Effects of metal ions on concentration of DNA in high-conductivity media by capillary electrophoresis

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Abstract

On-line concentration and separation of DNA prepared in low- or high-conductivity media has been demonstrated using poly(ethylene oxide) (PEO) solution in the presence of electroosmotic flow. DNA fragments migrating against EOF stacked at the boundary between the sample zone and PEO solutions, mainly because of sieving and increases in the viscosity. Unlike conventional methods, the large DNA fragments were detected earlier toward the cathode end in this study. The limit of detection (LOD) at a signal-to-noise ratio=3 for φX174 RF DNA-Hae III digest prepared in 50 mM Tris–borate, pH 10.0, was down to 0.171 ng/ml, with an 860-fold improvement (compared to that obtained by 10-s injection at 25 V/cm) in the sensitivity, when injecting about 2.58 μl. By applying a short plug (2.3 cm) of 0.5 mM AgNO$_3$ prepared in 1.5% PEO solution after sample injection, the analysis of up to 0.75 μl DNA prepared in phosphate-buffered saline (PBS) has been carried out without any tedious desalting processes. This results in an LOD of 6.86 ng/ml for the DNA sample and a 155-fold improvement in the sensitivity. Moreover, this method has allowed the analysis of 0.75 μl of polymerase chain reaction products amplified after 18 cycles with good reproducibility.
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Keywords: Electroosmotic flow; DNA; Metal cations; Poly(ethylene oxide)

1. Introduction

Capillary electrophoresis (CE) compared to high-performance liquid chromatography suffers from low concentration sensitivity, despite its advantages of rapidity, high-resolving power, and lesser amounts of sample and reagents used [1–4]. This is mainly due to small injection volumes (nl) and short optical path lengths (e.g. 75 μm). To enlarge the optical length, Z-shaped and bubble-cell capillaries have been used [5,6]. Owing to the limit of the size of the bubble

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cell, diffusion and imperfect distribution of the electric field strength inside the capillary that might cause loss of resolution, sensitivity improvement was not great (only several tens fold). Thus, these methods are not quite useful for DNA analysis such as DNA sequencing and single nucleotide polymorphism (SNP), wherein high-resolving power is of extreme importance [7–10].

Although DNA can be amplified by polymerase chain reaction (PCR), techniques allowing improvements in the sensitivity are always welcomed with respect to speed and cost [11]. So far, a number of friendly on-line electrophoretic concentration techniques have been developed in CE, including iso-

tachophoretic (ITP) analysis [12,13] and field ampli-
fication [14,15]. Although, more than 100-fold im-
provements in the sensitivity for DNA prepared in
low-conductivity media are easily achieved by these
techniques, the analysis of DNA prepared in high-
conductivity media remains problematic, including
the loss of resolution, a poor stacking efficiency, and
irreproducibility [16–18]. To partially overcome
these shortages, the separation could be performed at
low electric field strengths or the conductivity of the
sample zone must be decreased. The former is not
attractive because of slowness and poor stacking
efficiency. Titrating tris(hydroxymethyl) amino-
methane (Tris) cations used to prepare DNA samples
with hydroxide ions was found effective to reduce
the conductivity, leading to DNA stacking [19]. One
shortage of this method is a low sensitive improve-
ment (less than 100-fold) because the injection
length was limited in order to provide a high-resolv-
ing power.

   Recently, we have developed methods for the
separation of large volumes of proteins and DNA in
poly(ethylene oxide) (PEO) solution using bare
fused-silica capillaries [20–22]. In the presence of
electroosmotic flow (EOF), PEO enters the capillary
filled with Tris–borate (TB) buffer from the anode
end after DNA injection. With small migration
mobilities (EOF mobility minus the electrophoretic
mobility of DNA), DNA fragments migrate in PEO
solutions, resulting in decreases in the electropho-
retic mobilities. As a result, DNA stacks at the
interface between the sample zone and PEO solu-
tions. In contrast to conventional methods (in the
absence of EOF), large DNA fragments are detected
earlier in the cathode end because DNA migrates
against EOF. We have pointed out that poor con-
centration, irreproducibility, and loss of resolution
are problematic when injecting large-volume samples
prepared in low-conductivity media. These problems
are mainly due to PEO adsorption on the capillary
wall and have been partially overcome by using high
concentrations of TB buffers to fill the capillary and
to prepare PEO solution, and by preparing DNA in a
buffer with a suitable conductivity [22,23]. More
recently, we have demonstrated the separation of
DNA up to 5 μl, with a sensitivity improvement up
to 450-fold, using a 150-μm capillary [24].

   Our methods work well for the analysis of large

volumes of DNA or proteins prepared in low-con-
ductivity media, but not for that prepared in high-
conductivity media [22]. This is mainly due to a
small DNA migration in high-conductivity media. To
overcome this shortage, techniques based on switch-
ing the polarity after removing large amounts of
background electrolytes have been developed
[25,26]. Alternatively, we described a method similar
to our previous ones for the analysis of up to about
0.75 μl DNA prepared in high-conductivity media
[20–22]. We have found the importance of applying
a plug of $AgNO_3$ solution between the sample zone
and PEO solution. To show the feature of this new
method, the analysis of large volumes of polymerase
chain reaction (PCR) product was also demonstrated.

2. Materials and methods

2.1. Apparatus

   The basic design of the separation system has been
previously described [27]. Briefly, a high-voltage
power supply (Gamma High Voltage Research, Or-
mond Beach, FL, USA) was used to drive electro-
phoresis. 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 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 10$\times$ objective (numeric
aperture=0.25). One RG 610 cut-off filter was used
to block scattered light before the emitted light
reaches the photomultiplier tube (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. Capil-
laries (Polymicro Technologies, Phoenix, AZ, USA)
of 75 μm I.D. and 365 μm O.D. were used for DNA
separations without any further coating process. The
capillary length was 60 cm (50 cm of effective
length).

2.2. Materials

   PEO with molecular mass 8,000,000 and other

chemicals for preparing buffer solutions were from Aldrich (Milwaukee, WI, USA). Ethidium bromide (EtBr) was obtained from Molecular Probes (Eugene, OR, USA). Please note that wearing gloves is required when handling EtBr because it is a highly carcinogenic compound. TB buffers prepared from Tris were adjusted with boric acid to pH 10.0 and 9.0, respectively. Unless otherwise noted, an X mM TB buffer herein means a buffer containing X mM Tris adjusted with suitable amounts of boric acid. Phosphate-buffered saline (PBS), pH 7.4, was prepared by dissolving 8.0 g of NaCl, 0.2 g of KH_2PO_4, 0.46 g of Na_2HPO_4, and 0.2 g of KCl in 1 l of H_2O. PEO solutions were prepared in the TB buffers containing 5 μg/ml EtBr. ϕX174 RF DNA-Hae III digest (500 μg/ml) was purchased from Pharmacia Biotech (Uppsala, Sweden). QIAamp DNA Blood mini kit was purchased from QIAGEN (Hilden, Germany). PCR kits were obtained from Promega (Madison, WI, USA).

2.3. Preparation of PEO solutions

Certain amounts of PEO were gradually added into the TB buffer, pH 9.0, in a beaker stirring in a water bath at 85 to 90 °C. During the addition of PEO, a magnetic stirring rod was used to produce a well homogeneous suspension. After addition was complete, the suspension was stirred for at least one 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 3 days.

2.4. Stacking and separation

New capillaries were treated with 0.5 M NaOH overnight. Prior to analysis, the base was flushed out with 400 mM TB buffer, pH 10.0, by pressure means for 20 s. DNA samples were injected into the capillary filled with 400 mM TB buffers, pH 10.0, by electrokinetic means at 25 or 250 V/cm for certain times (10–120 s). The estimated injection volume was calculated from the injection length and the capillary I.D. [22]. When a short plug of $AgNO_3$ was applied after sample injection as shown in Fig. 1, electrokinetic injection was carried out at 250 V/cm for 60 s. During the separation at 333 V/cm, PEO
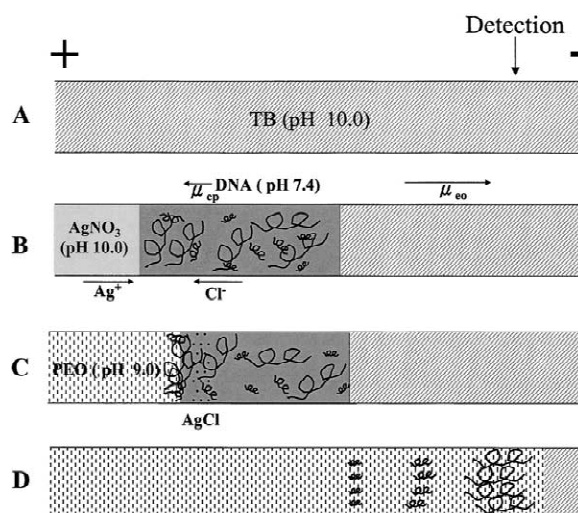

Fig. 1. Schematic of on-line concentration of DNA using PEO under discontinuous conditions. (A) Capillary is filled with 400 mM TB, pH 10.0, prior to DNA injection. (B) Discontinuous plugs are existent in the capillary after subsequent injections of DNA and a short plug of $AgNO_3$. (C) AgCl particles form and DNA stacks mainly because of sieving and increases in the viscosity. (D) Separation of the stacked DNA takes place based on the sieving mechanism in CE. μ_{eo} and μ_{ep} represent the EOF mobility and the electrophoretic mobility of the DNA fragments, respectively.

solutions entered the capillary from the anode end by EOF and acted as sieving matrices. After each run, capillaries were washed with 0.5 M NaOH at 25 V/cm for 10 min to remove PEO solutions and refresh the capillary wall. This treatment has been shown useful for reproducibility, with a relative standard deviation (RSD) of EOF less than 2.0%.

2.5. DNA extraction and PCR products

The blood sample was from a normal male. Human genomic DNA from buffy coat was extracted using the QIAamp DNA blood mini kit in accordance with the manufacturer's instructions. Amplification of the DNA sample was conducted as suggested by the manufacturer. Briefly, 22.5 μl PCR master mix component was prepared by mixing 17.45 μl sterile water, 2.50 μl STR 10$\times$ buffer, 2.50 μl TH01 10$\times$ primer pair, and 0.05 μl *Taq* polymerase (5 U/μl). To the PCR master mix, 2.5 μl of human genomic DNA (25 ng) were added. PCR was

conducted as: initial incubation at 96 °C for 2 min; cycling for the first 10 cycles at 94 °C for 1 min, at 64 °C for 1 min, and at 70 °C for 1.5 min; and cycling for the last eight or 22 cycles at 90 °C for 1 min, at 64 °C for 1 min, and at 70 °C for 1.5 min.

3. Results and discussion

3.1. Matrix effect

The on-line concentration of DNA in the presence of EOF described here is based on the decrease in the electrophoretic mobility of DNA mainly due to sieving and increases in the viscosity when migrating from the sample zone to the polymer solution [20,22]. It is thus important to select a suitable polymer solution for optimum concentration and separation. One other critical consideration when applying this method is adsorption of polymer molecules on the capillary wall mainly due to hydrogen bonding and hydrophobic patches, leading to a small EOF mobility and difficulty of refreshing the capillary wall. In this aspect, use of hydrophilic polymers such as PEO and hydroxyethylcellulose is

more appropriate [28]. It has been shown that the adsorption is profound at low ionic strengths and sample matrix plays a crucial role in determining migration time, resolution, and stacking when injecting a large volume of DNA [22]. Table 1 shows the results when using a number of TB buffers, pH 7.0 to 11.0, to prepare DNA samples. At the same pH, migration times became shorter due to reduced PEO adsorption, while resolution decreased with increasing ionic strength (concentration). In terms of sensitivity improvement (injection volume up to about 1.86 μl) and resolution, TB buffers of 5–10 mM, pH 10.0 and 11.0, are superior. On the other hand, the injection volumes were much less when DNA samples were prepared in TB buffers, pH 7.0, 8.0, and 9.0 (10 and 25 mM Tris). This indicated the use of high amounts of boric acid to prepare DNA samples is not suitable. The limit of injection volume is also due to the small electrophoretic mobility of the DNA fragments at low pH (or high ionic strength). Please note that the analysis is also not favored at low pH because of the relatively low quantum yields of intercalated DNA fragments at pH 7.0 and 8.0.

As the goal of this work aimed to the development of a method allowing sensitivity improvement for the

Table 1
Effect of the sample matrix on injection volume, peak height, bandwidth, resolution, and migration time using a 60-cm capillary

pH	TB (mM)	Borate (mM)	V^a (μl)	Peak height (mV)			Bandwidth (min)			Resolution			Migration time (min)		
				1353^b	271^b	72^b	1353	271	72	1353/1078	271/281	118/72	t_0^c	t_{1353}^d	t_{72}^d
7.0	5.0	26.5	0.46	140.5	4.9	2.0	0.02	0.11	0.22	1.84	0.96	12.12	19.51	22.27	31.11
	10.0	53.0	0.02	20.1	1.4	1.0	0.03	0.03	0.07	1.22	6.29	62.34	14.12	16.86	24.97
8.0	5.0	9.0	0.62	155.0	3.7	2.7	0.01	0.08	0.08	5.02	6.91	18.24	30.04	32.75	42.24
	10.0	18.0	0.15	67.6	1.8	1.7	0.02	0.05	0.06	2.36	6.76	55.17	16.42	19.25	28.06
	25.0	45.0	0.05	13.1	1.3	0.7	0.03	0.07	0.09	1.95	5.25	14.37	14.87	17.57	26.15
9.0	5.0	4.1	1.86	574.8	20.1	18.8	0.03	0.02	0.04	4.99	5.40	55.03	70.85	74.35	85.63
	10.0	8.2	0.93	859.9	10.0	8.6	0.02	0.07	0.05	3.54	1.90	27.43	56.93	59.83	70.94
	25.0	20.5	0.46	151.1	3.9	1.8	0.03	0.11	0.16	1.45	1.09	20.45	24.27	27.67	37.97
10.0	5.0	0.3	1.86	1223.2	21.9	40.3	0.04	0.03	0.04	3.33	3.89	43.27	65.56	66.77	75.12
	10.0	0.6	1.86	1224.3	54.5	53.6	0.03	0.02	0.03	2.18	5.44	54.23	50.18	51.30	58.37
	25.0	1.5	1.86	1184.7	444.3	48.7	0.04	0.02	0.04	0.96	6.65	56.15	43.03	45.98	56.74
11.0	5.0	0.3	1.86	1203.3	67.8	35.1	0.02	0.04	0.03	3.29	4.35	56.98	63.57	66.16	75.05
	10.0	0.6	1.86	1205.5	122.2	40.2	0.02	0.03	0.03	2.78	4.86	53.32	51.22	53.60	60.13
	25.0	1.5	1.86	1208.2	523.1	45.4	0.01	0.03	0.05	0.73	8.17	40.62	34.97	37.72	46.38

[a] Maximum injection volume.

[b] 1353, 271, and 72 represent the 1353-, 271-, and 72-bp fragments, respectively.

[c] Time when the baseline shifted.

[d] Migration times for the 1353- and 72-bp fragments, respectively.

analysis of DNA in biological samples without sample pretreatment, it is required to optimize the condition for increasing injection volumes at pH 7.4. Although Table 1 shows that DNA stacked when injecting DNA up to 0.46 µl at pH 7.0, the maximum injection volumes for biological samples should be different, depending on matrix. To explore the impact of salts on concentration and separation of DNA, 5 mM TB buffers, pH 7.4, containing different salts, including sodium chloride, sodium phosphate, and sodium acetate, were tested. Table 2 shows that the separation became faster with increasing the concentrations of salts when injecting 0.62 µl DNA. With increasing the concentration of salts, PEO adsorption decreased, leading to a greater EOF mobility. It should be pointed out that higher and sharper peak profiles were achieved at higher concentrations of salts when adding the same species. This indicated that ITP occurred. In addition, greater quantum yields of intercalated DNA fragments at high salts should be taken into account [29]. However, peak splitting was problematic when using TB buffers containing 50.0 mM sodium phosphate or sodium acetate. To this end, we should point out that the effect of pH changes on the stacking efficiency should not be ignored [30].

3.2. Effect of metal ions

Next, we tested the analysis of 0.75 µl DNA prepared in PBS by this proposed method, with a disappointing result as shown in Fig. 2A. Possible reasons include a significantly differential conductivity between the sample zone and PEO as well as small electrophoretic mobilities of the DNA fragments in high-conductivity media (the major salt is NaCl, 138 mM). To reduce the effect of Cl^-, we tested the possibility of forming AgCl particles with Ag^+ inside the capillary during the analysis. This was done by injecting a short plug (3.25 cm) of $AgNO_3$ prepared in 25 mM TB, pH 10.0, after DNA injection. Fig. 2B and C show very impressive results, while 2D and E demonstrate less successfully. It is interesting to note that the peak heights for the 72–310 base pair (bp) fragments increased with increasing the concentration of $AgNO_3$ up to 1 mM, while some peaks split and declined at greater than 1 mM. We inferred that the short plug of $AgNO_3$ should play a significant role in affecting stacking and resolution. The plug acted as a water plug that has been shown in other studies to increase the stacking efficiency [31,32], wherein the velocity of the DNA fragments accelerated when migrating from the sample zone to the low-conductivity plug. However, we have to mention that applying a short plug of water did not work by this method. In addition, pH changes and sample self stacking may be contributors [16,30]. It is also very possible that AgCl particles formed once Ag^+ from the plug interacted with Cl^- from the sample zone, with a support of the existence of a small, wide and noisy peak after the baseline shifted (not shown). As a result, the DNA fragments might interact with AgCl particles, leading to very sharp peaks and enhanced stacking efficiency. At high concentrations, greater amounts of particles might form and thus interact strongly with DNA, which caused peak splitting for the large DNA fragments. To support our reasoning, we injected a short plug of 0.5 mM $NaNO_3$ at concentrations ranging 0–2 mM instead of $AgNO_3$. In the range of

Table 2
Effect of salts on peak height, bandwidth, resolution, and migration time using a 60-cm capillary

|		Peak height (mV)			Bandwidth (min)			Resolution			Resolution		
		1353	271	72	1353	271	72	1353/1078	271/281	118/72	t_0	t_{1353}	t_{72}
Sodium chloride (mM)	1.0	124.6	8.9	3.3	0.04	0.13	0.22	4.42	1.16	72.18	25.72	28.71	39.48
	10.0	335.2	34.1	15.8	0.04	0.06	0.04	1.75	5.41	65.72	22.40	25.50	36.40
	50.0	1180.8	386.7	12.7	0.02	0.01	0.05	2.71	11.21	40.54	17.10	22.59	32.35
Sodium phosphate (mM)	1.0	501.7	11.2	4.3	0.02	0.08	0.10	1.57	2.19	27.37	27.08	30.32	42.13
	10.0	1229.0	552.5	22.2	0.02	0.02	0.04	4.33	13.73	58.52	24.84	29.52	42.49
Sodium acetate (mM)	1.0	136.2	3.5	1.3	0.04	0.12	0.23	2.51	2.97	68.53	26.99	30.04	41.28
	10.0	865.6	226.4	15.0	0.03	0.03	0.04	4.25	6.64	61.88	24.98	28.60	41.17
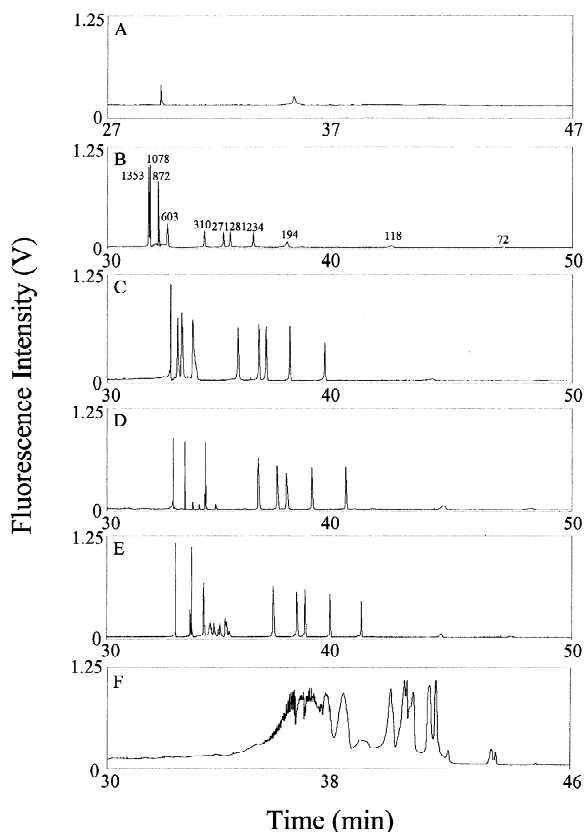
Fig. 2. Effect of the plug composition on DNA stacking at 333 V/cm using 1.5% PEO solution prepared in 200 m*M* TB, pH 9.0, containing 5 μg/ml EtBr. Capillary: 60 cm of total length, 50 cm of effective length, 75 μm of inner-diameter, and filled with 400 m*M* TB, pH 10.0. One μg/ml ϕX174 RF DNA-Hae III digest prepared in PBS was injected at 250 V/cm for 120 s (0.75 μl). Plugs of AgNO₃ prepared in 25 m*M* TB, pH 10.0, were injected at 250 V/cm for 60 s after sample injection, with [AgNO₃]=0 m*M* in A, 0.1 m*M* in B, 0.5 m*M* in C, 1 m*M* in D, and 2 m*M* in E. A plug of 0.5 m*M* NaNO₃ prepared in 25 m*M* TB, pH 10.0, was injected at 250 V/cm for 60 s after sample injection in F.

Fig. 3. Effect of the length and composition of AgNO₃ plug on DNA concentration and separation. (A) AgNO₃ prepared in 25 m*M* TB, pH 10.0, was injected at 250 V/cm for 90 s; (B) AgNO₃ prepared in 1.5% PEO dissolved in 200 m*M* TB, pH 9.0, was injected at 250 V/cm for 60 s. Other conditions were as in Fig. 2.

0–0.5 m*M*, the sensitivity for small DNA fragments increased, while the loss of resolution was problematic. Fig. 2F represents one of the best results using NaNO₃ plugs, with broad and unresolved peaks for the large DNA fragments that indicate poor stacking. When the concentrations of NaNO₃ were further increased from 0.5 to 1.0 m*M*, the resolution decreased and only a very broad peak was found at 1.0 m*M*, again indicating poor stacking.

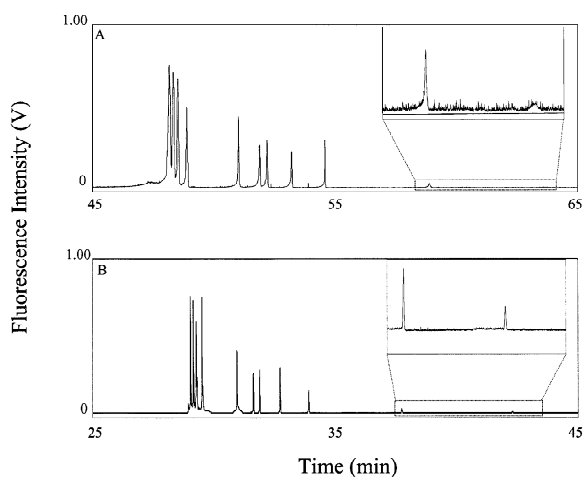To further evaluate the effect of AgNO₃ on DNA stacking, we increased the plug length of 0.5 m*M*

AgNO₃ prepared in 25 m*M* TB, pH 10.0. Compared to Fig. 1C, Fig. 3A shows that increases in the plug length did not provide better stacking, but caused a longer separation time (64 min). This is because PEO adsorption increased when the low-conductivity plug of AgNO₃ was increased. On the other hand, the separation time was shortened to 44 min shown in Fig. 3B when injecting 0.5 m*M* AgNO₃ prepared in 1.5% PEO dissolved in 200 m*M* TB, pH 9.0. The result indicates that our first reasoning about the role of AgNO₃ plug in increasing injection volumes addressed above is not the main reason. In comparison to broad and unresolved peaks obtained without applying such a plug (not shown), the result also shows the important role that AgNO₃ played in determining stacking. It is important to emphasize that the peak corresponding to the small DNA fragments were sharper and greater (about 10 times higher for the 72-bp fragments) when compared to that applying a plug of 0.5 m*M* AgNO₃ prepared in 25 m*M* TB buffer, pH 10.0. With a faster separation result, this again supports that PEO adsorption is more pronounced at low ionic strengths.

3.3. Linearity

When DNA samples prepared in 50 m*M* TB, pH

Table 3
Linearity, reproducibility, and efficiency for the analysis of DNA prepared in 50 m*M* TB, pH 10.0 using an 80-cm capillary

	DNA (bp)		
	1353	271	72
Linear regression[a]	$y = 679.85x + 3.23$	$y = 574.26x + 2.44$	$y = 99.34x + 1.39$
R^2	0.997	0.998	0.997
% RSD (peak height, $n=5$)[b]	3.6	3.8	2.9
% RSD (migration time, $n=5$)[b]	3.4	3.9	3.2
Theoretical plates[b]	5.7×10^6	3.1×10^6	1.7×10^6

[a] *y*, peak height (mV); *x*, injection volume (μl).

[b] 2.58 μl of 1.0 μg/ml φX174 RF DNA-Hae III digest injected.

10.0, were electrokinetically injected, the peak height is proportional to the injection volume up to 2.58 μl as shown in Table 3. For example, linear relationships between the injection volume and the peak heights corresponding to 1353-, 271-, and 72-bp fragments were obtained, with $R^2 > 0.997$. The reproducibility of this method was reasonable in terms of migration time and peak height (RSD<4.0%). The fact that the theoretical plates for all the DNA fragments were greater than 1 million while injecting 2.58 μl DNA further suggests that all DNA stacked without significant loss of the separation efficiency. On the basis of the peak height corresponding to the 72-bp fragment, the limit of detection (LOD) at a signal-to-noise ratio $(S/N) = 3$ for the DNA sample was 0.171 ng/ml. This was about an 860-fold sensitivity improvement compared to that by a conventional injection (10-s injection). However the analysis was slow (about 140 min). Fig. 4 depicts linearity between the peak height and the injection volume when injecting 0.5 m*M* AgNO$_3$ at 250 V/cm for 60 s after injection of DNA prepared in PBS. Linearity with $R^2 > 0.97$ was obtained for the 1353-, 271-, and 72-bp fragments, over the volume range of 0–0.75 μl. As 0.75 μl DNA was injected, the LOD was 6.86 ng/ml on the basis of the peak height corresponding to the 72-bp fragment. This was about a 155-fold sensitivity improvement compared to that by a conventional injection.

3.4. Stacking of PCR products

To show the feasibility of this method with a short plug of AgNO$_3$ for the analysis of real biological samples, we injected and separated 0.75-μl PCR products that were amplified after 18 cycles. Fig. 5 clearly shows that there are two peaks between 24 and 25 min. The RSD values ($n=3$) for the peak height and migration time for the first one were 3.1 and 2.2%, respectively. It is important to note that the PCR cycle performed in this study was much less than the one (32 cycles) suggested by the manufacturer. Advantages over greater amplification cycles include a shorter analysis time (PCR, concentration and separation), shortening from 122.7 min to 92.5 min, less costs (less consumption of primer, template, and other reagents), and a low risk of contaminants [33,34]. To identify the two peaks shown in Fig. 5, we spiked φX174 RF DNA-Hae III digest into the PCR product and performed the analysis (result not shown). Comparing the migration times for these two peaks to that for 234- and 194-bp fragments from the standard, we assigned these two
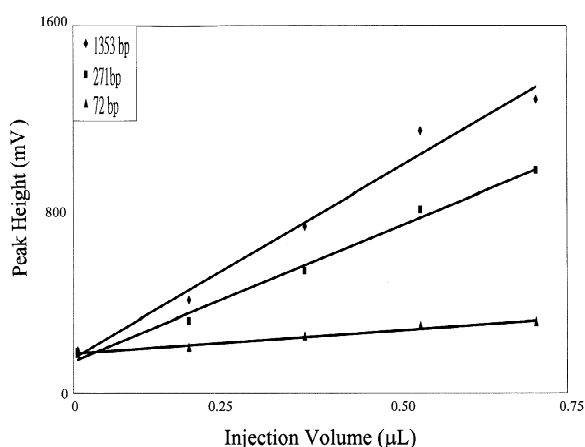

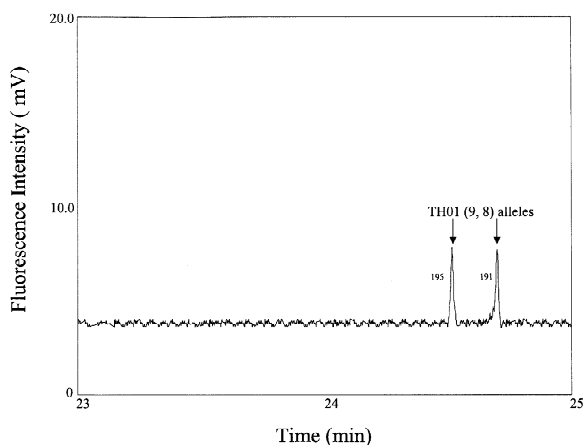Fig. 4. Linearity between peak height and injection volume. Conditions were as in Fig. 3B.

Fig. 5. Separation of 0.75-μl PCR products amplified after 18 cycles at 333 V/cm using 1.5% PEO prepared in 200 m*M* TB solution, pH 9.0. Other conditions were as in Fig. 3B.

peaks to TH01 (9, 8) allele, with 195- and 191-bp fragments, respectively.

4. Conclusions

We have found that matrices containing suitable amounts of salts like NaCl are essential for large-volume injection and rapid analysis, due in part to reduced PEO adsorption. Salts not only affect resolution, peak asymmetry, migration time, and stacking efficiency, but also affect the interactions with the capillary wall and the fluorescence characteristics of intercalated DNA fragments. However, large amounts of salts would cause small electrophoretic mobilities of the DNA fragments migrating from the sample zone to PEO solution and peak splitting, thereby limiting the injection volume. These problems have been partially solved by injection of a short plug of $AgNO_3$ after sample injection. As a result, it allows the analysis of up to 0.75-μl DNA samples prepared in PBS or PCR products without desalting. The results have proved this method provides the advantages of simplicity, robustness, reproducibility, and rapidity. Together with a 155-fold improvement in the sensitivity, this method is quite promising for analysis and/or recovery of gene amplified after PCR products.

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