EOF, resolution was also affected by the change in ionic strength. As a result of small EOF coefficients, resolution values were generally higher using 10 m*M* free TB buffers because of larger differential migration times between any two adjacent peaks ($>$271 bp). Regarding resolution and speed, the optimal concentration of TB free buffers used to fill the capillary is higher than 200 m*M* and that used to prepare PEO solutions is between 100 and 200 m*M*.

3.3. Effect of PEO concentration

To examine the effect of EOF and sieving on DNA separation, we performed DNA separation in a variety of PEO solutions at different pH. Table 5 shows the effect of PEO concentration on the bulk EOF and resolution. The bulk EOF coefficients decreased with increasing PEO concentration as a result of increases in the viscosity and the PEO adsorption. On the other hand, the effect of PEO solutions on decrease in the bulk EOF coefficient

Table 2
Effect of the concentration and pH of free TB buffers on resolution

F.B.[d] (m*M*)	Resolution[a]																							
	pH[b] 7.0												pH[b] 8.2											
	872/603				271/281				118/72				872/603				271/281				118/72			
	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0
10	31.0	15.5	N.D.	N.D.	10.3	5.3	N.D.	N.D.	77.5	53.2	N.D.	N.D.	22.1	19.3	21.6	14.7	2.3	9.2	4.6	3.8	56.5	57.4	47.0	35.9
50	9.2	8.9	3.0	23.2	7.5	3.8	15.8	4.8	73.9	64.4	39.3	26.1	8.1	8.4	11.3	13.4	1.8	6.4	5.4	5.8	61.6	58.5	50.9	54.6
100	12.7	7.9	10.0	15.9	7.6	3.7	21.0	3.4	70.9	52.2	34.0	31.7	9.7	6.8	8.6	9.7	5.6	5.3	5.1	4.5	56.0	54.2	53.6	41.0
200	7.2	9.3	3.0	4.9	4.9	3.8	8.5	3.3	50.4	31.5	44.3	41.3	3.0	5.3	2.6	5.3	6.8	3.3	1.6	3.4	19.7	43.3	48.9	41.9
400	5.1	9.8	N.D.	10.4	2.8	3.4	N.D.	4.0	40.9	36.8	N.D.	45.5	3.3	4.1	4.9	5.6	6.6	2.8	2.1	4.3	35.5	30.8	30.1	51.7

	pH[b] 9.0											
	872/603				271/281				118/72			
	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0	pH[c] 7.0	pH[c] 8.2	pH[c] 9.0	pH[c] 10.0
10	8.6	6.1	23.0	7.0	4.7	5.2	7.0	1.8	52.2	51.4	59.0	17.7
50	3.0	4.5	7.7	7.4	4.5	4.7	4.5	3.7	36.4	44.5	48.6	40.3
100	5.5	3.2	6.8	12.7	3.1	0.2	5.3	3.5	18.0	35.5	51.9	38.8
200	4.3	2.1	5.1	3.4	0.5	1.3	4.1	2.6	8.3	16.6	36.7	27.1
400	1.9	2.6	2.3	3.0	1.4	1.3	1.5	2.3	19.2	7.4	37.6	26.8

[a] Resolution of the 872/603, 271/281, and 118/72-bp fragments are 2.58, 1.56, 30.35, respectively, using 1× TBE buffers.
[b] pH of 1.5% PEO solutions prepared in 100 m*M* TB buffers.
[c] pH of 100 m*M* free TB buffers.
[d] Concentration of TB free buffers.
N.D.: Not detected.

Table 3
Effect of the pH and concentration of free TB buffers on the bulk EOF and separation window in DNA separation window (w)

F.B.[c] (m*M*)	pH[a] 7.0								pH[a] 8.2								pH[a] 9.0							
	pH[b] 7.0		pH[b] 8.2		pH[b] 9.0		pH[b] 10.0		pH[b] 7.0		pH[b] 8.2		pH[b] 9.0		pH[b] 10.0		pH[b] 7.0		pH[b] 8.2		pH[b] 9.0		pH[b] 10.0	
	t_0[d]	w[e]	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w
10	21.8	17.4	25.6	14.8	N.D.	N.D.	N.D.	N.D.	18.2	8.8	18.1	9.3	33.8	11.9	40.6	10.6	11.5	5.4	11.1	5.5	21.9	9.5	23.1	4.9
50	7.1	12.0	7.8	12.4	10.4	9.9	35.4	9.9	6.0	6.7	5.9	7.3	8.8	8.4	24.3	7.7	4.3	3.8	4.3	4.2	6.7	5.4	15.4	4.0
100	4.9	10.9	4.8	9.0	6.3	9.0	20.6	5.4	4.1	7.4	3.8	6.1	5.4	6.7	15.7	5.5	4.0	4.7	2.7	3.1	4.0	4.7	12.0	3.8
200	3.2	7.7	3.4	8.6	4.6	12.2	11.9	4.0	3.3	7.3	2.7	5.3	3.7	6.6	9.9	3.6	3.1	4.1	2.2	2.9	2.8	4.2	6.9	2.4
400	2.2	6.8	2.3	8.3	N.D.	N.D.	8.4	6.5	2.4	9.3	2.2	4.0	2.5	5.8	7.2	5.3	2.4	4.1	1.8	2.9	2.5	6.1	4.8	3.3

[a] pH of 1.5% PEO solutions.
[b] pH of TB free buffers.
[c] Concentration of TB free buffers.
[d] The time (min) at which the baseline shifted.
[e] Separation window (min), the time range between the first and last peak.

was varied with pH changes. As the concentration of PEO higher than 1.0%, the bulk EOF was higher at pH 8.2 and 9.0. However the bulk EOF was higher using 1.0% PEO solutions at pH 7.0. This is probably because the PEO adsorption (dynamic) on the capillary took place more profoundly at higher PEO concentrations and lower pH. Thus, the effect of PEO concentration on resolution was inconsistent at different pH, presumably due to the pH effect on the PEO adsorption, DNA conformation, and the stability of the complexes between EB and DNA. In general, the optimum PEO concentration is 1.5%. It

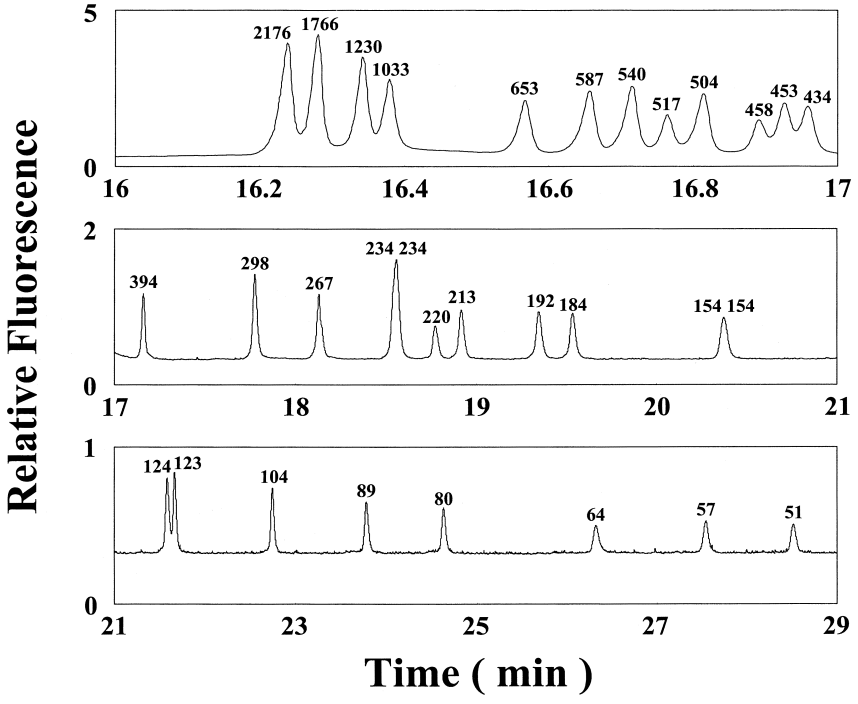

Fig. 4. Separation of 25 μg/ml DNA markers V and VI at 20 kV in a capillary filled with 10 m*M* free TB buffer, pH 7.0, using 1.5% PEO solutions prepared in 100 m*M* TB buffers, pH 7.0. Other conditions as in Fig. 1.

Table 4
Effect of the pH and concentration of TB buffers used to fill the capillary and prepare PEO solutions on the bulk EOF and separation window in DNA separation window (w)

F.B.[c] (m*M*)	pH[a] 7.0								pH[a] 8.2								pH[a] 9.0							
	P.S.[b] 89 m*M*		P.S.[b] 125 m*M*		P.S.[b] 150 m*M*		P.S.[b] 200 m*M*		P.S.[b] 89 m*M*		P.S.[b] 125 m*M*		P.S.[b] 150 m*M*		P.S.[b] 200 m*M*		P.S.[b] 89 m*M*		P.S.[b] 125 m*M*		P.S.[b] 150 m*M*		P.S.[b] 200 m*M*	
	t_0[d]	w[e]	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w	t_0	w
10	15.6	6.9	18.9	11.5	22.0	6.8	28.8	35.2	19.3	8.4	23.0	10.2	14.8	12.5	33.1	19.5	14.9	10.0	25.2	9.2	25.2	6.6	25.3	7.4
50	5.9	7.9	6.8	11.1	8.2	10.9	10.7	31.4	5.9	7.5	7.2	10.4	4.7	6.6	10.0	17.0	3.6	4.4	7.0	5.3	7.6	4.9	7.6	6.1
100	3.9	7.2	5.3	19.5	5.8	17.6	6.6	23.9	4.1	7.2	4.8	10.2	3.4	6.1	6.3	17.9	3.4	3.8	4.8	4.7	4.7	4.3	4.7	4.7
200	2.8	8.1	3.3	14.3	3.7	16.8	4.0	23.3	2.9	5.6	4.0	6.6	2.3	3.3	4.4	17.0	2.5	3.2	2.5	4.4	3.4	3.7	3.4	4.2
400	2.5	10.0	2.4	16.4	2.5	N.D.	2.6	17.1	1.9	3.7	2.4	4.4	1.9	3.2	3.0	16.5	1.8	2.7	2.2	3.6	2.3	3.2	2.3	6.1

[a] pH of 1.5% PEO solutions.
[b] Concentration of TB buffers used to prepare PEO solutions.
[c] Concentration of free TB buffers.
[d] The time (min) at which the baseline shifted.
[e] Separation window (min).

Table 5
Effect of PEO concentration on resolution when pH values of free TB buffers and PEO solutions were the same

DNA (bp)	Resolution											
	pH[a] 7.0				pH[a] 8.2				pH[a] 9.0			
	1% PEO, S.P.[b] 3.57 min	1.5% PEO, S.P.[b] 3.97 min	2% PEO, S.P.[b] 4.69 min	2.5% PEO, S.P.[b] 4.85 min	1% PEO, S.P.[b] 3.79 min	1.5% PEO, S.P.[b] 3.81 min	2% PEO, S.P.[b] 3.92 min	2.5% PEO, S.P.[b] 4.77 min	1% PEO, S.P.[b] 3.66 min	1.5% PEO, S.P.[b] 3.79 min	2% PEO, S.P.[b] 3.90 min	2.5% PEO, S.P.[b] 4.08 min
1353/1078	2.24	4.82	0.90	1.92	1.03	4.01	1.56	2.04	0.41	4.57	1.66	0.49
1078/872	2.15	6.25	1.21	1.97	1.01	4.13	1.13	2.11	3.30	5.01	1.97	0.68
872/603	3.65	12.69	1.63	3.72	1.52	6.84	2.41	3.49	2.66	6.79	3.80	0.98
603/310	23.45	52.21	7.82	17.37	16.45	42.01	12.66	20.27	3.20	32.33	19.69	6.29
310/271	7.67	20.72	3.13	9.26	9.98	17.40	6.65	13.81	8.77	13.87	13.49	5.25
271/281	2.82	7.60	0.92	3.50	3.31	5.31	2.31	4.24	3.02	5.31	4.89	1.16
281/234	9.95	23.47	2.97	13.49	11.12	14.95	7.88	130.3	10.18	17.40	13.72	3.26
234/194	12.91	31.57	4.30	17.54	17.21	19.41	12.21	8.68	12.78	21.83	20.43	3.42
194/118	40.64	80.79	17.01	48.78	36.37	58.20	38.41	22.99	33.75	55.46	61.95	9.16
118/72	35.43	70.93	32.72	47.44	29.84	54.24	38.08	44.44	29.77	51.92	46.98	24.80

[a] A capillary filled with 100 m*M* free TB buffers and PEO solutions prepared in 100 m*M* TB buffers was used.
[b] The time (min) at which the baseline shifted.

is interesting to note that at pH 7.0, 1.0% PEO solutions (relatively low viscosity) was better than that of 2.0% and 2.5% ones for separating DNA fragments in terms of speed and resolution.

3.4. Separation

Fig. 4 depicts the separation of 25 μg/ml DNA markers V and VI in the presence of EOF at 20 kV. Eight resolved peaks corresponding to 434- to 653-bp fragments, which were not resolved by performing a stepwise technique in our previous study, were obtained in 29 min [26]. The high resolving power of this technique resulted from the facts of opposite directions of the bulk EOF and electrophoretic migration of DNA fragments and a small bulk EOF. The relative standard deviation (RSD) of the migration of the 51-bp fragments was 2.5% in three runs. The RSD can be further lowered to 2% if we normalized the migration times based on the time when the baseline shifted due to the detection of PEO zones.

4. Conclusions

We have found that control of the bulk EOF by varying pH, ionic strength of polymer and/or free solutions is important to achieve optimized resolution and speed. The optimal PEO concentration for separating DNA fragments is dependent on pH and ionic strength, suggesting that the separation performance may be further optimized by controlling pH, ionic strength and so on. It is also possible to further optimize resolution and separation time by applying a number of gradient techniques, such as using different concentration and/or species of polymer solutions and electrolytes. As it has been shown that resolution of DNA separation can be further optimized at high pH, we are also interested in finding suitable polymer solutions for DNA separation under such conditions.

There are several advantages of this method over conventional CE methods for DNA separation, including no need of filling capillaries with polymer solutions; the possibility of using highly viscous polymer solutions and small capillaries; and performing gradient techniques and regulating the bulk EOF for optimized resolution and speed. These developing techniques may become popular in analysis of biopolymers in the very near future.

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