

# Analysis of double-stranded DNA by capillary electrophoresis using poly(ethylene oxide) in the presence of hexadecyltrimethylammonium bromide

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## Abstract

The impact of hexadecyltrimethylammonium bromide (CTAB) on the separation of ds-DNA by capillary electrophoresis in conjunction with laser-induced fluorescence (CE–LIF) detection using poly(ethylene oxide) (PEO) solution is described. The use of CTAB for improved separation reproducibility and efficiency of DNA has not been demonstrated although it is widely used for controlling the magnitude and direction of electroosmotic flow in CE. With increasing CTAB concentration, the interactions of DNA with ethidium bromide (EtBr) and with the capillary wall decrease. For the separation of DNA fragments with the sizes ranging from several base pairs (bp) to 2176 bp, a polymer solution consisting of 0.75% poly(ethylene oxide), 100 mM TB buffer (pH 8.0), 25  $\mu\text{g}/\text{mL}$  EtBr, and 0.36  $\mu\text{g}/\text{mL}$  CTAB is proper. Using the PEO solution, we separated a mixture of DNA markers V (pBR 322/*Hae*III digest) and VI (pBR 328/*Bgl*I digest and *pBR* 328/*Hinf*I digest) within 8 min at  $-375$  V/cm, with the limit of detection of 2.0 ng/mL based on the peak height for the 18-bp DNA fragment. The method is highly efficient ( $>10^6$  plate/m), repeatable (RSD of the migration times  $<1.5\%$ ), and sensitive. In addition, it is convenient to fill a capillary (75  $\mu\text{m}$  in diameter) with such a low-viscosity PEO solution by syringe pushing.

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**Keywords:** DNA separation; Hexadecyltrimethylammonium bromide; Stacking; Capillary electrophoresis; Laser-induced fluorescence

## 1. Introduction

Rapid, efficient, and sensitive separation methods are highly demanded for DNA analysis including genetic mutation detection and characterization of polymorphisms after the post-genome era [1–6]. Capillary electrophoresis (CE) provides the advantages over slab gel electrophoresis for DNA analysis, including high speed, ease of automation, and minute amounts of sample and reagents used [5–9]. The polymer solutions such as poly(ethylene oxide) (PEO) and linear poly(acrylamide) used in CE are replaceable after each run, allowing numerous runs in one capillary without loss in resolution and reproducibility. In conjunction with laser-induced fluorescence (LIF) detection, CE using polymer solutions has established a powerful tool for DNA sequencing and for the analysis of polymerase chain reaction (PCR) products [10–13].

Owing to small sizes of capillaries, poor concentration sensitivity and difficulty of filling viscous polymer solution still remain two important issues to be solved in CE. To improve the sensitivity for DNA analysis, a number of friendly stacking techniques have been developed in CE, including isotachopheric analysis [14–16] and field amplification [17,18]. Although these techniques provide greater than 100-fold improvements in sensitivity, a difficulty of filling viscous polymer solutions to small capillaries (e.g. 75  $\mu\text{m}$  in diameter) occurs. To prevent from the use of a high-pressure system, we demonstrated DNA separation in the presence of electroosmotic flow (EOF) [19–22]. Viscous PEO solution is introduced from the anodic inlet to the capillary via EOF. Because DNA molecules migrate against EOF, they enter PEO (neutral) zone during electrophoretic separation. When migrating from the sample zone to PEO zone, DNA molecules are stacked at the boundary as a result of sieving, increases in viscosity, decreases in electric field, and interaction with ethidium bromide (EtBr). This approach allows injection of DNA samples up to 5.0  $\mu\text{L}$ , with the sensitivity improvement greater than 450-fold [23]. One drawback of the separation of DNA in the presence of EOF is that EOF gradually decreases due

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to PEO adsorption on the capillary wall. PEO molecules interact with the capillary wall (SiOH) through hydrogen bonding and hydrophobic patches [24,25]. In order to generate high and reproducible bulk EOF, treating capillaries with 0.5 M NaOH at 1 kV for 10 min after each run is required. At high pH, PEO molecules are under hydrolysis and thus are removed from the capillary wall. The treatment takes time that limits capillary electrophoresis in conjunction with laser-induced fluorescence (CE-LIF) and array CE-LIF systems for high-throughput analysis.

Our aim of this study was at the development of a sensitive and fast method for the analysis of DNA by CE-LIF using low concentrations of PEO solutions. In our previous study, we demonstrated DNA separation using extremely low-viscosity PEO solution containing gold nanoparticles (AuNPs) or PEO modified AuNPs [8,26,27]. In the case of using PEO containing AuNPs, we suggested that improved DNA separation resolution and reproducibility is due to suppression of the interaction of DNA with the capillary wall and changes in the morphology of PEO matrixes. Herein, we tested the separation of DNA by using low-viscosity PEO solutions containing hexadecyltrimethylammonium bromide (CTAB) that has the interactions with DNA [28–31] and PEO molecules [32–34]. We carefully evaluated the impacts that CTAB, PEO, and ethidium bromide have on DNA separation. Using PEO solutions containing optimum amounts of CTAB, and EtBr, we conducted fast analyses of large-volume (0.2  $\mu$ L) DNA by CE-LIF.

## 2. Experimental

### 2.1. Apparatus

The basic design of the separation system has been previously described [35]. 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. High-voltage end of the separation system was put in a laboratory-made plexiglass box for safety. A 1.5-mW He-Ne laser with 543.6 nm output from Melles Griot (Irvine, CA, USA) was used for excitation. The light was collected with a 10X objective (numeric aperture = 0.25). One RG 610 cut-off filter was used to block scattered light before the emitted light reached the photomultiplier tube (Hamamatsu R928, Hamamatsu Corporation, Bridgewater, NJ). The amplified currents were transferred directly through a 10-k $\Omega$  resistor to a 24-bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a personal computer. Capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75  $\mu$ m I.D. and 365  $\mu$ m O.D. were used for DNA separation. The total length and effective length of the capillary are 40 and 30 cm, respectively. The fluorescence intensities ( $\lambda_{\text{ex}} = 543$  nm and  $\lambda_{\text{em}} = 605$  nm) of the solutions containing DNA and EtBr in the presence and absence of CTAB were recorded using a fluorometer (Aminco-Bowman Series 2, ThermoSpectronic, Pittsford, NY, USA). A viscometer (DV-E, Middleboro, MA, USA) was used to measure the viscosity values of the PEO solutions.

### 2.2. Materials

PEO with molecular weight 8,000,000 (entanglement threshold concentration 0.07%) and other chemicals for preparing buffers were from Aldrich (Milwaukee, WI, USA). EtBr was obtained from Molecular Probes (Eugene, OR, USA). Please note that wearing gloves is required when handling EtBr that is a highly carcinogenic compound.  $\phi$ x-174-RF DNA/*Hae*III digest was purchased from Pharmacia Biotech (Uppsala, Sweden). DNA markers V (pBR 322/*Hae*III digest) and VI (pBR 328/*Bgl*II digest and pBR 328/*Hinf*I digest) were purchased from Roche Molecular Biochemicals (Mannheim, Germany). Equal volumes of DNA markers V and VI were mixed and used in this study.

### 2.3. Preparation of PEO solution

Tris-borate (TB) solution (100 mM, pH 8.0) was prepared from Tris and boric acid that was used to adjust the pH. In this study, the molarity of Tris represents the concentration of TB solution. Appropriate amounts of CTAB were then added to the TB solution (50.0 mL), with the final concentrations ranging over 0–5  $\mu$ M (0–1.83  $\mu$ g/mL). While stirring in a water bath at 85–90  $^{\circ}$ C, different amounts (0–0.5 g) of PEO were separately and gradually added to the as-prepared TB solutions containing different concentrations of CTAB in beakers. After addition was completed, the suspensions were stirred for at least one more hour. Finally, polymer solutions were degassed with a vacuum system in an ultrasonic tank.

### 2.4. Stacking and separation

Prior to use, the capillaries were treated with 1.0 mg/mL CTAB at ambient temperature and pressure for 1 h. Fresh deionized water was applied to flush out the CTAB in the capillaries for 10 min. Finally, the capillaries were filled with PEO solutions (0.3–1.0%, pH 8.0) containing 0–1.83  $\mu$ g/mL CTAB and 5.0–50.0  $\mu$ g/mL EtBr at room temperature by syringe pushing. The electrokinetic injection was performed at –10 kV for a certain time over the period of 12–60 s. The separations were carried out at applied voltages of –375 V/cm (–15 kV). After each run, PEO solution was flushed out and fresh PEO solution was filled in.

## 3. Results and discussion

### 3.1. EOF in TB solution and PEO solution containing CTAB

Although CTAB has been commonly used to control the direction and magnitude of EOF [36], to the best of our knowledge, it has not been used in DNA separation by CE using polymer solution. When using a capillary filled with TB solution (pH 8.0) containing CTAB but without PEO, the magnitude of the EOF toward anodic end increases with increasing CTAB concentration before reaching a plateau [36]. However, when the capillary was filled with PEO solution containing CTAB and

EtBr, the magnitude of EOF is independent of CTAB concentration and its value is extremely small ( $<1 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) as a result of PEO adsorption. Although CTAB did not affect the EOF in the presence of PEO, we found that pretreatment of the capillary with 1.0 mg/mL (3.0 mM) CTAB solution is extremely important. When using a capillary without being treated with 3.0 mM CTAB solution, the separation of  $\phi\chi$ -174-RF DNA/*Hae*III digest using 0.75% PEO containing 25.0  $\mu\text{g/mL}$  EtBr was not repeatable (RSD values for the migration times were  $>7.3\%$ ). Since the EOF is negligible in the presence of PEO, we suggested that PEO molecules were adsorbed on the CTAB treated capillary wall mainly through hydrophobic interaction [32,33].

### 3.2. DNA separation using PEO solution containing EtBr and CTAB

The effects of PEO and EtBr on DNA separation are well known [37]. With increasing PEO concentrations,  $\mu_{ep}$  of DNA toward the anodic end decreases as a result of sieving and increases in viscosity. Similarly, upon increasing the concentration of EtBr,  $\mu_{ep}$  of DNA decreases as a result of the formation of a DNA–EtBr complex that possesses a less negatively charged density than does DNA. However, the effects may be more complicated when using a capillary filled with PEO solution containing EtBr and CTAB, mainly because CTAB molecules interact with DNA and PEO molecules. Thus, the roles that the concentrations of PEO, EtBr, and CTAB play on affecting the separation resolution and speed of DNA molecules need to be carefully evaluated.

First, we investigated the effect of CTAB on DNA separation by CE–LIF using 0.75% PEO solutions. The separation was unsuccessful using 0.75% PEO in the absence of CTAB, which is in good agreement with literature [35,38]. Fig. 1 shows the separation of  $\phi\chi$ -174-RF DNA/*Hae*III digest by CE–LIF using 0.75% PEO (pH 8.0) containing 0–1.83  $\mu\text{g/mL}$  (0–5.0  $\mu\text{M}$ ) CTAB and 25.0  $\mu\text{g/mL}$  EtBr. In the absence of EOF, DNA migrated toward the anodic end, indicating that the DNA fragments possess negative charges. The separation order suggests that the separation is according to the sieving mechanism [39,40]; smaller DNA fragments migrated faster through the PEO matrix because it collides less frequently with the gel fibers. For the larger DNA ( $>300$  bp), it crawls as an unraveled coil from head to tail in a snake-like fashion. The electrophoretic mobility is inversely proportional to the solute size at low electric field. When compared to the electropherograms, we observed a striking result that the migration times of the DNA fragments became shorter with increasing CTAB concentration. The negatively charged density of DNA molecules decreased once they interacted with the cations such as CTAB and EtBr [30,37]. The fact that the migration times for the DNA fragments were shorter in the presence of CTAB suggested that CTAB affected the intercalation of DNA with EtBr. The interaction between DNA and CTAB is mainly through electrostatic attractions, leading to changes in DNA conformation and thus decreases in the intercalation of DNA with EtBr. Our suggestion is supported by the fact that the fluorescence intensity of the solutions contain-

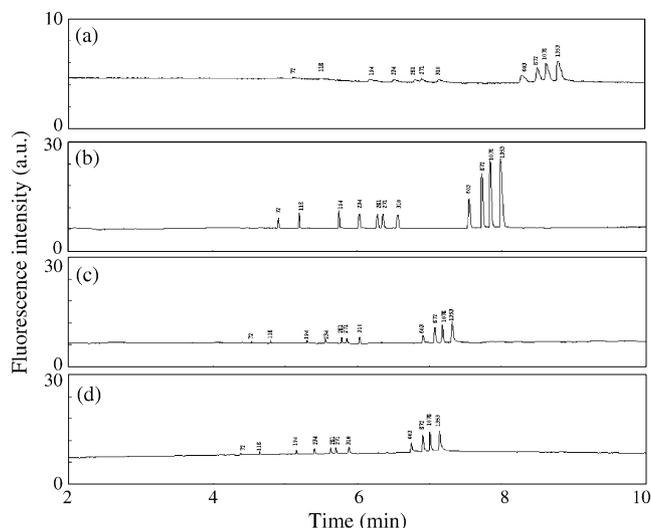


Fig. 1. The separations of 0.5  $\mu\text{g/mL}$   $\phi\chi$ -174-RF DNA/*Hae*III digest at: (a) 0; (b) 0.36; (c) 1.08; and (d) 1.83  $\mu\text{g/mL}$  CTAB by CE–LIF at  $-375 \text{ V/cm}$ . Capillaries were filled with 0.75% PEO (8 MDa) prepared in 100 mM TB (pH 8.0) containing 25.0  $\mu\text{g/mL}$  EtBr and different concentrations of CTAB. Injections were conducted at  $-10.0 \text{ kV}$  for 12 s. The two vials were filled with 0.75% PEO prepared in 100 mM TB (pH 8.0) containing 25.0  $\mu\text{g/mL}$  EtBr and 0.036  $\mu\text{g/mL}$  CTAB. The numbers in the electropherograms denote the DNA sizes (bp). The capillary length is 40 cm (30 cm in effective length). The fluorescence intensity is plotted as an arbitrary unit (a.u.).

ing  $\phi\chi$ -174-RF DNA/*Hae*III digest and EtBr decreased with increasing CTAB concentration above 0.36  $\mu\text{g/mL}$  as seen in Fig. 1 (CE–LIF) and in Fig. 2 (fluorometer; under a static condition). The electropherograms depicted in Fig. 1 indicate that the optimum solution is 0.75% PEO containing 25.0  $\mu\text{g/mL}$  EtBr and 0.36  $\mu\text{g/mL}$  CTAB. In the presence of 0.36  $\mu\text{g/mL}$  CTAB, the peak profiles for the DNA fragments are symmetric and sharp; suggesting that DNA interactions with the capillary wall and/or with PEO were weakened. Table 1 further summa-

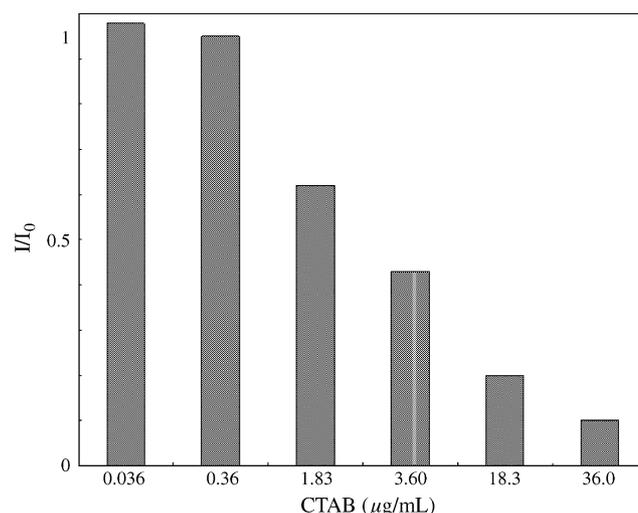


Fig. 2. The fluorescence intensities of the mixtures of 0.5  $\mu\text{g/mL}$   $\phi\chi$ -174-RF DNA/*Hae*III digest, 100 mM TB (pH 8.0), 25.0  $\mu\text{g/mL}$  EtBr and different concentrations of CTAB. The excitation and emission wavelengths are 543 and 605 nm, respectively. The fluorescence intensities of the solutions in the absence and presence of CTAB are denoted to be  $I_0$  and  $I_F$ , respectively.

Table 1  
Repeatability of the migration times ( $t_m$ ) and fluorescence intensities ( $I_F$ ) for the DNA fragments at different CTAB concentrations

DNA (bps)	CTAB concentration ( $\mu\text{g/mL}$ )							
	0		0.36		1.08		1.83	
	$t_m$ (min) (RSD %) <sup>a</sup>	$I_F$ (a.u.) (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) <sup>b</sup> (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) (RSD %)
72	5.1 (1.2)	0.1 (1.2)	4.9 (0.8)	2.8 (1.2)	4.5 (1.1)	0.5 (1.2)	4.4 (2.5)	0.4 (3.2)
118	5.5 (1.3)	0.1 (1.5)	5.2 (0.9)	4.3 (1.5)	4.8 (1.3)	0.8 (1.5)	4.6 (2.7)	0.8 (5.5)
194	6.2 (1.5)	0.2 (1.5)	5.7 (1.0)	4.9 (1.1)	5.3 (1.4)	0.9 (1.4)	5.2 (2.4)	1.1 (3.2)
234	6.5 (1.7)	0.2 (1.4)	6.0 (1.2)	3.8 (1.2)	5.6 (1.6)	1.2 (1.1)	5.4 (2.2)	1.3 (2.2)
281	6.8 (1.6)	0.2 (1.9)	6.1 (1.4)	3.7 (1.3)	5.8 (1.4)	1.6 (1.2)	5.6 (2.9)	1.5 (3.1)
271	6.9 (1.5)	0.2 (1.8)	6.3 (1.3)	3.9 (1.1)	5.9 (1.8)	1.5 (2.1)	5.7 (3.5)	1.5 (4.4)
310	7.2 (1.4)	0.2 (1.1)	6.5 (1.5)	3.7 (1.0)	6.0 (1.9)	1.7 (2.2)	5.9 (3.4)	1.6 (4.8)
603	8.3 (1.2)	0.7 (1.7)	7.4 (1.3)	8.0 (1.2)	6.9 (1.9)	2.2 (1.9)	6.8 (3.6)	2.7 (5.2)
872	8.5 (1.1)	1.2 (2.1)	7.7 (1.2)	14.8 (1.7)	7.1 (2.0)	4.2 (2.1)	6.9 (3.9)	4.5 (5.6)
1078	8.6 (1.4)	1.5 (2.2)	7.8 (1.4)	18.3 (1.4)	7.2 (1.9)	4.9 (2.0)	7.0 (4.0)	5.3 (6.1)
1358	8.8 (1.3)	1.8 (2.0)	8.0 (1.2)	18.8 (1.2)	7.3 (1.9)	5.8 (2.2)	7.1 (4.5)	5.4 (6.5)

<sup>a</sup>  $n=3$ .

<sup>b</sup> a.u.: arbitrary unit.

rizes the impacts of CTAB on separation repeatability when the DNA sample was injected at  $-10.0$  kV for 12 s. The repeatability values of the migration times for the DNA fragments are all less than 1.5% ( $n=3$ ) in the presence of  $0.36$   $\mu\text{g/mL}$  ( $1.0$   $\mu\text{M}$ ) CTAB. We point out that the LOD for the DNA sample was about  $10.0$  ng/mL based on the peak height for the 118-bp fragment. The repeatability was relatively poor (up to 4.5 and 6.5% for the migration times and peak heights, respectively) in the presence of CTAB at the concentration greater than  $1.83$   $\mu\text{g/mL}$ , mainly because of changes in DNA conformation [41]. At such a high CTAB concentration, the changes in PEO morphology and capillary surface might also cause relatively poor repeatability [32,33]. We point out that the detrimental effect caused by Joule heating is negligible, which was supported by the fact that the currents were all small ( $<8$   $\mu\text{A}$ ) under different conditions. The variation of EOF is ruled out because EOF values were extremely low in this study.

To further investigate the interaction between EtBr and DNA in the presence of CTAB, we conducted the DNA separation

by using 0.75% PEO solutions (pH 8.0) containing  $0.36$   $\mu\text{g/mL}$  CTAB and different concentrations of EtBr ( $5.0$ – $50.0$   $\mu\text{g/mL}$ ). Fig. 3 displays that the migration times and peak heights for the DNA fragments increased with increasing EtBr concentration. The electrophoretic mobilities of the DNA fragments decrease once they intercalate with EtBr. With increasing EtBr concentration, DNA fragments possess less negative charges and their structures become more rigid. Table 2 further summarizes the repeatability of the migration times and peak heights ( $n=3$ ). Although the effect of EtBr on the migration times and repeatability was smaller than was CTAB, it had a profound effect on the fluorescence intensities. In terms of sensitivity, EtBr at the concentration range  $25.0$ – $50.0$   $\mu\text{g/mL}$  is proper. Since EtBr is a potential carcinogenic reagent, we conducted the following separations at  $25.0$   $\mu\text{g/mL}$  EtBr.

It has been suggested that higher concentration of PEO solution is required for the separation of small DNA fragments [22,35]. On the basis of our own experience, at least 1.5% PEO is required for completely resolving the 11 DNA frag-

Table 2  
Repeatability of the migration times ( $t_m$ ) and fluorescence intensities ( $I_F$ ) for the DNA fragments by CE–LIF at different EtBr concentrations

DNA (bps)	EtBr concentration ( $\mu\text{g/mL}$ )							
	5.0		10.0		25.0		50.0	
	$t_m$ (min) (RSD %) <sup>a</sup>	$I_F$ (a.u.) (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) (RSD %)	$t_m$ (min) (RSD %)	$I_F$ (a.u.) (RSD %)
72	4.5 (0.9)	0.3 (0.9)	4.6 (0.6)	0.9 (0.9)	4.8 (0.8)	1.7 (1.5)	4.9 (1.9)	2.2 (1.9)
118	4.8 (1.4)	0.6 (1.1)	4.9 (0.5)	1.5 (0.4)	5.1 (0.9)	2.5 (1.9)	5.2 (1.8)	3.4 (1.9)
194	5.2 (2.2)	0.7 (1.8)	5.4 (0.4)	2.1 (0.8)	5.7 (1.0)	3.3 (1.6)	5.8 (2.2)	4.8 (2.1)
234	5.5 (1.9)	0.9 (0.9)	5.7 (0.3)	2.3 (0.4)	5.9 (1.2)	3.5 (1.9)	6.1 (1.7)	5.3 (1.9)
281	5.8 (1.4)	1.0 (1.5)	5.9 (0.2)	2.3 (0.6)	6.2 (1.4)	3.7 (1.7)	6.4 (1.8)	6.2 (1.8)
271	5.9 (1.1)	1.1 (1.9)	6.0 (0.3)	2.5 (0.2)	6.3 (1.3)	3.9 (1.9)	6.5 (1.8)	6.0 (1.5)
310	6.1 (1.0)	1.0 (1.1)	6.2 (0.3)	2.8 (0.4)	6.5 (1.5)	4.0 (1.3)	6.7 (1.8)	6.6 (2.1)
603	7.0 (0.8)	1.6 (0.9)	7.2 (0.2)	4.3 (0.3)	7.6 (1.3)	6.8 (1.2)	7.9 (2.1)	9.2 (2.9)
872	7.2 (1.3)	2.4 (1.5)	7.4 (0.2)	6.1 (0.5)	7.8 (1.2)	10.6 (1.4)	8.1 (2.1)	14.0 (2.7)
1078	7.3 (1.3)	2.7 (1.4)	7.6 (0.2)	6.8 (0.3)	7.9 (1.4)	11.9 (1.2)	8.3 (2.1)	17.0 (2.9)
1358	7.4 (1.0)	3.7 (1.1)	7.7 (0.2)	7.9 (0.5)	8.1 (1.2)	13.6 (1.1)	8.4 (2.0)	17.3 (2.8)

<sup>a</sup>  $n=3$ .

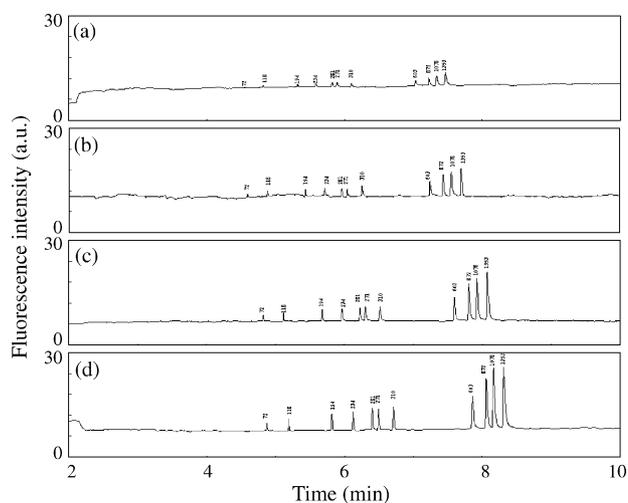


Fig. 3. The separations of 0.25 µg/mL  $\phi$ x-174-RF DNA/*Hae*III digest at: (a) 5.0; (b) 10.0; (c) 25.0; and (d) 50.0 µg/mL EtBr by CE-LIF at  $-375$  V/cm. Other conditions are the same as in Fig. 1.

ments of  $\phi$ x-174-RF DNA/*Hae*III digest in the absence of EOF [35]. By using high concentrations of PEO solutions, we have experienced a difficulty of filling the capillary with the viscous matrixes and long separation times. The electropherograms exhibited in Fig. 4 show the migration times for the DNA fragments become longer with increasing PEO concentration. The values of  $\mu_{ep}$  for the DNA fragments were smaller at higher PEO concentrations because of greater sieving effect and higher viscosity. At 0.3% PEO, the separation was completed within 7.0 min. We point out that filling such a PEO solution (viscosity  $25 \pm 5$  cp) to a small capillary is quite easy, preventing from the use of high-pressure means. However, the peaks are smaller and broader, mainly because of slight adsorption of DNA molecules on the capillary wall. When using 0.75% PEO solution containing 25 µg/mL EtBr and 0.36 µg/mL CTAB (viscosity  $930 \pm 73$  cp), the plate numbers for the 72-,

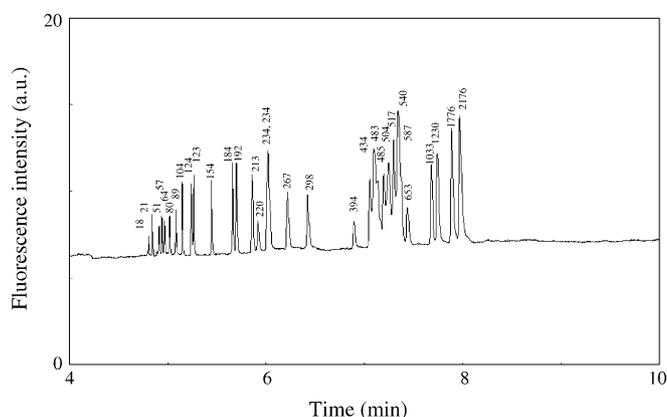


Fig. 5. Electropherogram of 0.1 µg/mL DNA markers V and VI by CE-LIF at  $-375$  V/cm. The capillary was filled with 0.75% PEO that was prepared in 100 mM TB (pH 8.0) containing 25 µg/mL EtBr and 0.36 µg/mL CTAB. Injection was conducted at  $-10$  kV for 60 s. Other conditions are the same as in Fig. 1.

310, and 1358-bp DNA fragments were  $8.8 \times 10^6$ ,  $1.9 \times 10^6$ , and  $1.6 \times 10^6$  plates/m. The plate numbers for the 72-, 310-, and 1358-bp DNA fragments were  $9.2 \times 10^6$ ,  $4.9 \times 10^6$ , and  $3.4 \times 10^6$  plates/m, respectively, when using 1.0% PEO solution containing 25 µg/mL EtBr and 0.36 µg/mL CTAB (viscosity  $4500 \pm 80$  cp). The results also indicate that small diffusion of the DNA fragment in high-viscosity matrixes is also a reason for great efficiencies. Although PEO solutions at the concentration greater than 1.0% should provide better resolving power, it was not tested because of a difficulty of filling the solution to a small capillary and longer separation times.

### 3.3. Separation of DNA markers V and VI

To further show the merit of using 0.75% PEO solution containing 0.36 µg/mL CTAB and 25 µg/mL EtBr, we conducted the separation of a mixture of DNA markers V and VI (both 0.1 µg/mL) that was injected at  $-10$  kV for 60 s. As exhibited in Fig. 5, the separation was completed within 8 min and two small DNA fragments (18 and 21 bp) were clearly detected. We estimated the LOD for the DNA sample was to be about 2.0 ng/mL, based on the peak height for the 18-bp fragment. Although the injection volume was about 0.2 µL [23], the theoretical plate numbers for 154-, 267-, 504-, 1033-, and 2176-bp DNA fragments were  $1.07 \times 10^7$ ,  $3.5 \times 10^6$ ,  $5.0 \times 10^6$ ,  $2.5 \times 10^6$  and  $2.9 \times 10^6$  plates/m, respectively. The sharp peak profiles indicated that stacking occurred. When DNA fragments migrated from aqueous solution to PEO solution, they slowed down and were stacked as a result of sieving, increases in viscosity, and decreases in electric field. In addition, stacking was also partially due to the interaction of the anionic DNA fragments with cationic EtBr and CTAB. To further support the stacking of DNA fragments, we plot the fluorescence intensities of the 154-, 267-, 504-, 1033, and 2176-bp against the injection volume up to 0.2 µL. Good linearity ( $R^2 > 0.98$ ) exhibited in Fig. 6 shows efficient stacking of the DNA fragments. Although the improved sensitivity is not profound when compared to our previous one (in the presence of EOF) [23], the present method is simple (no

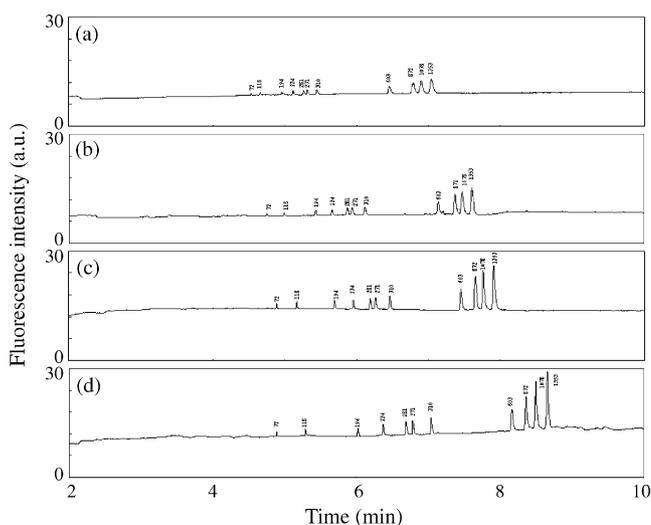


Fig. 4. The separations of 0.25 µg/mL  $\phi$ x-174-RF DNA/*Hae*III digest at: (a) 0.3%; (b) 0.5%; (c) 0.75%; and (d) 1.0% PEO solution by CE-LIF at  $-375$  V/cm. Other conditions are the same as in Fig. 1.

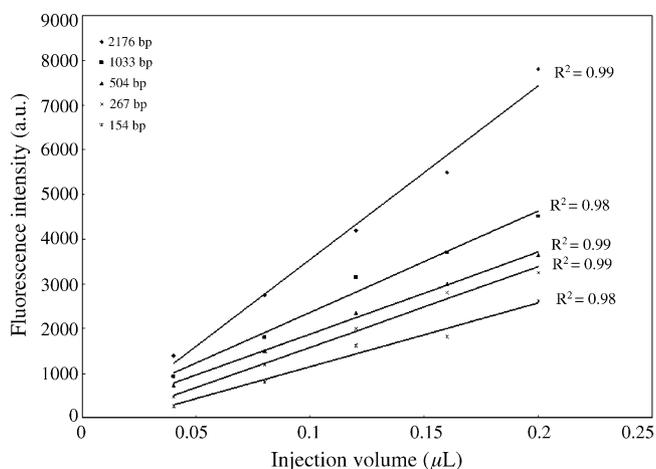


Fig. 6. Linearity between the peak heights for some of the DNA fragments in a mixture of DNA markers V and VI and injection volumes. Other conditions are the same as in Fig. 5.

need for equilibration after each run), which holds great potential for automation. We note that the sensitivity can be further improved by using solutions containing greater amounts of PEO and EtBr if required.

#### 4. Conclusions

We have demonstrated a fast, sensitive, and reproducible separation method for the analysis of DNA by CE–LIF using PEO solutions containing EtBr and CTAB. Although PEO offers self-coating capability, the present result suggests that treating the capillary wall prior to PEO coating is required for achieving high resolution and reproducibility of DNA separation by CE using low-viscosity PEO matrices. DNA adsorption on the capillary wall was suppressed in the presence of CTAB and PEO, leading to improved efficiency, reproducibility, and sensitivity. Owing to minimum DNA adsorption, 0.75% PEO solution was effective for the separation of DNA such as a mixture of DNA markers V and VI. By using such a low-viscosity PEO solution, high-pressure means is not required for filling the sieving matrix to a small capillary.

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