Influence of the Sample Volume upon Injection on Capillary Gel Electrophoresis for the Separation of Small DNA Fragments

Tomoka Nakazumi and Yusuke Hara

***Abstract*—In this study, we evaluated the influence of the sample volume (10 μL and 20 μL) upon the injection to a developed capillary gel electrophoresis (CGE) system designed using a short-length capillary (total length of 15 cm and effective length of 7.5 cm). The fused silica capillary was coated with an acrylamide linear polymer chain to prevent the electroosmotic flow in the direction opposite to the movement of DNA samples. The sieving polymer solution used was hydroxyethyl cellulose with a molecular weight of 1,300,000. Using this CGE system for the separation of a 100-bp DNA Ladder sample, the sample injection volume had a substantial effect on the electropherogram results. The error bars for migration time, mobility, and resolution length were much larger when using 20 μL of the sample than using 10 μL. The use of 10 μL of the sample provided highly reliable results. These findings indicated that developers of CGE equipment should pay close attention to the sample injection volume in order to measure small DNA samples with high accuracy.**

***Index Terms*—Capillary gel electrophoresis, injection, point-of-care testing, polymer solution.**

I. Introduction

Capillary gel electrophoresis (CGE) is recognized as a very useful tool for the separation of products after polymerase chain reaction (PCR), with greater reliability compared to slab gel electrophoresis (SGE) [1]-[4], owing to its speed, requirement of a very small amount of DNA, and higher accuracy. Recently, there has been great worldwide interest for an immediate compact analysis tool in point-of-care testing (POCT) to detect diseases and bacteria that can be used in bedside applications such in hospitals. To meet this demand, we are challenging development of a compact CGE system for the analysis of PCR products. To apply the CGE method to a compact system for POCT, the length of the fused silica capillary needs to be shortened while maintaining a high accuracy and resolution. To separate a DNA sample using the fused silica capillary, the capillary should first be coated because the capillary walls cause electroosmotic flow (EOF) in an opposite direction to the flow of the DNA samples due to the negative charge. Indeed, when using an untreated capillary, the DNA samples cannot be inserted into the capillary because of the inhibition by the EOF. Therefore, a coating method for a fused silica capillary has been extensively investigated [5]-[10]. In this study, to prevent the EOF during the process of introducing the DNA samples, a non-ionic acrylamide polymer chain was adopted via chemical bonding to the fused silica wall. In addition, a sieving polymer is needed to separate small DNA samples [11]-[13]. Since the DNA samples and the sieving polymer chain entangle, the migration time of the DNA with different lengths are different [14], [15]. To separate DNA samples clearly, we adopted hydroxyethyl cellulose (HEC) with a molecular weight of 1,300,000.

In our experiments, to separate a 100-bp DNA Ladder sample accurately with the developed CGE system, we evaluated the influence of the DNA samples volume upon the injection on the electropherogram results based on comparisons of the migration time, mobility, and resolution length.

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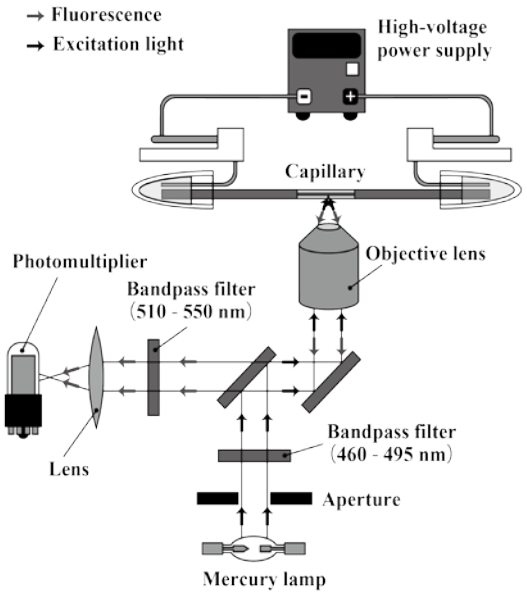

Fig. 1. Schematic illustration of the capillary gel electrophoresis system.

II. Experimental Section

A. CGE Measurement System

The CGE system (Fig. 1) consisted of a microscope with epi-illumination (IX73; Olympus, Tokyo, Japan) and a high-voltage power supply (HJPQ-10P3; Matsusada, Osaka, Japan). The excitation light source was a mercury lamp using an optical filter (U-FBWA; Olympus, Tokyo, Japan). For this study, SYBR Green II, which conjugates to the DNA samples,

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was used as the fluorescent chemical compound. The fluorescence from the DNA samples was captured using a 60× objective lens (UPlanFLN; Olympus), and the fluorescence signal was detected using a photomultiplier tube (H8249-101; Hamamatsu Photonics, Hamamatsu, Japan). The signal was converted to digital data for quantification with the National Instrument NI USB-6341. The diameter of the fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was 75 μm, with a total length of 15 cm and an effective length of 7.5 cm. The DNA samples were injected into the capillary at a voltage of 1.5 kV for 1 s, and the separation process was performed at 100 V/cm.

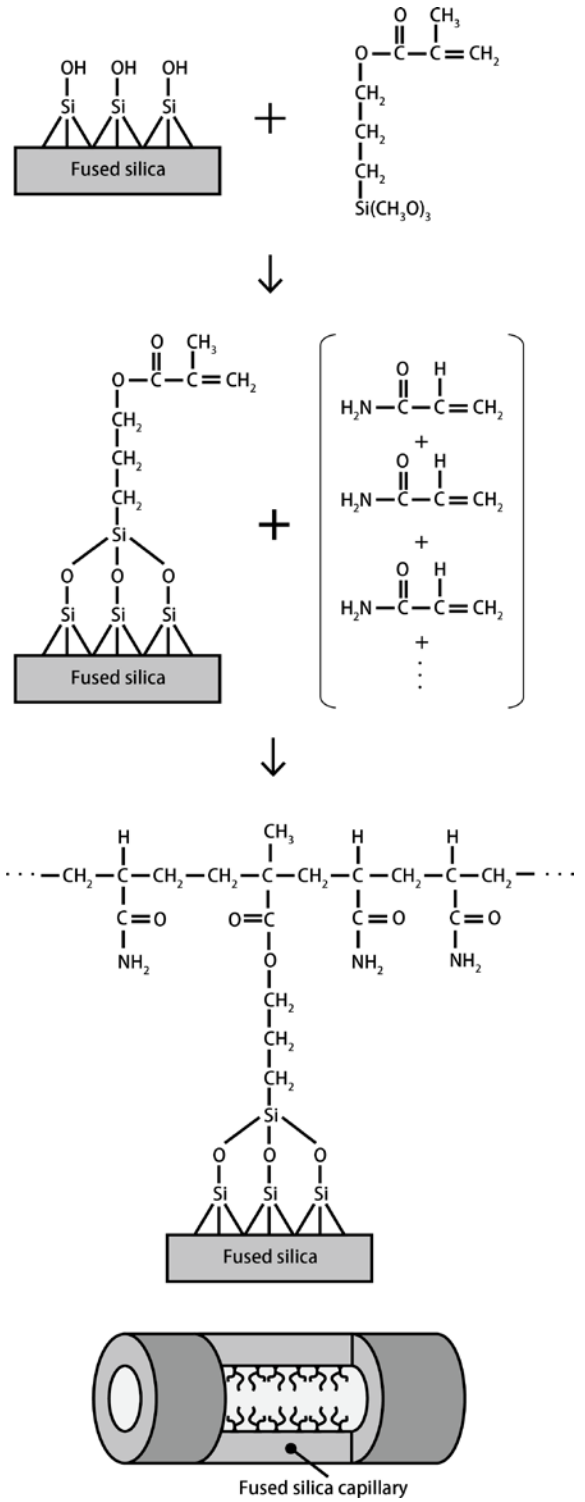


Fig. 2. Schematic illustration of the polymer coating method.

B. Coating Method for the Fused Silica Capillary and CGE Chemicals

An overview of the overall process of the coating method for the fused silica capillary was shown in Fig. 2. Firstly, the capillary was washed using 1 N NaOH (15 min), water (15 min), and methanol (15 min). Then, a solution (total 20 mL) containing 80 μL of 3-methacryloxypropyltrimethoxysilane (Shin-Etsu Chemical, Tokyo, Japan), 1 mL of methanol, and one drop of acetic acid was flowed into the capillary to coat the capillary walls. In the next step, the monomer solution (total 20 mL), including acrylamide monomer (0.7 g), ammonium persulfate (20 mg), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (20 μL), was flowed into the capillary for 2 h. Before flowing the monomer solution, the solution was bubbled with nitrogen gas for 30 min. The 100-bp DNA Ladder (TAKARA BIO, Shiga, Japan) consisting of 11 double-stranded fragments with lengths of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1500 bp was selected as the separation target. The DNA ladder sample (130 μg/mL) was used after it was diluted 10 times with ultrapure water. For the present evaluation, the DNA ladder sample solutions were prepared at two volumes (10 μL and 20 μL) with the same DNA concentration (13 μg/mL) as the injection samples. A 0.5× Tris-borate-ethylenediaminetetraacetic acid buffer was selected as the running buffer.

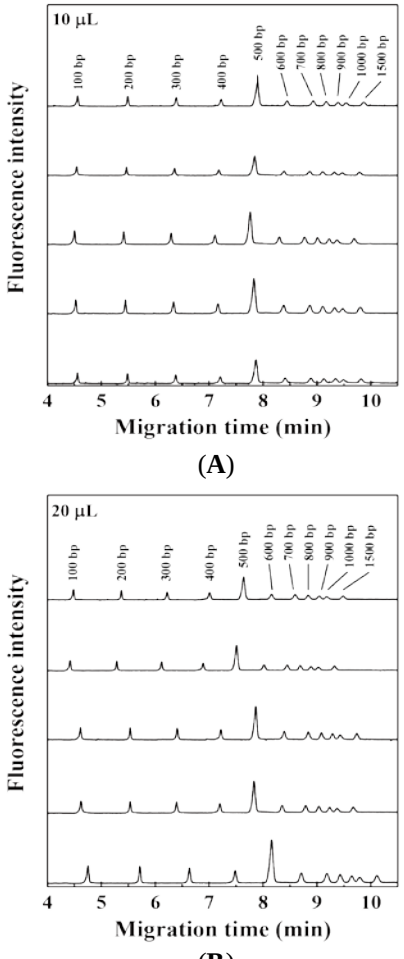
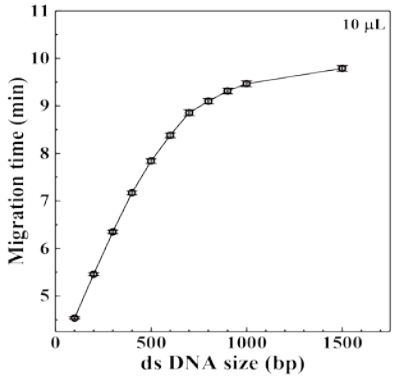


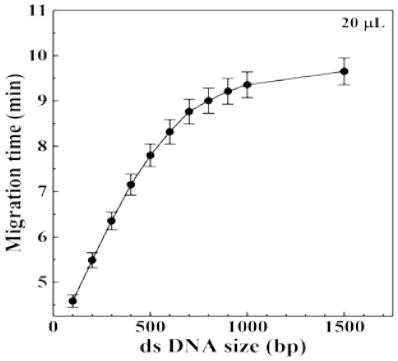
Fig. 3. Separation of the 100-bp DNA Ladder sample using different DNA sample volumes upon injection: (A) 10 μL and (B) 20 μL.

III. Results and Discussion

Fig. 3 shows the results of the separation of 100 bp DNA Ladder samples with two different volumes (10 μL and 20 μL) upon the injection. The electropherograms showed that the experimental condition was suitable for clearly separating the 100-bp DNA Ladder sample. However, the migration time differed depending on the sample volume upon injection. In particular, when 20 μL of the sample was used, the migration time varied among CGE measurements. As shown in Fig. 4, the error bars were relatively large at all points for 20 μL, whereas the error bars were small for 10 μL of the sample (Fig. 4A vs. Fig. 4B). This trend indicated that the sample volume upon injection substantially affected the injection of DNA samples when using the short-length capillary in this CGE process. The main cause of this phenomenon is that the DNA position in the capillary was not stable after insertion since the applied state of the voltage at the injection is affected by the sample volume. Similarly, the error bars for mobility were higher for 20 μL than for 10 μL (Fig. 5).

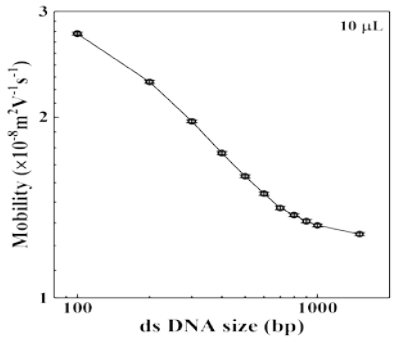


(**A**)

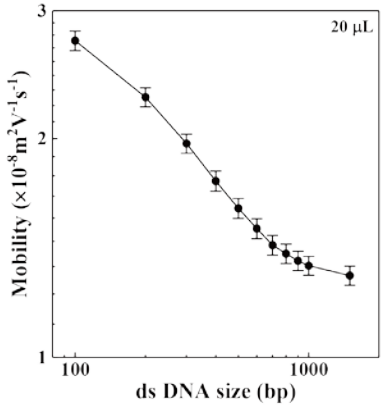


(**B**)

Fig. 4. Relationship between migration time and dsDNA size (bp) using different DNA sample volumes upon injection: (A) 10 μL and (B) 20 μL.



(**A**)



(**B**)

Fig. 5. Relationship between mobility and dsDNA size (bp) using different DNA sample volumes upon injection: (A) 10 μL and (B) 20 μL.

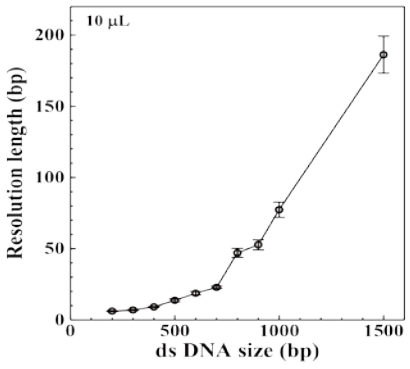
In addition, Fig. 6 shows the relationship between the resolution length (RSL) and DNA size. The resolution (Rs) was calculated according to the following equation:

$$Rs = 1.18 \times \Delta t/(w_{0.5}(1) + w_{0.5}(2)) \quad (1)$$

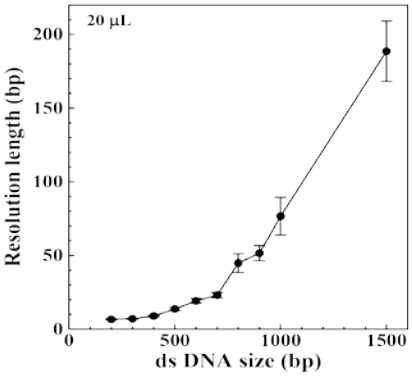
where Δt is the difference in the migration time between two consecutive peaks in the electropherograms, and $w_{0.5}$ is the full width at half maximum of the peak in the graph. The RSL was determined using the following equation:

$$\text{RSL} = \Delta n/Rs, \quad (2)$$

where Δn is the DNA length difference for adjacent peaks in the graph, and Rs is the resolution value.



(**A**)



(**B**)

Fig. 6. Relationship between resolution length (bp) and dsDNA size (bp) using different DNA sample volumes upon injection: (A) 10 μL and (B) 20 μL.

As shown in Fig. 6, there was much greater variation in the RSL when using 20 μL of sample than using 10 μL of the sample. This result indicated that the experimental condition of 20 μL did not allow for a stable DNA flow rate, whereas flow stability was maintained using 10 μL as the injection volume. We considered that this difference is likely caused by the position and spread of the DNA in the capillary after insertion. Based on these results, we concluded that developers of CGE systems need to pay close attention to setting the appropriate sample amount for injection in order to accurately measure small DNA fragments.

IV. Conclusion

In this study, we measured the effect of 100-bp DNA Ladder sample volume (10 μL and 20 μL) on the CGE process using a developed CGE system. To measure small DNA samples, a short-length capillary (total length of 15 cm and effective length of 7.5 cm) coated with a non-ionic acrylamide polymer chain was adopted to prevent inhibition from the EOF. HEC with a molecular size of 1,300,000 was adopted as the sieving polymer solution. Measurement of the CGE at different volumes (10 μL and 20 μL) upon the injection showed that the sample volume had a substantial effect on the electropherogram results. Variation in migration time, mobility, and RSL were much greater for the larger sample volume than for the smaller volume. These results indicated that the DNA sample volume upon injection has a large influence on the position and spread of DNA in the capillary. This observation indicated that the applied voltage state during injection differs depending on the sample volume. Thus, a developer of a compact CGE system should pay close attention to setting the sample volume upon the injection so as to ensure separating DNA samples with accuracy.

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