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DNA Concentration Effect of Various Hydroxide Compounds on Stacking in Capillary Electrophoresis

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The effects of various hydroxide compounds on base stacking (BS) were investigated for pre-concentration of DNA molecules in capillary electrophoresis (CE). In BS, hydroxide ions (OH^-) were electrokinetically introduced after DNA sample injection. A neutralization reaction occurred between the OH^- and $Tris^+$ of the running buffer, which resulted in a zone of lower conductivity. Within the low conductivity zone of the high electric field, the DNA molecules moved more rapidly and were concentrated in front of the low conductivity zone. At the same BS conditions of CE, the enhanced sensitivity of the DNA samples was dependent on the kind of multivalent cations in the hydroxide compounds. Except for LiOH, the hydroxide compounds with monovalent cations showed more effective BS than those with divalent cations because of solubility, ionic strength and electronegativity. The order of hydroxide compounds that enhance the detection sensitivity of DNA molecules was as follows: NaOH > NH_4OH > KOH > $Ba(OH)_2$ > $Sr(OH)_2$ > LiOH > $Ca(OH)_2$ > $Mg(OH)_2$. NH_4OH, KOH and $Ba(OH)_2$ proved to be efficient hydroxide compounds to use as effective BS reagents in CE instead of NaOH.

Key Words : Base stacking, Capillary electrophoresis, DNA concentration, Hydroxide compounds

Introduction

Capillary electrophoresis (CE) has become one of the most powerful tools for DNA analysis because of its high separation efficiency, fast analysis, small injection volume, on-capillary detection, simplicity and miniaturization.[1-9] Although CE has its merits, it also has serious flaws. For example, CE has low detection sensitivity due to the small sample volume.[8-11] Recently, various on-column pre-concentration techniques such as pH-mediated base stacking,[4,5] micellar electrokinetic chromatography (MEKC),[6] field amplification or enhancement,[9-11] moving reaction boundary (MRB),[12] micelle to solvent stacking (MSS)[13] and base stacking (BS)[14,15] have been developed and applied to eliminate these constraints. Some methods combine two concentration techniques, such as field-enhanced sample injection (FESI) and sweeping for the on-line concentration of analyte.[7] This combination was termed cationic selective exhaustive injection-sweeping (CSEI-sweep). As an example, Wang *et al.* developed a large-volume sample stacking using an electroosmotic flow (EOF) pump with anion-selective exhaustive injection (LVSEP-ASEI), which was sweeping plus large volume sample stacking.[8] These pre-concentration techniques improved the detection sensitivity of CE. However, they involve complicated processes.

The base stacking (BS) method in CE was developed to increase the sensitivity enhancement for DNA fragments analysis. On-column concentration of DNA molecules in CE was achieved simply by an electrokinetic injection of hydroxide ions (OH^-) before the DNA sample was injected, which was first reported in capillary-based DNA sequencing.[5] This method allowed direct injection of unpurified products of dye-primer sequencing reactions onto capillaries. The BS method can enhance the detection sensitivity without modification of the device or instrument. In addition, the total analysis time is even shorter because the chemical derivatization or pretreatment is not required.

However, previous BS studies were considered and performed with only sodium hydroxide (NaOH),[4,5,14,15] although there are many other hydroxide compounds. In this study, the pre-concentration effect of DNA molecules on the BS was investigated using various hydroxide compounds such as lithium hydroxide (LiOH), potassium hydroxide (KOH), ammonium hydroxide (NH_4OH), magnesium hydroxide ($Mg(OH)_2$), calcium hydroxide ($Ca(OH)_2$), strontium hydroxide ($Sr(OH)_2$) and barium hydroxide ($Ba(OH)_2$).

Experimental Section

Chemical and Reagents. The 1× TE (50 mM Tris-HCl, 2 mM EDTA disodium, pH 8.0) buffer was prepared by dissolving Tris-base, Tris-HCl, (Amresco, Solon, OH, USA) and EDTA disodium salt (Sigma, St. Louis, MO, USA) in water. Water was purified with the ultra-pure water system HUMAN POWER I^+ from Human Corporation (Seoul, KOREA). Deionized water (18 MΩ) was used for preparation of the sample and buffer solutions. The capillary sieving matrix was made with 2.0% (w/v) poly(vinylpyrrolidone) (PVP, relative molecular mass (M_r) = 1 000 000) (Polyscience, Warrington, England) diluted in 1× TE buffer containing 0.5 μg/mL ethidium bromide (EtBr, Molecular Probes, Eugene, Oregon, USA). The mixture was shaken for

1 min and left to stand for 2 h in order to release the bubbles. The 50-bp DNA ladder was purchased from Invitrogen (Carlsbad, USA). The DNA sample was diluted with 1× TE buffer prior to use.

Preparation of Hydroxide Compound Solution. Various multivalent hydroxides were dissolved in deionized water. Sodium hydroxide (NaOH) was purchased from SHOWA (Tokyo, Japan). Lithium hydroxide (LiOH), calcium hydroxide ($Ca(OH)_2$), magnesium hydroxide ($Mg(OH)_2$), strontium hydroxide ($Sr(OH)_2$) and barium hydroxide ($Ba(OH)_2$) were purchased from Duksan (Ansan, Korea). Potassium hydroxide (KOH), acetic acid (CH_3COOH) and ammonium hydroxide (NH_4OH) were purchased from Sigma (St, Louis, MO, USA). All of the reagents were prepared by dissolved in deionized water until the concentration becomes 0.1 M.

Capillary Electrophoresis. The experimental home-made CE system with a laser-induced fluorescence (LIF) detector was similar to that described previously (Figure 1).[16] Briefly, a diode-pumped solid-state laser ($\lambda_{ex} = 532$ nm; Power Technology Inc., Alexander, AR, USA) was used as the light source in the CE system, which was coupled to an LIF detector with a photomultiplier tube (Hamamatsu Photonics K.K., Japan). A Bertan ARB 30 high-voltage power supply (Bertan High Voltage Inc., New York, USA) was used to drive the electrophoresis. A bare fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 35 cm (effective length of 21 cm) and an I.D. of 50 μm was used for the separation. The running buffer was made of 1× TE buffer containing 0.5 μg/mL of EtBr. The sieving matrix was hydrodynamically injected at one end of the capillary through a syringe. After the sample was injected electrokinetically at 50 V/cm for 45 s, sample separation was performed in an electric field of 200 V/cm. After each run, the capillary was reconditioned before the next analysis by rinsing it successively in water, 0.1 M NaOH, water and running buffer. The data was recorded as a function of time and was saved on a Samsung notebook computer (1.6-GHz Intel Celeron M 370) at 2 Hz. Data output and analysis were performed using Lab view (version 6.1, National Instruments, Austin, TX, USA).

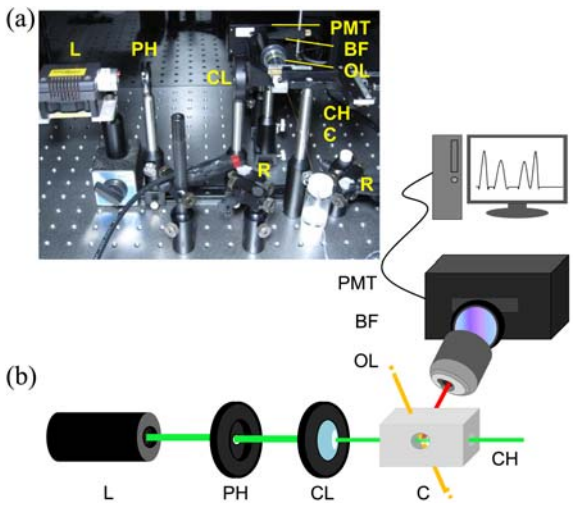

Figure 1. (a) Picture and (b) schematic diagram of the custom-built CE-LIF system. Indication: L, laser; PH, pinhole; CL, convex lens; C, capillary; CH, capillary holder; OL, objective lens; BF, band-pass filter; PMT, photo-multiplier tube; R, running buffer.

Sensitivity Enhancement of DNA Molecules at BS. The BS procedure used the conventional electrokinetic injection method and was executed in the following three steps (Figure 2): (1) the DNA sample was injected into the capillary (1× TE buffer) by applying 50 V/cm from the sample inlet reservoir, which changed with the OH^- inlet reservoir; (2) OH^- was injected into the capillary by applying 200 V/cm at 0.01 - 1.0 M NaOH for 0 - 210 s and BS started immediately; (3) separation was started by applying 200 V/cm at the buffer reservoir to ground the buffer reservoir.

Results and Discussion

The BS of DNA molecules in CE was caused by injecting hydroxide compounds. The formation of a low-conductivity zone was confirmed, and the current was measured during the DNA fragment separation.[14] A neutralization reaction between the OH^- ions of various hydroxide compounds and the $Tris^+$ ions of the running buffer (1× TE buffer) resulted in a zone of lower conductivity, within which the field focusing occurred. Since OH^- ions were immediately neutralized by the $Tris^+$ ions of the running buffer, the maximum current gap appeared at the initial separation step. After injection of

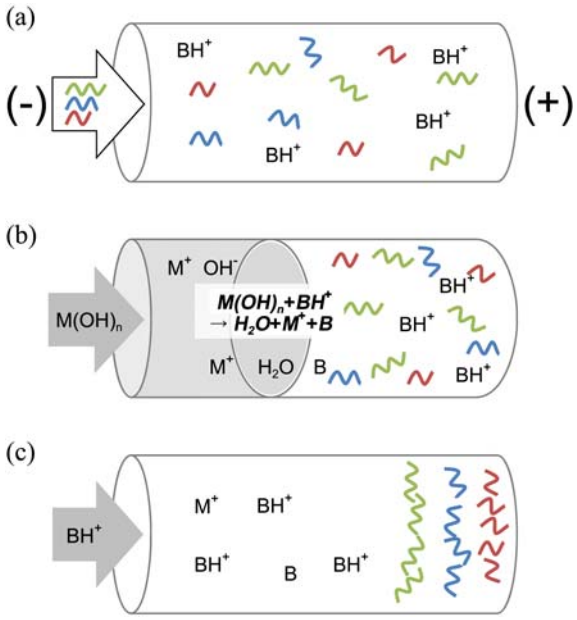

Figure 2. Schematic mechanism of base stacking during the CE separation of DNA molecules. (a) A diluted DNA sample was injected into the capillary. (b) Neutralization reaction and formation of low-conductivity zone started with injection of hydroxide ions. When the stacking was complete, the low-conductivity zone disappeared. (c) As a result of BS, DNA fragments moved and stacked rapidly. CE separation occurred continuously. The capillary total length and effective length were 35 and 21 cm (50 μm I.D.), respectively. BH^+, charged species of $Tris^+$ buffer ion; B, uncharged species of buffer ion; M^+, cation of hydroxide.

OH^- ions, the BS was completed. When the separation of DNA fragments was initiated, the low-conductivity zone dissipated and the running buffer was restored to a homogeneous state. Therefore, the current gradually returned to its original value in a smooth pattern, due to ion migration into the low-conductivity zone until the DNA fragment separation was complete.

As shown in Figure 2, the BS mechanism is a simple process to concentrate DNA molecules because it only requires the introduction of OH^- ions after injecting the DNA sample. First, the DNA sample was injected into the capillary (Figure 2(a)). Then the OH^- ions of various hydroxide compounds were introduced into the capillary in order to produce a neutralization reaction between the OH^- ions of the hydroxide compounds and the $Tris^+$ buffer ions (Figure 2(b)). The neutralization reaction created a low conductivity zone at the interface zone of DNA molecules and OH^- ions. The stacking procedure began simultaneously introducing OH^- ions within the zone of the high electric field, and the DNA fragments moved more rapidly than those in the untreated zone. The BS process was confirmed by monitoring the current according to a previous method.[14] The DNA molecules were stacked in front of the low conductivity zone. Separation of DNA fragments started with a high electric field, and the low conductivity zone dissipated. The CE separation proceeded in the PVP sieving matrix of 2.0% (w/v) PVP (M_r = 1 000 000) in the 1× TE buffer (Figure 2(c)).

A comparison of CE separation with BS using 0.1 M NaOH and without BS at the optimum CE separation condition is shown in Figure 3. After analyzing various concentrations (0.05 - 1.0 M) and injection times (0 - 210 s) of NaOH, the 90 s injection of 0.1 M NaOH was selected as an optimum BS condition for the DNA sample injection of 45 s on the 50 μm I.D. of capillary.

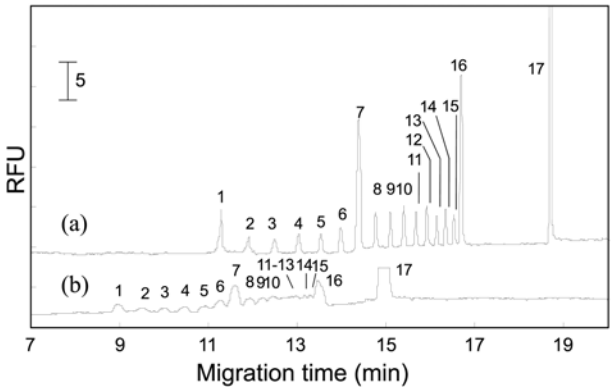

Figure 3. Comparison of CE separation (a) with BS using 0.1 M NaOH and (b) without BS. CE condition: sample, 50-bp DNA ladder; running buffer, 1× TE buffer (pH 8.0) with 0.5 ppm EtBr; sieving matrix, 2.0% PVP (M_r = 1 000 000); injection electric field, 50 V/cm for 45 s; OH^- injection electric field, 200 V/cm for 0 - 90 s; separation electric field, 200 V/cm; capillary total length, 35 cm (50 μm I.D.); capillary effective length, 21 cm. Indication: 1, 50; 2, 100; 3, 150; 4, 200; 5, 250; 6, 300; 7, 350; 8, 400; 9, 450; 10, 500; 11, 550; 12, 600; 13, 650; 14, 700; 15, 750; 16, 800; 17, 2652-bp DNA.

Under CE separation without BS (Figure 3(b)), most DNA fragments showed weak sensitivities and broadening peaks. However, at the CE separation with BS (CE-BS), all DNA fragments of the 50-bp DNA ladder were separated within 19 min without significant loss in baseline resolution and with high efficiency (Figure 3(a)). The average calculated

Table 1. Comparison of peak heights and relative peak height ratios of DNA fragments with various NaOH injection times

DNA fragment (bp)	Peak height (peak height ratio[a]) for NaOH injection time				
	0 s	30 s	60 s	90 s	120 s
50	0.94 (1.00)	3.55 (3.78)	5.10 (5.43)	5.35 (5.69)	5.14 (5.47)
100	0.46 (1.00)	0.37 (0.80)	1.34 (2.91)	1.90 (4.13)	1.82 (3.96)
150	0.50 (1.00)	0.37 (0.74)	1.41 (2.82)	1.78 (3.56)	1.56 (3.12)
200	0.71 (1.00)	0.60 (0.85)	1.75 (2.46)	2.39 (3.37)	2.18 (3.07)
250	0.47 (1.00)	0.61 (1.30)	1.77 (3.77)	2.41 (5.13)	2.07 (4.40)
300	0.72 (1.00)	1.15 (1.60)	2.70 (3.75)	3.13 (4.35)	2.93 (4.07)
350	2.53 (1.00)	12.03 (4.75)	15.83 (6.26)	16.25 (6.42)	15.76 (6.23)
400	0.81 (1.00)	1.40 (1.73)	3.90 (4.81)	4.37 (5.40)	4.29 (5.30)
450	0.58 (1.00)	1.41 (2.43)	3.92 (6.76)	4.36 (7.52)	4.25 (7.33)
500	0.47 (1.00)	1.65 (3.51)	4.50 (9.57)	5.00 (10.64)	4.86 (10.34)
550	0.51 (1.00)	1.38 (2.71)	3.92 (7.69)	4.26 (8.35)	4.09 (8.02)
600	0.51 (1.00)	1.64 (3.22)	4.40 (8.63)	4.89 (9.59)	4.57 (8.96)
650	0.51 (1.00)	1.14 (2.24)	3.27 (6.41)	3.69 (7.24)	3.40 (6.67)
700	0.35 (1.00)	1.48 (4.23)	4.01 (11.46)	4.52 (12.91)	4.20 (12.00)
750	0.36 (1.00)	1.14 (3.17)	4.00 (11.11)	3.61 (10.03)	3.26 (9.06)
800	2.26 (1.00)	17.65 (7.81)	20.10 (8.89)	20.81 (9.21)	19.65 (8.69)
2652	11.23 (1.00)	73.25 (6.52)	81.98 (7.30)	99.73 (8.88)	94.94 (8.45)

[a]Height ratio calculated as the peak height of BS divided by the peak height without BS (n = 3). CE separation condition: Sample, 50-bp DNA ladder; running buffer, 1× TE buffer (pH 8.0) with 0.5 ppm EtBr; sieving matrix, 2.0% PVP (M_r = 1 000 000); injection electric field, 50 V/cm for 45 s; separation electric field, 200 V/cm; capillary total length, 35 cm (50 μm I.D.); capillary effective length, 21 cm.

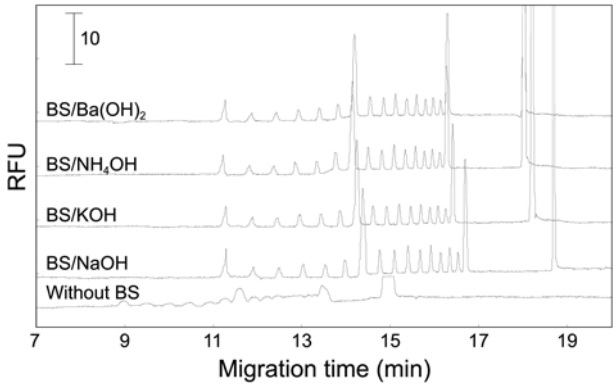

Figure 4. Comparison of representative CE separations with the BS using various hydroxide compounds. CE condition: injection electric field of hydroxide compounds, 200 V/cm for 90 s. The other CE conditions were identical to those in Figure 3.

signals (n = 3) based on the peak height measurements were enhanced 3.37 - 12.91-fold in the CE-BS separation with a 90 s injection time of NaOH compared to those of the normal CE (Table 1). In particular, the intensities of the 700-bp and 750-bp DNA fragments were enhanced about 10 - 13-fold by the BS method using 0.1 M NaOH. Compared to conventional CE separation without BS, the CE-BS method easily increased the detection sensitivity for the analysis of the 50-bp DNA ladder (50 - 2650-bp DNAs) by simply using NaOH.

The effects of various hydroxide compounds such as LiOH, KOH, NH_4OH, $Mg(OH)_2$, $Ca(OH)_2$, $Sr(OH)_2$ and $Ba(OH)_2$ on the detection sensitivity of DNA molecules caused by BS were compared to that of BS with 0.1 M NaOH (Figure 4). Each hydroxide compound with different cations showed different BS effects such as sensitivity enhancement of DNA molecules (i.e., increasing peak height) at the same conditions (Table 2). The sensitivity enhancement of hydroxide compounds in descending order is as following: NaOH > NH_4OH > KOH > $Ba(OH)_2$ > $Sr(OH)_2$ > LiOH > $Ca(OH)_2$ > $Mg(OH)_2$.

According to Reijenga's theory, the effective electrophoretic mobility of ions can be affected by ionic strength as follows:[17]

$$\mu_{eff} = \mu_0 e^{-0.5 z^{1.78} I^{0.5}}$$

where μ_0 represents the absolute mobility, z is the charge on the ion, and I is the ionic strength of the solution. As shown in the equation, the effective mobility of ions depends on the ionic strength, which is related to the conductivity of the solution. The ion is the current carrier in solution, which means the ionic strength increases with the conductivity of the solution. In the BS method, the concentration of DNA fragments was related to the changing conductivity. The ion strength of each hydroxide compound changed the conductivity of the solution. The presence of a high concentration of sodium ions had more efficient shielding of negative charges.[18] Therefore, the BS effect of NaOH was the largest compared to those of the other hydroxide compounds with a monovalent cation. Other monovalent hydroxide compounds such as NH_4OH, KOH and LiOH, also showed sensitivity enhancement of DNA molecules.

Monovalent hydroxide compounds had higher solubility in water compared to those with divalent cations (Table 3), which was sufficient to produce fully ionized OH^- ions of 0.1 M hydroxide compounds. Therefore, most monovalent hydroxide compounds showed 5.32 - 12.91-fold more effective BS of DNA molecules with increasing peak heights

Table 2. Comparison of the ratios of DNA peak heights of BS with NaOH to those of other hydroxide compounds

DNA fragment (bp)	Peak height (peak height ratio[a])						
	NaOH	KOH	NH_4OH	LiOH	$Ca(OH)_2$	$Sr(OH)_2$	$Ba(OH)_2$
50	5.35 (1.00)	3.89 (0.73)	3.61 (0.67)	3.53 (0.66)	3.96 (0.74)	3.85 (0.72)	4.08 (0.76)
100	1.90 (1.00)	1.79 (0.94)	1.75 (0.92)	1.56 (0.82)	1.14 (0.60)	1.49 (0.78)	1.59 (0.83)
150	1.78 (1.00)	1.69 (0.95)	1.66 (0.93)	1.39 (0.78)	1.23 (0.69)	1.54 (0.87)	1.53 (0.86)
200	2.39 (1.00)	2.30 (0.96)	2.38 (0.87)	1.88 (0.79)	1.54 (0.64)	2.15 (0.90)	2.04 (0.86)
250	2.41 (1.00)	2.38 (0.99)	2.24 (0.93)	1.92 (0.80)	1.49 (0.62)	2.61 (1.08)	2.25 (0.94)
300	3.13 (1.00)	2.93 (0.93)	3.22 (0.96)	2.29 (0.73)	1.91 (0.61)	2.88 (0.92)	2.69 (0.86)
350	16.25 (1.00)	15.85 (0.98)	16.47 (0.95)	13.45 (0.83)	7.16 (0.44)	13.80 (0.85)	15.42 (0.95)
400	4.37 (1.00)	3.54 (0.81)	4.18 (0.96)	2.77 (0.63)	2.37 (0.54)	3.19 (0.73)	3.60 (0.82)
450	4.36 (1.00)	3.43 (0.79)	3.54 (0.81)	2.53 (0.58)	2.23 (0.51)	2.55 (0.59)	3.29 (0.76)
500	5.00 (1.00)	3.76 (0.75)	4.65 (0.93)	2.85 (0.57)	2.49 (0.50)	3.53 (0.71)	3.97 (0.79)
550	4.26 (1.00)	3.39 (0.80)	3.49 (0.82)	2.35 (0.55)	2.15 (0.50)	2.42 (0.57)	3.17 (0.74)
600	4.89 (1.00)	3.53 (0.72)	4.12 (0.84)	2.73 (0.56)	2.31 (0.47)	3.27 (0.67)	4.02 (0.82)
650	3.69 (1.00)	2.92 (0.79)	3.22 (0.87)	2.17 (0.59)	1.90 (0.51)	2.61 (0.71)	3.08 (0.83)
700	4.52 (1.00)	3.24 (0.72)	3.56 (0.79)	2.50 (0.55)	2.06 (0.46)	2.82 (0.62)	3.31 (0.73)
750	3.61 (1.00)	2.68 (0.74)	2.81 (0.78)	2.01 (0.56)	1.73 (0.48)	2.38 (0.66)	2.77 (0.77)
800	20.81 (1.00)	18.28 (0.88)	19.04 (0.91)	14.61 (0.70)	8.14 (0.39)	16.25 (0.78)	18.93 (0.91)
2652	99.73 (1.00)	75.63 (0.76)	79.69 (0.80)	60.77 (0.61)	44.74 (0.45)	71.38 (0.72)	88.33 (0.89)

[a]Height ratio calculated as the peak height of BS with various hydroxide compounds divided by the peak height of BS with NaOH (n = 3). CE-BS condition: OH^- injection electric field, 200 V/cm for 90 s. The other CE conditions were the same as those in Table 1.

Table 3. The average peak height ratios of DNA fragments based on the solubility and cation mobility of various hydroxide compounds

Hydroxide	Solubility[a]	Cation mobility ($m^2s^{-1}V^{-1}$)	Peak height ratio[b]
NaOH	1.09×10^2	5.19×10^{-8}	1.00
LiOH	1.23×10	4.01×10^{-8}	0.67
KOH	1.12×10^2	7.62×10^{-8}	0.84
NH_4OH	7.02×10^2	7.61×10^{-8}	0.87
$Mg(OH)_2$	9.63×10^{-4}	5.46×10^{-8}	0.12
$Ca(OH)_2$	1.73×10^{-1}	6.12×10^{-8}	0.54
$Sr(OH)_2$	1.17	6.11×10^{-8}	0.76
$Ba(OH)_2$	3.89	6.59×10^{-8}	0.83

[a]Unit of solubility: g/100 g H_2O. [b]Peak height ratio indicates the ratio of the BS with other hydroxide compounds to BS with NaOH (n = 3). The CE conditions are identical to those in Table 2.

(Tables 2 and 3). Although LiOH is a monovalent hydroxide compound, it showed a weak BS effect because of its low solubility. In divalent hydroxide compounds such as $Ba(OH)_2$, $Sr(OH)_2$, $Ca(OH)_2$ and $Mg(OH)_2$, few hydroxide ions were produced because of the relatively low solubility compared to that of monovalent hydroxide compounds. This caused a weak BS effect of DNA fragments in CE (Tables 2 and 3). Since $Ba(OH)_2$ has a higher solubility than the other hydroxide compounds of divalent cations, it showed the most effective enhancement of DNA peak height in the divalent hydroxide compounds.

The electronegativity is also affected by the strength of ionic bonding in ionic compounds and the BS effect. Generally, the electronegativity decreases as the radius of the ion increases. The descending order of electronegativity is as follows: Mg^{2+} (1.31) > Ca^{2+} (1.00) > Li^+ (0.98) > Sr^{2+} (0.95) > Na^+ (0.93) > Ba^{2+} (0.89) > K^+ (0.82).[19] In LiOH, the enhancement of peak height was lower than that of $Ba(OH)_2$ due to the higher electronegativity of the Li^+ ion, which influenced the neutralization reaction between OH^- and $Tris^+$. In addition, the cation mobility of hydroxide compounds did not influence the BS effect in CE.

Conclusion

For analyzing trace DNA molecules, the CE method has some flaws such as low detection sensitivity. To increase the detection sensitivity, the simple BS technique was added to CE, which proved to be effective because the introduction of OH^- ions did not alter the device or instrument. At optimum conditions, the CE-BS method with a 90 s injection of 0.1 M NaOH enhanced the detection sensitivity of the 50-bp DNA ladder by 12.91-fold and separated the products in 19 min without a significant loss of baseline resolution. Others hydroxide compounds such as NH_4OH, KOH and $Ba(OH)_2$ also showed excellent BS effects. This suggests that hydroxide compounds can be used as effective BS reagents in CE instead of NaOH. The increasing efficiency and sensitivity of DNA fragments analysis by the CE-BS method were dependent on the ionic strength, solubility and electronegativity of hydroxide compounds. However, the cation mobility of hydroxide compounds did not influence the BS effect in CE.

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References

1. Lee, M.; Cho, K.; Yoon, D.; Yoo, D. J.; Kang, S. H. *Electrophoresis* **2010**, *31*, 1.
2. Go, H.; Lee, M.; Oh, D.; Kim, K.-S.; Cho, K.; Yoo, D. J.; Kang, S. H. *Bull. Korean Chem. Soc.* **2009**, *30*, 2141.
3. Jeon, S.; Lee, M.-J.; Park, J.; Kang, S. H. *J. Chromatogr. B* **2007**, *854*, 268.
4. Hoque, M. E.; Arnett, S. D.; Lunte, C. E. *J. Chromatogr. B* **2005**, *827*, 51.
5. Xiong, Y.; Park, S.-R.; Swerdlow, H. *Anal. Chem.* **1998**, *70*, 3605.
6. Lin, K.-C.; Hsieh, M.-M.; Chang, C.-W.; Lin, E.-P.; Wu, T.-H. *Talanta* **2010**, *82*, 1912.
7. Quirino, J. P.; Iwai, Y.; Otsuka, K.; Terabe, S. *Electrophoresis* **2000**, *21*, 2899.
8. Wang, Z.; Liu, C.; Kang, J. *J. Chromatogr. A* **2011**, *1218*, 1775.
9. Zhao, Y.; Lunte, C. E. *Anal. Chem.* **1999**, *71*, 3985.
10. Shihabi, Z. K. *J. Chromatogr. A* **2005**, *1066*, 205.
11. Zhang. J.; Wang, S.; Chen, X.; Hu, Z.; Ma, X. *Anal. Bioanal. Chem.* **2003**, *376*, 210.
12. Cao, C.; Zhang, W.; Fan, L.; Shao, J.; Li, S. *Talanta* **2011**, *84*, 651.
13. Guidote Jr, A. M.; Quirino, J. P. *J. Chromotogr. A* **2010**, *1217*, 6290.
14. Kim, D.-K.; Kang, S. H. *J. Chromatogr. A* **2005**, *1064*, 121.
15. Kang, S. H.; Jang, S.; Park, S.-K. *Bull. Korean Chem. Soc.* **2006**, *27*, 1346.
16. Lee, M.; Yoon, D.; Jeon, J.-T.; Eo, S. K.; Kang, S. H. *Bull. Korean Chem. Soc.* **2009**, *30*, 2655.
17. Duso, A. B; Chen, D. D. Y. *Anal. Chem.* **2002**, *74*, 2938.
18. Okafo, G.; Harland, G.; Camilleri, P. *Anal. Chem.* **1996**, *68*, 1982.
19. Mann, J. B.; Meek, T. L.; Allen, L. C. *J. Am. Chem. Soc.* **2000**, *122*, 2780.