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High-sensitivity capillary gel electrophoretic analysis of DNA fragments on an electrophoresis microchip using electrokinetic injection with transient isotachophoretic preconcentration

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Abstract

The research adopted a single-channel microchip as the probe, and focused electrokinetic injection combined with transient isotachophoresis preconcentration technique on capillary electrophoresis microchip to improve the analytical sensitivity of DNA fragments. The channel length, channel width and channel depth of the used microchip were 40.5 mm, and 110 and 50 μm, respectively. The separation was detected by CCD (charge-coupled device) (effective length=25 mm, 260 nm). A 1/100 diluted sample (0.2 mg/l of each DNA fragment) of commercially available stepladder DNA sample could be baseline separated in 120 s with S/N=2–5. Compared with conventional chip gel electrophoresis, the proposed method is ideally suited to improve the sensitivity of DNA analysis by chip electrophoresis.

Keywords: Chip technology; Injection methods; Isotachophoresis; DNA

1. Introduction

Microchip electrophoresis is becoming a popular and effective technique for DNA analysis, which has been applied to polymerase chain reaction (PCR) products analysis, forensic identification and DNA sequencing to achieve genetic and diagnostic research [1–6]. The most significant advantage of adopting a microchip is its high analysis speed that could lead to a significant improvement in throughput of DNA analysis compared to the conventional slab gel electrophoresis method. However, the smaller injection volume associated with microchips inherently limits the sensitivity, which hinders its application to trace analysis.

Many methods were developed to improve the sensitivity, including changing the design of the microchip to increase the injection amount and separation length, but these brought about other problems such as band broadening [6,7]. Detection sensitivity could be enhanced by intercalating a dye for fluorescence detection [1,3] and adopting laser-produced fluorescence (LIF) detection [2,4,8,9]. However, developing an on-line preconcentration technique which can be used for conventional commercial apparatuses is more desirable. Isotachophoresis is becoming an important preconcentration technique for other electrophoretic modes [10,11].

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Many applications of transient isotachophoresis (tr-ITP) preconcentration have been reported and reviewed [12–16], but there are no reports about DNA preconcentration on microchips.

The running buffer for DNA analysis is usually the TAE (Tris–acetic acid–EDTA) or TBE (Tris–boric acid–EDTA) system, and the effective mobility of DNA fragments in these buffers have been studied in detail [17]. In the present study, we have developed a DNA running buffer containing the leading ion to achieve the electrokinetic injection with tr-ITP (we named such a procedure electrokinetic supercharging [18]) and a sieving material [hydroxyethylcellulose (HEC)]. The microchip with a cross-column geometry is the structure that allows injection and separation to occur quickly and efficiently [1,8,19,20]. In order to achieve supercharging tr-ITP, however, a single-channel microchip, just as in a conventional capillary, was preferable to get good reproducibility.

In this study, the results of DNA analysis under capillary gel electrophoresis (CGE) mode using conventional cross-column geometry microchips were compared to those obtained under tr-ITP-CGE mode using the single-channel microchip. Operational conditions, such as the injection time of DNA sample and terminating electrolyte (TE) under tr-ITP-CGE mode, was optimized to be suitable for the chip size and getting good resolution for a commercial DNA stepladder sample [50–800 basepairs (bp)]. These demonstrations indicated that the supercharging transient isotachophoresis technique combined with chip gel electrophoresis could be widely applied to improve the analytical sensitivity for DNA fragments.

2. Experimental

2.1. Apparatus

The apparatus used was a Shimadzu (Kyoto, Japan) model MCE-2010 chip electrophoresis system. Fig. 1 shows a schematic of the MCE-2010

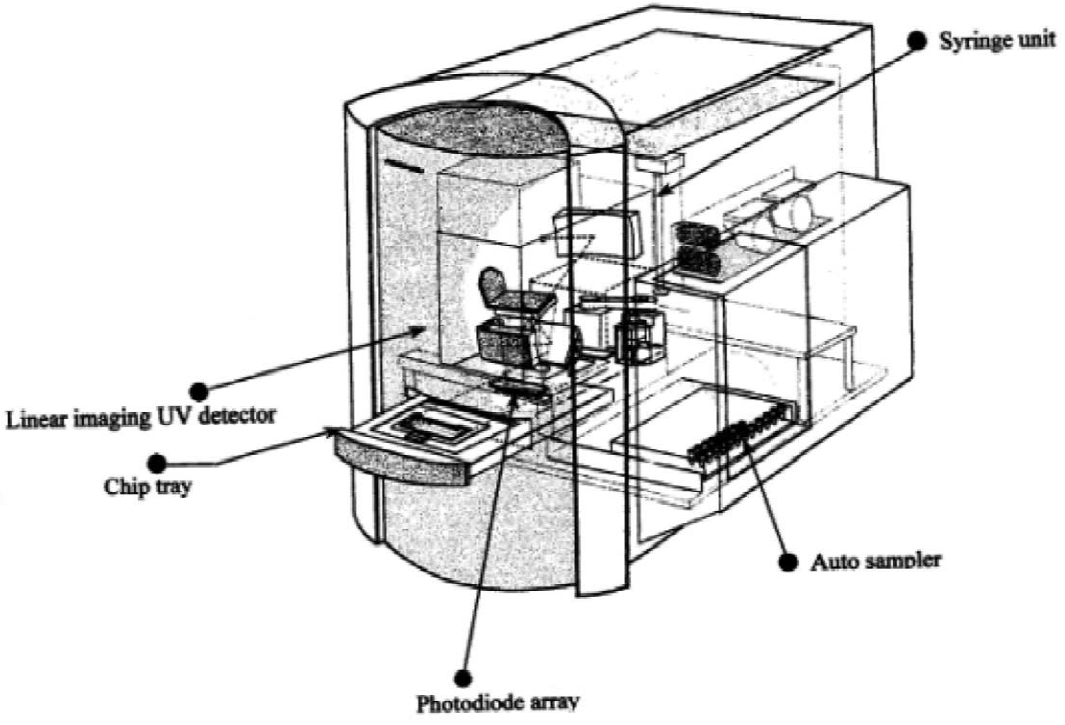


Fig. 1. Shimadzu microchip electrophoresis system (model MCE-2010). The system consisted of a linear imaging UV detector with a photodiode array, a syringe unit with drive mechanism, and an autosampler.

system. The system consisted of a linear imaging UV detector (photodiode array, effective length=25 mm), a syringe unit for the introduction and evacuation of the buffer and sample, a drive mechanism (chip tray) to move a chip to the specified position, and an autosampler. The light source was a deuterium ($^2H_2$) lamp. High-throughput analysis (1–2 min/sample) of non-labeled DNA fragments could be achieved on the chip system. The linear imaging UV detector could make the separation of the sample components monitored in real-time in the separation channel.

2.2. Types of microchips used

Two types of quartz microchips from Shimadzu were used, as shown in Fig. 2. One of the microchips had cross-column geometry, and was commercially available (code: type D [110×50]-C). The whole channel length, channel width and channel depth of the microchip were 40.5 mm, and 110 and 50 μm, respectively. On the chip, ports 1–4 were used as the analyte reservoir, the analyte waste reservoir, the buffer reservoir and the waste reservoir, respectively. The capacity of every reservoir was 3 μl. The single-channel microchip had only the channel between ports 3 and 4.

2.3. DNA sample and running buffers

A standard DNA stepladder marker (Promega, Madison, WI, USA) was used, which consisted of 16 DNA fragments ranging from 50 to 800 bp in exactly 50-bp increments. The sample concentration was 340 mg/l as a whole and was supplied in 10 m*M* Tris–HCl (pH 8.0), 1 m*M* EDTA according to specifications. The 800-bp band appeared 2–3 times more concentrated than the other bands, which were of approximately equal concentration. On the assumption that the 800-bp component was three times concentrated in comparison with the other components, the concentration of each DNA fragment (50–750 bp) would be about 19 mg/l. The original sample was diluted 1/10, 1/20, 1/50 and 1/100 in purified water to a lower concentration to test the preconcentration effect on the chip.

Although the typical sieving matrix for DNA separation was polyacrylamide [1,5,21], hydrophilic polymer was also used [4,22]. In the present study, 2% HEC (average M_r 250 000) from Aldrich (Milwaukee, WI, USA) was used, which can be injected and replaced by the syringe system. Table 1 shows two types of DNA running buffer system used. Running buffer 1 (TBE system) was from Shimadzu (code: Type D, 228-40597-91). Running buffer 2 was a set of electrolytes prepared by us to enable preconcentration by electrokinetic supercharging. In

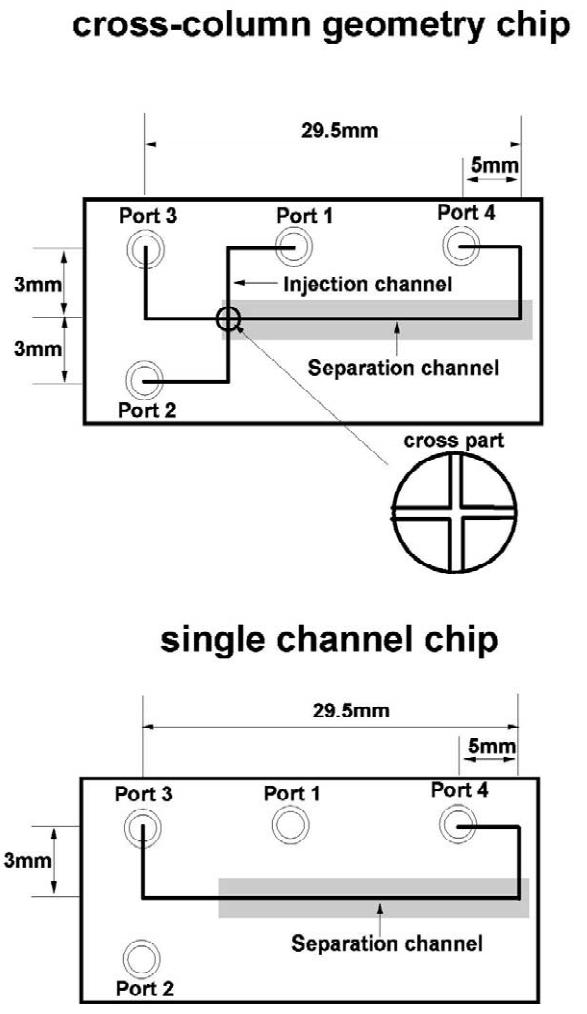


Fig. 2. Two types of microchip devised for MCE-2010. The channel length, channel width and channel depth of the microchip were 40.5 mm, and 110 and 50 μm, respectively. Effective length for CCD detection (hatched area) was 25 mm. Ports 1–4 were the analyte reservoir, the analyte waste reservoir, the buffer reservoir and the waste reservoir, respectively.

Table 1
DNA running buffer systems used in the present work

Type name		Components
DNA running buffer 1 (TBE system)		2% HEC, 445 m*M* Tris–borate (pH 8.3), 12.5 m*M* EDTA, 0.1% NaN_3
DNA running buffer 2	Buffer A (LE)	2% HEC, 50 m*M* HCl–Tris (pH 8.1)
	Buffer B (TE)	20 m*M* glycine–Tris (pH 8.1)
	Buffer C (LE)	50 m*M* HCl–Tris (pH 8.1)

the system buffers A and C were the leading electrolyte (LE), and buffer B was used as the terminating electrolyte (TE) for tr-ITP.

2.4. Analytical modes

2.4.1. Conventional CGE mode

The analytical procedure using cross-column geometry microchip has been reported by many researchers [1,2,23]. Fig. 3a shows the voltage-timing program of the standard DNA method supported by MCE-2010. At the beginning, the high voltage was applied between ports 1 and 2 to introduce the sample electrokinetically from the analyte reservoir (port 1) to the analyte waste reservoir (port 2). Then the high voltage was applied to the separation channel between the buffer reservoir (port 3) and the waste reservoir (port 4) to separate the sample at the cross part. Before sample introduction, the channel was thoroughly washed with water and then filled with running buffer 1.

2.4.2. Electrokinetic supercharging tr-ITP-CGE mode

In this case, DNA running buffer worked not only as polymer-sieving matrix but also as LE. As shown in Fig. 3b, firstly buffer A was introduced into the channel using the syringe unit, and then the DNA sample and TE (buffer B) were electrokinetically

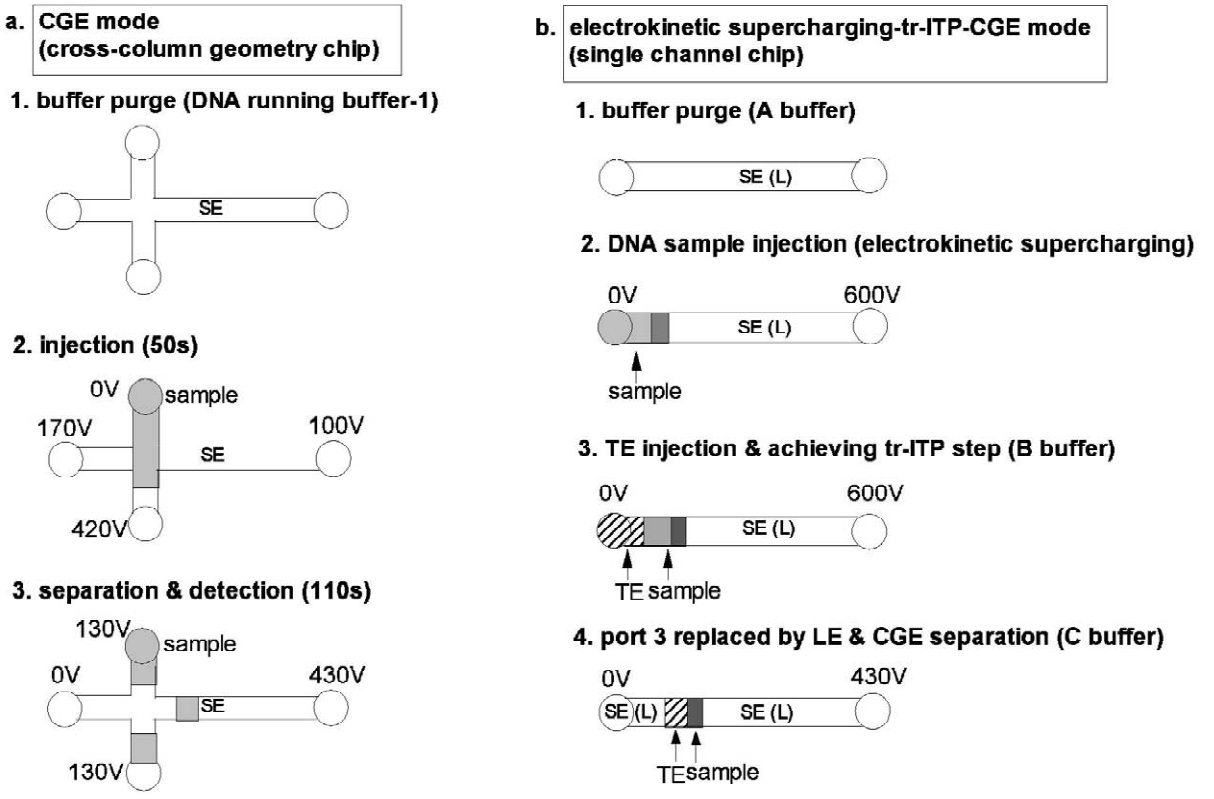


Fig. 3. Protocol of microchip electrophoresis. (a) CGE mode using a cross-column geometry microchip, (b) electrokinetic supercharging tr-ITP-CGE mode using a single-channel chip.

injected from port 3. At this stage, a greater amount of sample was introduced to the separation channel without deteriorating the peak shapes of the sample component due to isotachophoretic regulation. Finally, port 3 was replaced by LE (buffer C) again, which did not contain HEC for smooth replacement of electrolytes. Then the preconcentrated sample components were separated as usual CGE mode.

3. Results and discussion

3.1. Separation behavior in the cross-geometry chip under CGE mode using two buffer systems

Although the polymer-sieving matrix has the most significant function for DNA separation, the co-existing ions also play important roles affecting the resolution and effective mobility of DNA fragments. In this study, the resolution obtained using DNA running buffer 1 containing borate was better than that obtained by buffer A (50 m*M* HCl added as leading ion), which was attributed to the formation of a borate–DNA complex [17]. When buffer A was used, obviously from comparison between Fig. 4a and b, the effective mobility of DNA fragments was higher than that in TBE buffer. In Fig. 4b 50-, 100- and 150-bp fragments flowed into the waste reservoir in spite of the same separation time (110 s). This demonstrated that TBE system DNA running buffer acted better than Tris–HCl under CGE mode. We have tried another electrolyte system containing borate along with chloride, but it was unsuccessful because the effective mobility of borate was larger than those of the sample components.

When the original DNA sample was diluted to 1/10 in water, the peaks except for the 800-bp fragment, could not be detected under CGE mode in both buffer systems (Fig. 4c,d), which indicated that the low limit of detectable concentration (LLDC) for each DNA fragment (50–750 bp) using the cross-geometry microchip was about 4 mg/l.

3.2. Optimization of injection time of the DNA sample and TE under electrokinetic supercharging tr-ITP-CGE mode in a single-channel chip

The sensitivity of DNA analysis was enhanced by electrokinetic supercharging, where electrokinetically

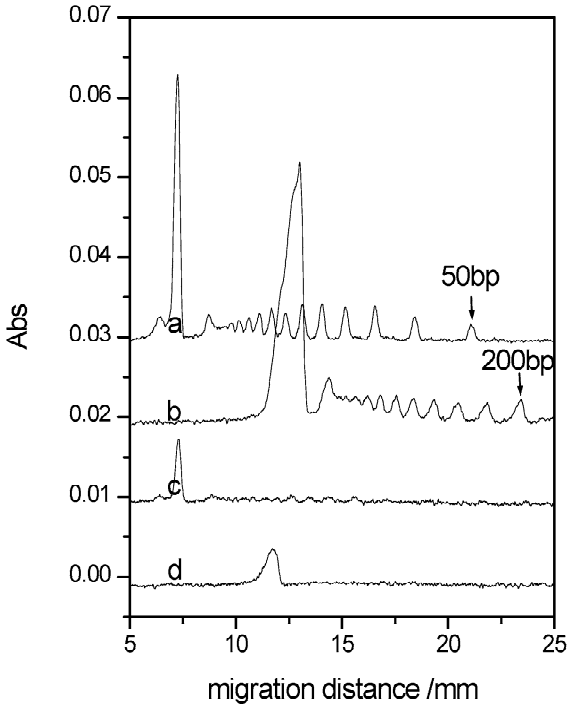


Fig. 4. Electropherograms obtained under CGE mode using a cross-column geometry microchip. The original sample (total conc. = 340 mg/l) was used for (a,b), and the 1/10 diluted sample (34 mg/l) was used for (c,d). DNA running buffer 1 was used for (a,c), and buffer A was used for (b,d). The protocol used was as in Fig. 3a.

injected sample zone was self-sharpened by tr-ITP technique using the single-channel microchip. Fig. 5 shows time dependence of supercharging on the amount of the injected sample. When only the electrokinetic supercharging was used without the tr-ITP step, the sample components migrated as a single zone without separation, although an increase of the sample amount was observed with the increase of injection time. An important conclusion was that high sensitivity could not be obtained only by increasing the sample amount, which was due to the short migration path of the used microchip.

The injection of TE after the sample was essential to obtain a good resolution. As shown in Fig. 6, however, the injection time of TE should be optimized according to the sample injection time and the sample amount [14–16]. If the injection time of TE was too long, transition from tr-ITP to CGE was too late to get sufficient resolution in the CGE stage. In

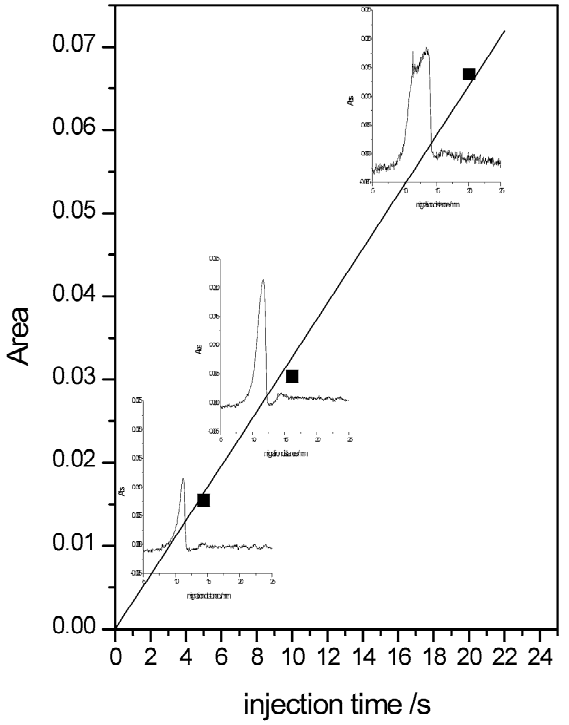


Fig. 5. Electropherograms of the 1/10 diluted sample (total conc. = 34 mg/l) obtained using the single-channel chip and the relationship between peak area and injection time. The sample injection time was 5 s (a), 10 s (b) and 20 s (c). The sample was injected by electrokinetic supercharging without subsequent injection of TE (without the tr-ITP step). Separation time, 120 s; running buffer, buffer 2.

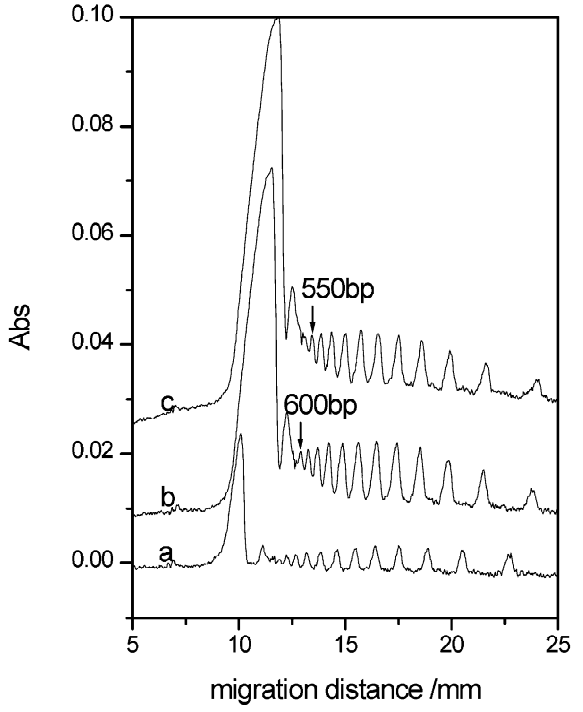


Fig. 6. Electropherograms obtained by varying sample injection time as 5 s (a), 20 s (b) and 30 s (c). The sample was the 1/10 diluted (total conc. = 34 mg/l). TE injection time was 15 s. The protocol used was as in Fig. 3b. Other conditions as in Fig. 5.

Fig. 6b the DNA fragments from 50 to 600 bp were separated when the DNA injection time was 20 s; however, in Fig. 6c the peak of 600 bp overlapped with successive fragments when DNA injection time was 30s (see Fig. 6c). The electropherograms also showed that the intensity of peaks remained virtually constant when the injection time increased from 20 to 30 s, suggesting that a 20-s injection was sufficient for introducing all of the DNA sample from the 3-μl reservoir to the separation channel.

Fig. 7 displays the obtained separation behavior when the DNA sample injection time was kept constant (20 s) and the injection time of TE was increased from 5 to 30 s. The better preconcentration effect was obtained with the longer injection time, but a few peaks of small fragments flowed into the waste reservoir, as shown in Fig. 7c (30-s injection). This is again due to the limitation of chip size. Obviously from Figs. 6 and 7, the injection time of both sample and TE should be optimized to obtain high preconcentration effect without loss of resolution. We found that the appropriate injection times of the present DNA sample and TE were 20 and 15 s, respectively.

3.3. Analysis of highly diluted DNA samples

By using electrokinetic supercharging tr-ITP-CGE mode, highly diluted DNA samples could be analyzed under the optimized injection time of the DNA sample and TE (20 and 15 s). Fig. 8b–e shows the electropherograms obtained for the 1/100, 1/50, 1/20 and 1/10 diluted samples, respectively. Fig. 8a shows the separation behavior of the original DNA sample analyzed under CGE mode. The S/N values

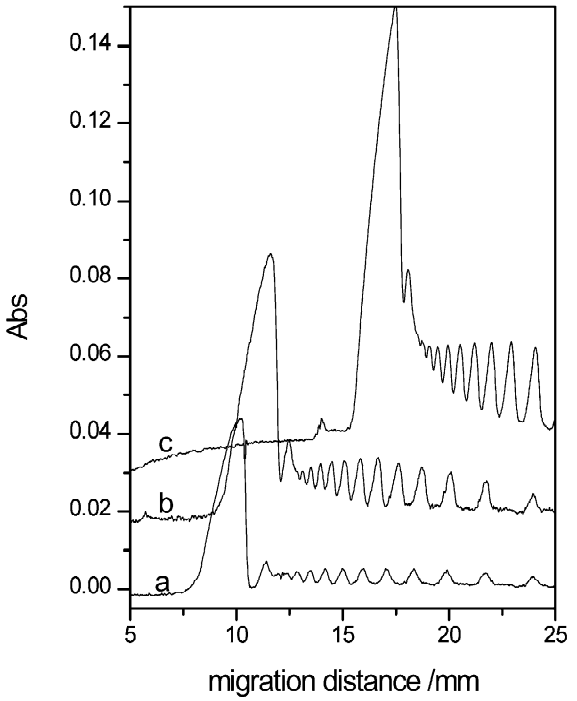


Fig. 7. Electropherograms obtained by varying TE injection time [5 s (a), 15 s (b) and 30 s (c)]. Sample injection time was 20 s. Other conditions as in Figs. 5 and 6.

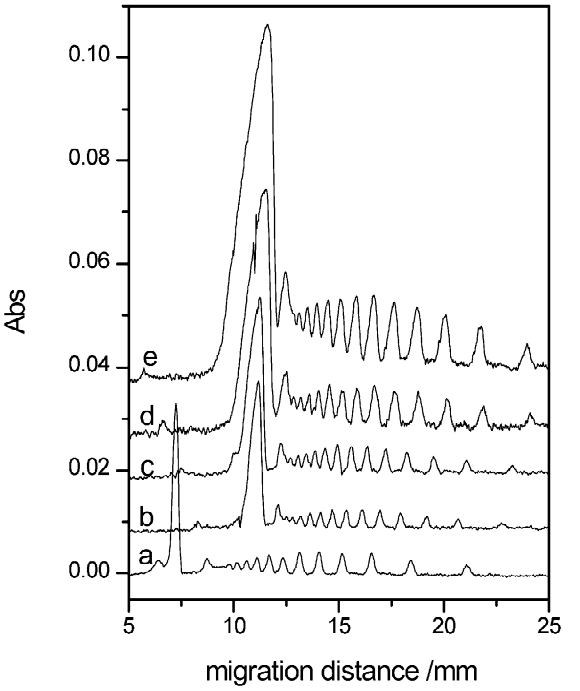


Fig. 8. Electropherograms of the diluted samples [1/10 (b), 1/20 (c), 1/50 (d) and 1/100 (e)] obtained under optimized conditions. The sample and TE injection times were 20 and 15 s, respectively, and the separation time was 120 s. Electropherogram (a) was for the original sample (total conc. = 340 mg/l) using cross-chip under CGE mode (running buffer 1).

of 50-, 150- and 300-bp fragments were calculated as shown in Table 2. The 1/100 diluted sample (0.2 mg/l of each DNA fragment) could be analyzed under electrokinetic supercharging tr-ITP-CGE with S/N values between 2 and 5. It should be noted that migration distance in Fig. 8b–e was stable for wide range on concentration under the same operational conditions, suggesting that the proposed method can be also used for the determination of the size of DNA fragments according calibration curve.

3.4. Size calibration curve

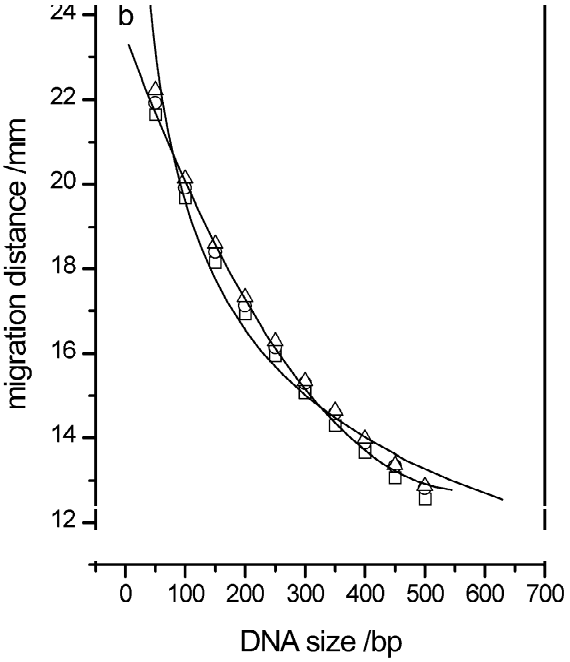
Since the qualitative analysis of DNA fragments is important for different aims, the DNA size calibration curve was created under supercharging tr-ITP-CGE mode for the 1/100 diluted 50-bp DNA stepladder sample. Fig. 9 showed the two size calibration curves under the optimized injection time and separation time. The relationship between effective mobility (μ) and DNA size (N) was fitted by the following equation [24]:

$$\mu \approx K \cdot \left(\frac{1}{N} + fE^2 \right) \quad (1)$$

where f is the function of ξ, q and stability of sample, and E is the field strength. Fig. 9a shows size calibration curve $\log L$ (L is the migration distance) and $\log N$. However obviously from Fig. 9a, the degree of fitting ($R^2 = 0.984$) was not suitable for accurate determination of the number of basepairs. This might be due to the relationship between DNA size and concentration of HEC. In the high concentration of sieving matrix (2% HEC) the migration behaviors of DNA fragments are considerable influenced by the length of the basepair chain, and other the small DNA fragments ease to disperse at the space of replacing TE and LE on port 3. On

Table 2
S/*N* values for some DNA fragments

Sample dilution	CGE mode, *S*/*N*			SC tr-ITP-CGE mode, *S*/*N*		
	50 bp	150 bp	300 bp	50 bp	150 bp	300 bp
Original	3.2	6.1	6.3			
1/10						



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