Influence of thickness of alkyl-silane coupling agent coating on separation of small DNA fragments in capillary gel electrophoresis

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Abstract. To simplify the process of coating capillaries with fused silica, we herein set out to develop a one-step procedure for coating capillaries to prevent electro-osmotic flow (EOF) during the separation of small DNA fragments. We selected a short capillary (total length = 15 cm; effective length = 7.5 cm) for use in a compact capillary gel electrophoresis (CGE) system. To develop a one-step coating procedure, we employed alkyltrimethoxysilane agents because they are cheap and can be easily acquired, in contrast to polyethylene glycol (PEG) silane coupling agents. We examined a 100-bp DNA Ladder sample using fused silica capillaries, which were coated with alkyltrimethoxysilane agents of five different molecular lengths (C4, C6, C8, C12, and C16). We found that a fused-silica capillary with C8 alkyltrimethoxysilane is optimal for separating small DNA samples.

1. Introduction

High-resolution capillary gel electrophoresis (CGE) has proven to be a reliable tool for separating small DNA samples in a short time [1–4]. CGE can clearly separate single- and double-stranded DNA fragments by utilizing a sieving polymer [5–12]. CGE has many advantages over slab gel electrophoresis (SGE): CGE can separate PCR products despite the use of smaller sample volumes, and in lesser time. In addition, by using CGE, we can detect the separation of small DNA fragments with high resolution and in real time more successfully than with SGE. However, the use of CGE to separate a DNA sample would lead to electro-osmotic flow (EOF) because the flow direction of the EOF in the capillary is opposite to that of the DNA samples [13–20]. Some studies have reported the use of capillaries coated with a non-ionic polymer chain to prevent EOF [21–23]. Unfortunately, the synthesis of polymer chains on the capillary walls is a complex and cumbersome procedure. Therefore, this process is not realistic for the mass production of capillaries coated with linear polymer chains.

In the present study, we set out to develop a simple method for coating the capillaries used in the separation of small DNA fragments. That is, we attempt to develop a one-step procedure to use capillary coating for preventing the EOF. At present, we are developing a compact analytical device for application to a CGE system for incorporation into a point-of-care-testing (POCT) system. To miniaturize the CGE equipment, the capillaries should be as short as possible. Therefore, in the present study, we adopted a short capillary (total length = 15 cm; effective length = 7.5 cm) that would be used with the developed compact analytical device. For our developed POCT apparatus, we planned to eliminate both the washing process and the need for injecting a sieving polymer into the

capillary when separating a DNA sample. To achieve this, we proposed the use of disposable short capillaries that can remove the need for both the washing process and sieving polymer solution filling work. To mass produce disposable capillaries, a simple and cost-effective means should be used for reducing the production cost and time. Therefore, we believe that the one-step coating of the capillary will make a significant contribution to realizing mass production, especially for short capillaries that can be used in a compact POCT device.

In the present study, to develop a one-step coating procedure for the fused silica capillary wall, we used alkyltrimethoxysilane agents with five different molecular lengths because they are cheap and easy to acquire, in contrast to polyethylene glycol (PEG) silane coupling agents. To optimize the lengths of the alkyltrimethoxysilane agents, we measured the CGE by using the fused silica capillaries with five different lengths of coating because the EOF strength is greatly affected by the length of the silane coupling agents. These CGE measurements allowed us to determine the optimal length of alkyltrimethoxysilane for separating small DNA samples with short fused-silica capillaries.

2.   Materials and methods

2.1. Butyltrimethoxysilane (BTS) coating for 15-cm long fused-silica capillary

After washing the capillary with 1 N NaOH (15 min), water (15 min), and then methanol (15 min), a total of 20 mL of methanol solution containing butyltrimethoxysilane (BTS) (80 µL) and one drop of acetic acid was passed through the capillary for 2 h at room temperature, so that it covalently bonded to the glass wall of the fused-silica capillary. Next, the BTS-coated capillary was washed with methanol (15 min) and water (15 min) at room temperature.
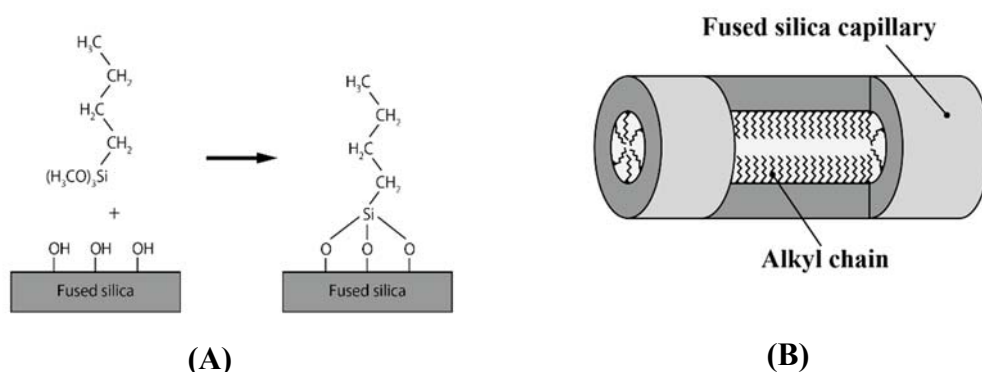

(A)   **(B)**

Figure 1. Schematic of (A) butyltrimethoxysilane coating procedure and (B) inner surface of fused-silica capillary, modified with alkyltrimethoxysilane.

2.2. Coating of 15-cm fused-silica capillary with four types of alkyltrimethoxysilane agents

First, the fused-silica capillary was washed with 1 N NaOH (15 min), water (15 min), and ethanol (15 min). Then, 20 mL of hexadecane solution containing 80 µL of alkyltrimethoxysilane coupling agent (hexyltrimethoxysilane (HTS), octyltrimethoxysilane (OTS), dodecyltrimethoxysilane (DDS), hexadecyltrimethoxysilane (HDS), or octadecyltrimethoxysilane (ODS)) was passed through the capillary for 2 h at room temperature, such that it covalently bonded to the glass wall of the fused-silica capillary. Next, the capillaries coated with the alkyltrimethoxysilanes were flushed with ethanol and water at room temperature.

Table 1. Alkyltrimethoxysilane coupling agents and their molecular chain lengths.

Silane coupling agent	Abbreviation	Length (nm)
Butyltrimethoxysilane	BTS (C4)	0.54
Hexyltrimethoxysilane	HTS (C6)	0.80
Octyltrimethoxysilane	OTS (C8)	1.05
Dodecyltrimethoxysilane	DDS (C12)	1.56
Hexadecyltrimethoxysilane	HDS (C16)	2.08

2.3. Equipment developed for capillary gel electrophoresis (CGE)
We used capillary gel electrophoresis (CGE) equipment developed in house for all the measurements. This equipment consisted of a microscope with epi-illumination (IX73, Olympus, Tokyo, Japan) and a high-voltage power supply (HJPQ-10P3, Matsusada, Kusatsu, Japan). To enable detection using this equipment, the DNA was conjugated using SYBR Green II. We used a mercury lamp with an optical filter (U-FBWA, Olympus, Tokyo, Japan), which allowed the passage of light of wavelength 460–495 nm. This was done because the DNA conjugated with SYBR Green II has an excitation wavelength in this region. The fluorescence of the DNA conjugated by SYBR Green II was collected by a 60× objective lens (UPlanFLN, Olympus, Tokyo, Japan). The corrected fluorescence signal could be detected by using a photomultiplier tube (PMT) (H8249-101, Hamamatsu Photonics, Hamamatsu, Japan). The detected signal was converted to a digital signal by utilizing a multifunction I/O device (NI USB-6341, National Instrument, Austin, TX, USA). For every measurement, we used the fused silica capillaries with a 75-μm circle (Polymicro Technologies, Phoenix, AZ, USA). To inject the 100-bp DNA Ladder sample, we applied 1.5 kV to the capillary for 1 s. The separation of the DNA samples was performed at 100 V/cm.

2.4. Chemicals
We used a 0.5× TBE buffer (44.5-mM Tris, 44.5-mM boric acid, 1.0-mM EDTA, pH 8.3) (Nippon Gene, Toyama, Japan) diluted with ultrapure water to act as the running buffer for all the measurements. Hydroxyethyl cellulose (HEC) (molecular size: 1300 k) was utilized as the sieving polymer for the CGE process. The HEC was adjusted to 0.5 w/v % and contained 0.5× TBE buffer and 2× SYBR Green II (Takara Bio Inc., Kusatsu, Japan). As the DNA samples, we selected a 100-bp DNA Ladder (Takara Bio Inc., Kusatsu, Japan) consisting of 11 double-stranded fragments with 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1500 bp as the measurement targets for all these measurements. The 130-μg/mL 100-bp DNA Ladder sample was used as the injection sample.

3. Results and discussion
To develop a simple and fast coating method, we used alkyltrimethoxysilane agents because they can coat a fused silica capillary wall in a single step after the completion of the washing process (see Figure 1). In addition, the alkyltrimethoxysilane agents can be acquired at a low cost compared to silane-coupling agents with a polyethylene glycol (PEO) chemical structure. To evaluate the effect of the molecular length of the alkyltrimethoxysilane agents on the separation of small DNA fragments, we measured five silane-coupling agents with different molecular lengths, namely, BTS (C4), HTS (C6), OTS (C8), DDS (C12), and HDS (C16) (see Table 1). Figure 2 shows the separation results for the 100-bp DNA Ladder sample as obtained by CGE. As shown in Figure 2(A), there was no peak in the separation graph. The calculated thickness of the electronic double layer was 1.44 nm, while that of BTS (C4) was about 0.54 nm, implying that the BTS (C4) coating cannot effectively prevent EOF. The flow direction of the EOF is opposite to that of the DNA samples. Therefore, the DNA samples cannot be injected into the capillary by disturbing the EOF. In contrast, for alkyltrimethoxysilane lengths of ≥C6, we measured the peak of the small DNA samples during CGE. The calculated molecular length of C6 is about 0.8 nm, implying that the C8 coating can prevent the EOF opposite flow more effectively than C6. As shown in Figure 2(C), we can obtain clear peaks for every DNA

sample length in the 100-bp DNA Ladder sample. The calculated molecular length of the OTS (C8) is about 1.05 nm. This trend indicates that the slightly different molecular length of the alkyltrimethoxysilane agent significantly affects the result of the separation for the small DNA samples in the CGE process. In contrast, when DDS (C12) and HDS (C16) are used (see Figure 2(D) and 2(E)), we cannot obtain a clear peak in the CGE graph again. We believe that this occurred because of the hydrophobic nature of the alkyltrimethoxysilane agents on the capillary wall. Because the strength of hydrophobicity increases with increase in the long chains of the alkyltrimethoxysilane agent, we believe that the flow in the capillary during CGE changed from plug flow (OTS (C8)) to laminar flow (DDS (C12), with HDS (C16)) (see Figure 3) owing to the change in the contact angle between the solution and the capillary wall. The laminar flow affected the measurement of small DNAs during CGE because DNA fragments of the same length scattered in the flow. That is, the density of DNA of the same length in the laminar flow was lower than that in the plug flow. When the density of the DNA of the same length increased, we obtained clear separation peaks in the electrophoregram.
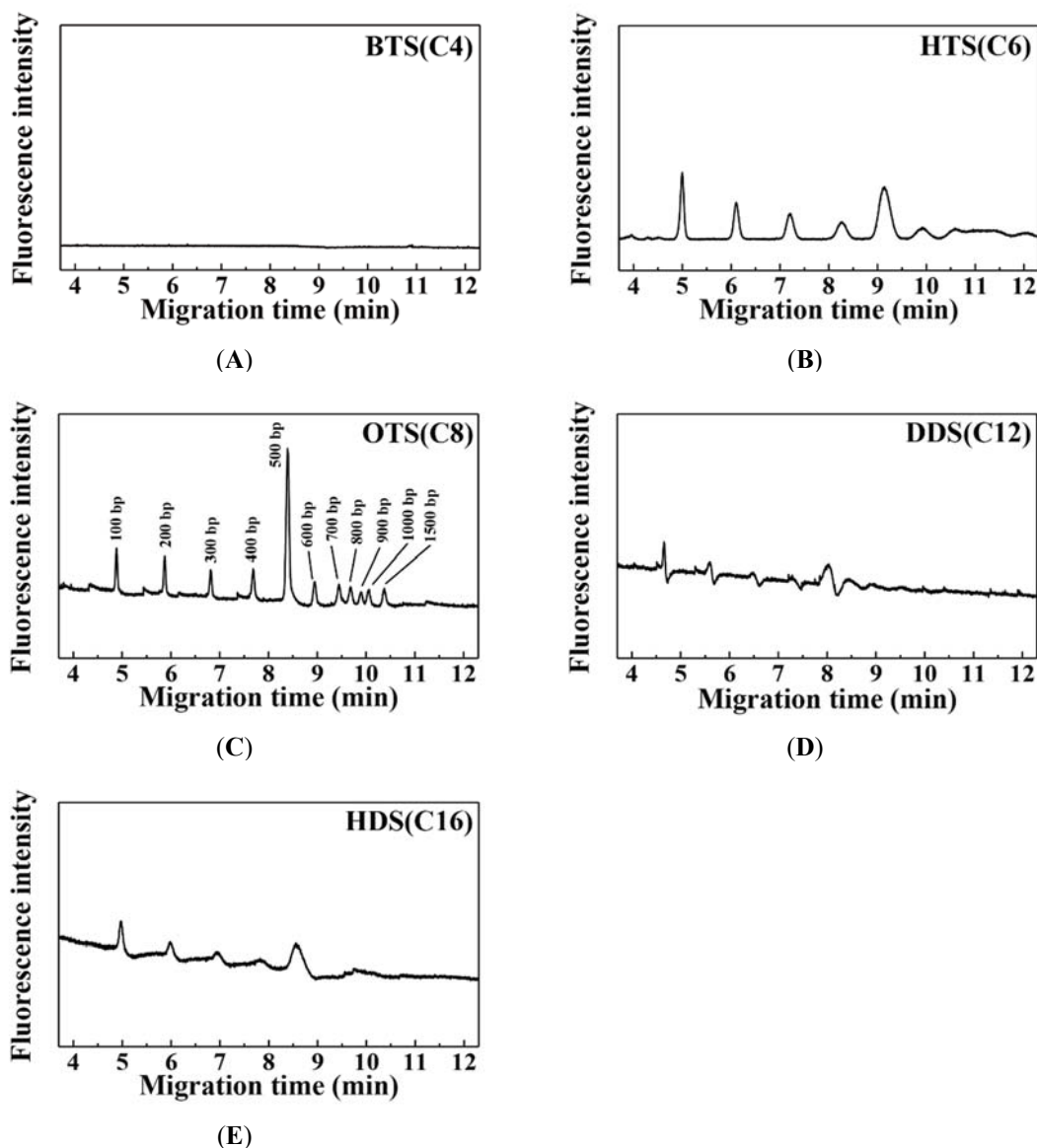

Figure 2 Separation of DNA samples on capillaries coated with (A) BTS (C4), (B) HTS (C6), (C) OTS (C8), (D) DDS (C12), and (E) HDS (C16).
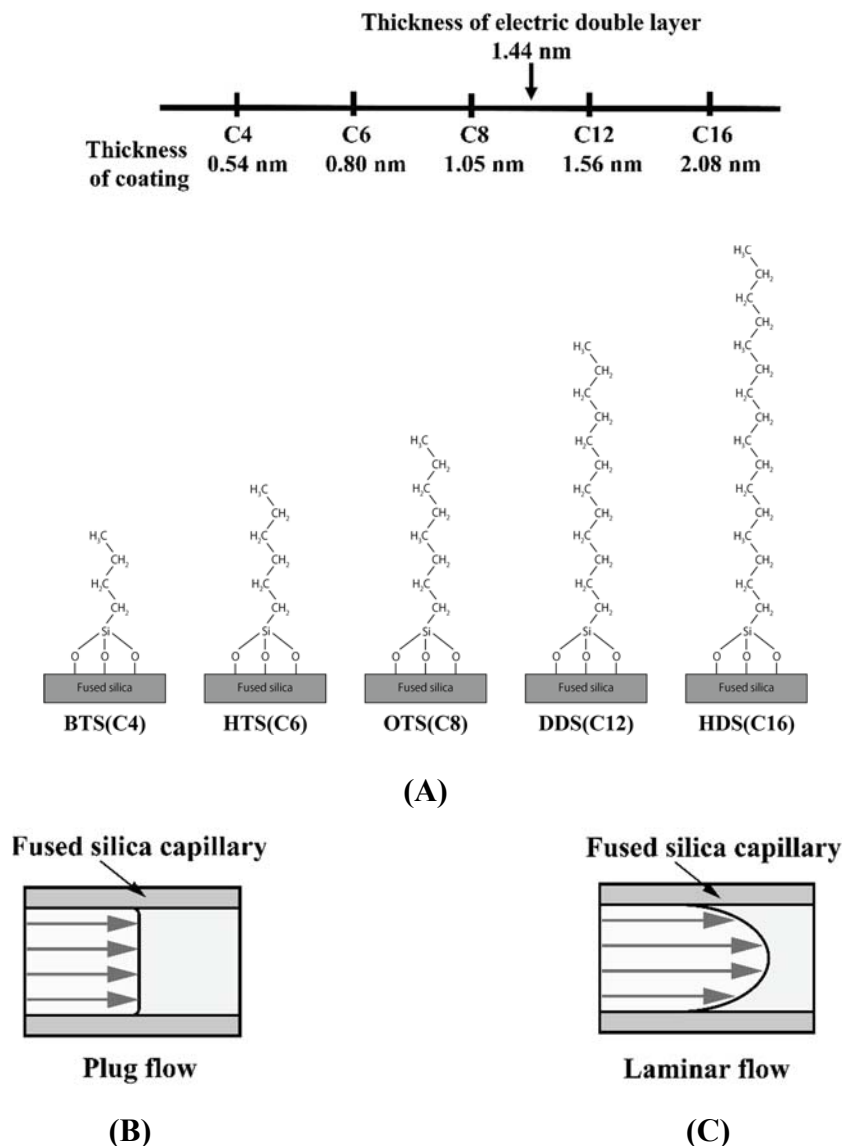
Figure 3. Schematic of capillaries coated with (A) OTS (C 8), (B) HDS (C12), and ODS (C16).

4. Conclusions

In the present study, to develop a simple method for coating a fused silica capillary, we selected alkyltrimethoxysilane agents with five different molecular lengths. CGE measurement revealed that the OTS (C8) can be detected in all the DNA samples in the 100-bp DNA Ladder sample. In the case of BTS (C4), there is no peak in the CGE graph because the DNA samples cannot be injected into the capillary due to EOF with a flow in the opposite direction to that of the DNA samples. As we used capillaries coated with DDS (C12) and HDS (C16), we could not separate the DNA fragments from the 100-bp DNA Ladder again. This trend indicates that the flow in the capillary changed from a plug flow (OTS (C8)) to a laminar flow (DDS (C12) and HDS (C16)) due to an increase in the hydrophobicity of alkyltrimethoxysilane agents on the capillary wall.

References
[1]   Mitchelson K R and Cheng J 2001 *Capillary Electrophoresis of Nucleic Acids: Introduction to the Capillary Electrophoresis of Nucleic Acids* (Springer Science & Business Media)
[2]   Mitchelson K R and Cheng J 2001 *Capillary Electrophoresis of Nucleic Acids Volume Ⅱ: Practical Application of Capillary Electrophoresis* (Springer Science & Business Media)
[3]   Paulus A, Gassmann E, Field M J 1990 *Electrophoresis* **11** 702
[4]   Yin H F, Lux J A, Shomburg G 1990 *J. High Resolut. Chromatogr.* **13** 624
[5]   Chu B J, Liang D H 2002 *J. Chromatogr. A* **966** 1–13
[6]   Manabe T, Chen N, Terabe S, Yohda M, Endo I 1994 *Anal. Chem.* **66** 4243
[7]   Cohen A S, Najarian D R, Paulus A, Guttman A, Smith J A, Karger B L 1988 *Proc. Natl. Acad. Sci. USA* **85** 9660
[8]   Wei W and Yeung E S 2000 *J. Chromatogr. B* **745** 221
[9]   Kim Y and Yeung E S 1997 *J. Chromatogr. A* **781** 315
[10]   Albarghouthi M N, Buchholz B A, Doherty E A, Bogdan F M, Zhou H, Barron A E 2001 *Electrophoresis* **22** 737
[11]   Song J M and Yeung E S 2001 *Electrophoresis* **22** 748
[12]   Barta C, Ronai Z, Sasvari-Szekely M, Guttman A 2001 *Electrophoresis* **22** 779
[13]   Horvath J, Dolník V 2001 *Electrophoresis* **22** 644–655
[14]   Lucy C A, MacDonald A M, Gulcev M D 2008 *J. Chromatogr. A* **1184** 81–105
[15]   Robb C S 2007 J. *Liq. Chromatogr. Relat. Technol.* **30** 729–759
[16]   Liu C-Y 2001 *Electrophoresis* **22** 612–628
[17]   Huhn C, Ramautar R, Wuhrer M, Somsen G W 2010 *Anal. Bioanal. Chem.* **396** 00297
[18]   Cifuentes A, Canalejas P, Diez-Masa J C 1999 *J. Chromatogr. A* **830** 423–438
[19]   Schmalzing D, Piggee C A, Foret F, Carrilho E, Karger B L 1993 *J. Chromatogr. A* **652** 149–159
[20]   Cifuentes A, de Frutos M, Santos J M, Diez-Masa J C 1993 *J. Chromatogr. A* **655** 63–72
[21]   Simò-Alfonso E, Gelfi C, Sebastiano R, Citterio A, Righetti P G 1996 *Electrophoresis* **17** 723
[22]   Chiari M, Dell'Ort N, Gelain A 1996 *Anal. Chem.* **68** 2731–2736