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Investigation of the mechanism of pH-mediated stacking of anions for the analysis of physiological samples by capillary electrophoresis

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Abstract

Capillary electrophoresis has been widely used for the analysis of physiological samples such as plasma and microdialysate. However, sample destacking can occur during the analysis of these high-ionic strength samples, resulting in poor separation efficiency and reduced sensitivity. A technique termed pH-mediated stacking of anions (base stacking) has previously been developed to analyze microdialysate samples and achieve on-line preconcentration of analytes by following sample injection with an injection of sodium hydroxide. In this work, the mechanism of base stacking was investigated. Peak efficiency was shown to be a function of background electrolyte and sample ionic strength. Analytes representing several classes of compounds with a wide range of mobilities were used to study the effects of multiple parameters on sample stacking. The length of hydroxide injection required for stacking was shown to be dependent on analyte mobility and the type of amine background electrolyte used. Combinations of electrokinetic and hydrodynamic injections of sample and hydroxide were examined and it was concluded that although stacking could be achieved with several injection modes, electrokinetic injection of both sample and hydroxide was most effective for sample stacking. The mechanism of pH-mediated stacking for each of these modes is presented.

Keywords

Capillary electrophoresis; Microdialysate; On-column sample preconcentration; pH-mediated stacking; Physiological samples

1 Introduction

Capillary electrophoresis (CE) has become a powerful analytical technique for the analysis of physiological samples such as plasma and microdialysate [1–6]. Because of the small sample volume requirement, CE is an excellent separation technique to couple to microdialysis sampling to achieve highly time-resolved sample analysis. A serious issue arises in CE, however, when attempting to separate analytes in high-ionic strength matrices such as microdialysate. Sample destacking can occur in the analysis of high ionic strength samples, resulting in poor peak efficiency and reduced sensitivity. This can be especially detrimental when using UV detection since the small capillary dimensions already place a limitation on concentration sensitivity. There have been several strategies developed to improve the concentration sensitivity of CE on-line, including field-amplified sample stacking [7–11], large-volume sample stacking [12,13], isotachopheresis [14,15], stacking of neutral analytes [16,17], dynamic pH junction [18,19], the transient moving chemical reaction boundary

method (tMCRBM) [20,21], and chromatographic preconcentration [22]. Field-amplified stacking is the simplest of these techniques and has therefore been the most commonly used approach for on-column pre-concentration. For field-amplified stacking, the sample must be in a matrix of lower ionic strength than the CE background electrolyte (BGE). When the low-conductivity sample zone is injected, the voltage drop across the sample zone is much steeper than that across the BGE. As the electrophoretic velocity of ions is proportional to the field strength, ions in the sample zone migrate much faster than those in the BGE. This results in analyte ions accelerating across the sample zone and concentrating at the interface of the sample zone and the BGE. Once the analytes have stacked at the boundary of the sample zone, they separate electrophoretically in the BGE. A significant limitation of this approach is that the sample matrix must be of considerably lower ionic strength than the BGE. This condition is not met for the analysis of microdialysis and other biological samples, which are normally of high ionic strength. For microdialysis samples, extraction into a low-ionic strength matrix is not practical due to the small volume of the sample.

In order to achieve field-amplified stacking of analytes in high-ionic strength samples without the need for a dilution or extraction step, we have developed a technique termed pH-mediated stacking [23–28]. pH-mediated stacking allows the on-line transformation of a high-ionic strength sample matrix to low ionic strength with only one additional injection step following the sample injection. It can be performed for either cationic or anionic analytes by “acid stacking” [23] or “base stacking” [24], respectively. The dynamic pH junction [18,19] and tMCRBM [20,21] techniques mentioned previously also possess several components similar to pH-mediated stacking. For base stacking, the EOF is reversed and the separation is performed with reverse polarity. The BGE consists of the salt of a weak base, such as the ammonium ion. The mechanism of base stacking has been proposed previously [24] and is partially illustrated in Fig. 1. Briefly, the sample injection is performed electrokinetically (step 1 in Fig. 1), during which cations from the BGE displace sample cations (such as sodium in a Ringer’s solution). An electrokinetic injection of basic solution follows (step 2), during which the swiftly-migrating hydroxide ions will titrate the BGE cations and create a zone of low conductivity. Field amplification then occurs in this titrated zone, and anionic analytes will be stacked and subsequently separated in the BGE as described earlier. Sensitivity enhancements of 100-fold have been demonstrated using base stacking for samples in Ringer’s solution relative to hydrodynamic injection [24].

pH-mediated stacking has been successfully used to analyze microdialysate samples [28]. However, the hypothesized mechanism has not been fully interrogated. In this work, experimental parameters affecting the peak efficiency of several classes of anionic analytes will be investigated in order to test the working hypothesis of the base stacking mechanism. Information gained in this study will be used to optimize separation conditions in order to maximize CE concentration sensitivity using base stacking.

2 Materials and methods

2.1 Chemicals

p-Hydroxybenzoic acid (HBA), vanillic acid, *p*-coumaric acid, glutathione disulfide, naproxen, 8-hydroxy-2'-de-oxyguanosine (8-OHdG), ammonium chloride, imidazole hydrochloride, tris (hydroxymethyl)aminomethane hydrochloride (Tris), methylamine hydrochloride, and tetradecyltrimethylammonium bromide (TTAB) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were reagent-grade or better and used as received. All solutions were prepared in distilled, deionized water (Lab-conco, Kansas City, MO, USA) and were filtered through a 0.22 μ m pore size membrane filter prior to use. BGE pH was adjusted with sodium hydroxide solution.

2.2 Capillary electrophoresis systems

Fused-silica capillary (50 μ m ID) was obtained from Poly-micro Technologies (Phoenix, AZ, USA). A high-voltage power supply (Spellman High Voltage Electronics, Haupauge, NY, USA) was used to drive electrophoresis at -12 kV (negative polarity mode). The cathodic capillary reservoir (inlet) and electrical connections were isolated in a Plexiglas safety box fitted with an interlock. Detection was performed with a Spectra-Physics (San Jose, CA, USA) SpectraSYSTEM UV1000 UV-vis detector (with a flow cell modified for CE) at 254 nm unless otherwise noted. Data was collected with a PCI-MIO-16XE-50 A/D computer card and a data acquisition program using LabView 5.1 software (National Instruments, Austin, TX, USA). This home-built CE system was used to generate all data except for that shown in Figs. 2 and 7, in which cases a Beckman P/ACE MDQ system with a UV detector was used (Beckman Coulter, Fullerton, CA, USA). TTAB was added to the BGE to reverse the EOF. Under these conditions, the electromigration of anions and the EOF are toward the detector. Capillaries were flushed with 0.1 M NaOH, water, and BGE before each injection at 20 psi. Experiments performed with the home-built system were at ambient temperature, while experiments performed with the Beckman were maintained at 25°C.

2.3 Standard preparation and injection protocol

A 1 mM stock solution of each analyte was prepared in water and standard solutions were diluted from stock in either BGE or Ringer's solution (145 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂) as noted. Standards prepared in Ringer's solution were injected electrokinetically at -12 kV, immediately followed by a 0.1 M NaOH injection at the same voltage. The minimum duration of NaOH injection required for stacking of standards was determined experimentally, as well as the maximum sample injection time allowed before degradation of the separation. Unless otherwise noted, injection times were varied by increments of 5 s. The term "injection ratio" will be used to refer to the ratio of sample injection time to NaOH injection time.

3 Results and discussion

3.1 Effect of sample ionic strength on peak efficiency and injection ratio

Table 1 shows the minimum length of NaOH injection required to stack HBA in a sample matrix of varying percent Ringer's solution and the HBA peak efficiency at the minimum NaOH injection length necessary for complete stacking. Efficiency is observed to increase and S/N to decrease as a function of sample ionic strength, which correlates with previous work by Zhao [24]. At higher sample ionic strength, it has been proposed that BGE cations displace sample cations more efficiently, resulting in greater differences in field strength between the titrated zone and the BGE. Analytes in higher ionic strength samples, however, experience lesser field strength during sample injection than in lower ionic strength matrices and therefore less analyte is injected, resulting in decreased S/N (and decreased sensitivity since stacking does not cause an increase in noise). In the current research, however, it was noted that the minimum NaOH injection time required for stacking also increases as a function of sample ionic strength. This may be explained by the fact that higher ionic strength samples require a greater amount of sample cation displacement by BGE cations, and therefore require a greater amount of hydroxide ions for titration of the sample zone.

3.2 Effect of BGE ionic strength on peak efficiency and injection ratio

Table 2 shows the minimum length of NaOH injection required to stack HBA in varying BGE ionic strengths and the HBA peak efficiency at the minimum NaOH injection length. Injection ratio was found to remain relatively constant for each BGE ionic strength tested. This is consistent with the idea that BGE cations displace sample cations to maintain electroneutrality

during sample injection, and that since the sample ionic strength is constant, there should be the same amount of BGE cations in the sample zone regardless of BGE ionic strength when the hydroxide titration begins. Efficiency and S/N increase as a function of BGE ionic strength due to the greater difference between ionic strengths at the interface of the titrated zone and the BGE with increasing BGE ionic strength. This trend in efficiency for BGE ionic strength is also in correlation with what was observed previously [24].

3.3 Effect of injection type (hydrodynamic or electrokinetic) on pH-mediated stacking

pH-mediated stacking has previously only been reported using electrokinetic sample injection followed by hydrodynamic acid/base injection, and this mechanism was discussed previously. Based on the discussion and mechanism illustrated in Fig. 1, it would seem that electrokinetic sample injection followed by electrokinetic hydroxide injection would be the most efficient for pH-mediated sample stacking. However, the efficacy of hydrodynamic (HD) injection alone or hydrodynamic injection in combination with electrokinetic (EK) injection has not yet been fully evaluated with pH-mediated stacking. Different injection mode combinations were therefore compared for their efficacy in sample stacking, and if sample stacking occurred in an injection mode combination, to deduce the mechanism involved. When developing a method using pH-mediated stacking, the acid/base injection length is varied in order to determine the minimal injection length needed for analyte stacking. The peak shape for the analyte(s), in this case HBA, when partially stacked gives insight into the mechanism of stacking in each of the modes, and electropherograms at different injection ratios will be shown for each mode to illustrate how peak shape relates to the corresponding mechanism. Some degree of stacking was observed with each of the injection modes, which first of all indicates that the EK/EK injection mode is not the only type of injection that will result in pH-mediated stacking for anions.

Figures 2A and B show an EK and HD injection of HBA only, without subsequent injection of NaOH and therefore no stacking. Figures 2C and D show electropherograms of EK injections of 100 μ M HBA in Ringer's solution followed by EK injection of 0.1 M NaOH. HBA is completely stacked in D, but is only partially stacked in C before the NaOH injection length is optimized. Note that in C the partially stacked peak has a leading shoulder. This corresponds with the mechanism in Fig. 1, where titration and stacking begin at the back of the sample zone. Incomplete stacking would end somewhere in the middle of the sample zone, leaving the front of the zone unstacked.

Figure 3 illustrates a proposed stacking process for the EK/HD mode. The first step (1) is analogous to step 1 of the EK/EK mechanism (Fig. 1). In the second step (2), NaOH solution is injected hydrodynamically and the sample zone is forced farther into the capillary before the start of the separation. Partial stacking of the HBA peak can be seen in Figs. 4A and B for this injection mode. The leading shoulder of the partially stacked peak indicates that the front of the sample zone was not fully stacked (compare to Fig. 4C), as similar to the partially stacked HBA peak in the EK/EK mode. If an insufficient amount of hydroxide ion is injected, the titrated zone will not overtake the sample zone and stacking will occur somewhere in the middle of the sample zone. After the separation voltage is applied, the same effect as in Fig. 1 is eventually achieved for the EK/HD mode.

Figure 5 illustrates a proposed stacking process for the HD/EK mode. Sample cations are not displaced by BGE cations during sample injection (1), but rather begin to be displaced during NaOH injection (2). In this injection scheme, the partially stacked HBA peak (seen in Fig. 6A) has a tailing shoulder instead of a leading shoulder, which implies the back of the sample zone is not fully titrated. This is due to only a partial displacement of sample cations by BGE cations such that the back of the sample zone contains mainly untitratable sample cations (Na^+), and the front contains mainly titratable BGE cations. The swiftly-migrating hydroxide ions will

have already passed the sample zone by the time the slower-migrating BGE cations completely enter it, therefore, only the front of the sample zone will be titrated. The HBA peak height in Fig. 6C decreases compared to Fig. 6B as the hydroxide injection is increased, which is a result of over-titration of the sample zone, as we have routinely observed.

Figure 7 illustrates a proposed stacking process for the HD/HD mode. Displacement of sample cations does not occur until after both sample and NaOH injection. Therefore, this should be the least efficient mechanism for titration of the sample zone, as evidenced by the poor peak shape at several different injection ratios (Figs. 8A–C).

Although pH-mediated stacking may be successful by the HD/EK or EK/HD injection modes, EK/EK injection would still be favorable for two reasons. First, BGE cations displace sample cations during the sample injection, allowing a more efficient titration of the sample zone upon hydroxide injection since the sample zone already consists of titratable BGE cations. Second, electrokinetic injection of hydroxide leads to a larger portion of the capillary available for the separation. This is because an electrokinetic injection introduces a higher concentration of hydroxide ions due to their high mobility, whereas a larger hydrodynamically injected plug of hydroxide solution would be necessary for an equivalent number of hydroxide ions. This would push analytes farther into the capillary before the separation begins. The inevitable loss of resolution is not evident in this single-analyte experiment, but would prove disadvantageous when trying to perform a complex separation.

3.4 Effect of injection ratio on peak shape as a function of analyte mobility

We have previously reported an injection ratio of 0.46 for base-stacking of anions [24]. The phenolic acids used to demonstrate the method had very similar mobilities, which made the injection ratio appear constant for all of these compounds. However, analysis of several other analytes using base-stacking has revealed that the minimum amount of NaOH required to stack may be different for different analytes and appears to correlate with analyte mobility. To test this hypothesis, a mixture of six anions in Ringer's solution was injected for 30 s followed by hydroxide injections of varying lengths (Fig. 9). The analytes represent several classes of compounds with a wide range of mobilities, including phenolic acids (HBA, vanillic acid, *p*-coumaric acid), a peptide (glutathione disulfide), an anti-inflammatory drug (naproxen), and a nucleoside (8-OHdG). All analytes are anionic in normal CZE above pH 7 (data not shown). Base stacking was effective for each of the six anions tested. The necessary length of hydroxide injection for complete stacking of each of the six analytes was determined by peak shape and was observed to increase as a function of analyte mobility (Fig. 10). Partially stacked or unstacked analytes appear as peaks with leading shoulders and diminished height. Only a 1-s injection of NaOH was required to fully stack 8-OHdG (lowest mobility) while a 15-s injection was necessary to fully stack HBA (highest mobility). Analyte mobility is therefore an important factor to consider when developing a pH-mediated stacking method for new analytes.

3.5 Effect of BGE type on peak efficiency and injection ratio

Table 3 shows both the minimum length of NaOH injection required to stack HBA and 8-OHdG, and the peak efficiency at the minimum NaOH injection length in four different amine BGEs. Peak efficiencies with base stacking for samples prepared in Ringer's solution are also compared to efficiencies without base stacking for analytes prepared in BGE. Despite the fact that stacked analytes are in a higher ionic strength matrix, efficiencies for stacked analytes are significantly higher than for analytes in BGE without stacking. HBA consistently required a longer NaOH injection compared to 8-OHdG, due to the higher mobility of HBA compared to 8-OHdG, as addressed previously. The minimum length of NaOH injection needed to stack each analyte varied greatly between BGE types. The injection ratio required for stacking in methylamine and ammonium BGEs was much higher than in Tris and imidazole BGEs. In

general, peak efficiencies were similar for both stacked analytes in all four BGEs (100–200 thousand plates), with the exception of 8-OHdG in imidazole at pH 7, which had a peak efficiency of over 4 million plates. Conditions responsible for the considerable efficiency enhancement for this particular analyte are currently under investigation.

4 Concluding remarks

pH-mediated stacking of anions, or base stacking, is a simple and effective method for on-line concentration of analytes in high-ionic strength matrices. Higher ionic strength sample matrices and the use of higher ionic strength BGEs result in higher peak efficiencies with base stacking. Base stacking can be achieved with several injection modes, although electrokinetic/electrokinetic injection is most efficient because it uses the least amount of capillary length to achieve stacking before separation, leading to better resolution and peak shape. The injection ratio required for stacking is dependent on analyte mobility and BGE type, and therefore must be optimized for specific analytes and BGEs. The observation of reproducible peak efficiency of over 4 million plates for one analyte warrants further study, as this level of peak efficiency greatly enhances concentration sensitivity.

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Abbreviations

EK	electrokinetic
HBA	<i>p</i> -hydroxybenzoic acid
HD	hydrodynamic
8-OHdG	8-hydroxy-2'-deoxyguanosine
TTAB	tetradecyltrimethylammonium bromide

References

1. Hernandez M, Aquilar C, Borrull F, Calull M. J Chromatogr B 2002;772:163–172.
2. Soetebeer UB, Schierenberg MO, Schultz H, Hempel G, Andresen P, Blaschke G. Anal Chem 2001;73:2178–2182. [PubMed: 11393838]
3. Arias R, Jiménez RM, Alonso RM, Télez M, Arrieta I, Flores P, Ortiz-Lastra E. J Chromatogr A 2001;916:297–304. [PubMed: 11382304]
4. Desiderio C, Rudaz S, Raggi MA, Fanali S. Electrophoresis 1999;20:3432–3438. [PubMed: 10608711]
5. Chen SH, Chen YH. Electrophoresis 1999;20:3259–3268. [PubMed: 10596828]
6. Denoroy L, Bert L, Parrot S, Robert F, Renaud B. Electrophoresis 1998;19:2841–2847. [PubMed: 9870378]
7. Mikkers FEP, Everaerts FM, Verheggen TPEM. J Chromatogr 1979;169:11–20.
8. Burgi DS, Chien RL. Anal Chem 1991;63:2042–2047.
9. Tan WG, Tyrrell DLJ, Dovichi NJ. J Chromatogr A 1999;853:309–319. [PubMed: 10486738]
10. Shihabi ZK. J Capil Electrophor 1995;2:267–271.

11. Shihabi ZK. J Chromatogr A 1999;853:3–9. [PubMed: 10486706]
12. Chien RL, Burgi DS. Anal Chem 1992;64:1046–1050.
13. McGrath G, Smyth WF. J Chromatogr B 1996;681:125–131.
14. Mazereeuw M, Tjaden UR, Reinhoud N. J Chromatogr Sci 1995;33:686–697.
15. Foret F, Szökö E, Karger BL. J Chromatogr 1992;608:3–12.
16. Quirino JP, Terabe S. Science 1998;282:465–468. [PubMed: 9774271]
17. Palmer J, Burgi DS, Munro NJ, Landers JP. Anal Chem 2001;73:725–731. [PubMed: 11248884]
18. Britz-McKibbin P, Wong J, Chen DDY. J Chromatogr A 1999;853:535–540. [PubMed: 10486763]
19. Britz-McKibbin P, Bebault GM, Chen DDY. Anal Chem 2000;72:1729–1735. [PubMed: 10784134]
20. Cao CX, He YZ, Li M, Qian YT, Gao MF, Ge LH, Zhou SL, Yang L, Qu QS. Anal Chem 2002;74:4167–4174. [PubMed: 12199589]
21. Cao CX, He YZ, Li M, Qian YT, Yang L, Qu QS, Zhou SL, Chen WK. J Chromatogr A 2002;952:39–46. [PubMed: 12064544]
22. Swartz ME, Merion M. J Chromatogr 1993;632:209–213.
23. Weiss DJ, Saunders K, Lunte CE. Electrophoresis 2001;22:59–65. [PubMed: 11197180]
24. Zhao Y, Lunte CE. Anal Chem 1999;71:3985–3991. [PubMed: 10500485]
25. Park S, Lunte CE. J Microcol Sep 1998;10:511–517.
26. Zhao Y, McLaughlin K, Lunte CE. Anal Chem 1998;70:4578–4585. [PubMed: 9823717]
27. Xiong Y, Park SR, Swerdlow H. Anal Chem 1998;70:3605–3611. [PubMed: 9737211]
28. Hadwiger ME, Torchia SR, Park S, Biggin ME, Lunte CE. J Chromatogr B 1996;681:241–249.

Figure 1.

EK/EK injection stacking mechanism. (1) Electrokinetic (EK) sample injection begins and anionic analytes ($\ominus$) travel towards the anode; BGE cations (NH_4^+) move toward cathode and replace sample cations (Na^+). (2) NaOH is injected electrokinetically and BGE cations in the sample zone are titrated by hydroxide ions to create a zone of low conductivity (shaded gray).

Figure 2.

EK/EK electropherograms at varying injection ratios and nonstacked controls. (A) Control, 15 s EK injection of sample only; (B) control, 5 s HD injection of sample only; (C) 15 s EK sample/ 5 s EK NaOH; (D) 15 s EK/10 s EK. Conditions: 100 μ M HBA in Ringer's followed by 0.1 M NaOH, 50 mM Tris at pH 8.0 with 0.5 mM TTAB, 50 μ m ID capillary $\times$ 40.2 (30) cm, separation and EK injection at -10 kV, HD injection at 1 psi.

Figure 3.

EK/HD injection stacking mechanism. (1) Electrokinetic sample injection begins and anionic analytes travel towards the anode; BGE cations move toward cathode and replace sample cations. (2) NaOH is injected hydrodynamically and sample zone is pushed farther into capillary. (3) High voltage is applied and BGE cations are titrated to create a zone of low conductivity as hydroxide ions migrate past the sample zone.

Figure 4. EK/HD electropherograms at varying injection ratios. (A) 15 s EK sample/5 s HD NaOH, (B) 15 s EK/10 s HD, (C) 15 s EK/20 s HD. Conditions as in Fig. 2.

Figure 5.

HD/EK injection stacking mechanism. (1) Sample is injected hydrodynamically. (2) NaOH is injected electrokinetically and sample BGE cations displace sample cations; BGE cations begin to be titrated by hydroxide ions in the middle of the sample zone where Na^+ has been replaced with NH_4^+ . Note that tailing will occur because the hydroxide ions will pass the sample zone by the time Na^+ at the back of the zone has been replaced by NH_4^+ .

Figure 6. HD/EK electropherograms at varying injection ratios. (A) 5 s HD sample/15 s EK NaOH, (B) 5 s HD/30 s EK, (C) 5 s HD, 45 s EK. Conditions as in Fig. 2.

Figure 7.

HD/HD injection stacking mechanism. (1) Sample is injected hydrodynamically. (2) NaOH is injected hydrodynamically and the sample zone is pushed farther into the capillary. (3) High voltage is applied and BGE cations replace sample cations; sample zone is titrated to low conductivity. Tailing also occurs in this mode as already described.

Figure 8. HD/EK electropherograms at varying injection ratios. (A) 5 s HD sample/5 s HD NaOH, (B) 5 s HD/10 s HD, (C) 5 s HD/15 s HD. Conditions as in Fig. 2.

Figure 9.

Effect of anion mobility on required injection ratio for base-stacking. (A) 30 s EK of sample/15 s EK injection of NaOH, (B) 30 s/10 s, (C) 30 s/5 s, (D) 30 s/3 s, (E) 30 s/1 s, (F) 30 s/0 s. Peak identities are: (1) HBA, (2) *p*-coumaric acid, (3) vanillic acid, (4) glutathione disulfide, (5) naproxen, (6) 8-OHdG. Conditions: 50 μM (for each anion) sample mixture in Ringer's followed by 0.1 M NaOH, 100 mM Tris at pH 8.0 with 0.5 mM TTAB, 50 μm ID capillary × 60 (45) cm, separation and injection at -12 kV.

Figure 10.

Effect of analyte mobility on injection ratio. Legend: (■) HBA, (▼) *p*-coumaric acid, (▲) vanillic acid, (●) glutathione disulfide, (*) naproxen, (◆) 8-OHdG, Tris BGE pH 8.0 (.....), NH₄OH BGE pH 9.0 (---), methylamine BGE pH 10.0 (—). All other conditions same as in Fig. 3. **p*-Coumaric acid and vanillic acid comigrate at this pH.

Table 1
Effect of sample ionic strength on injection ratio, S/N, and peak efficiency for HBA with base-stacking

Ringer's in sample	Min. NaOH (s)	S/N	$W_{1/2}$ (s)	Migration time (min)	Efficiency (N/1000)
25%	20	51	4.57 ± 0.14	11.16 ± 0.89	118 ± 11
50%	30	41	4.48 ± 0.13	11.57 ± 0.08	133 ± 7
90%	40	38	4.15 ± 0.03	11.37 ± 0.39	150 ± 8

Conditions: 30 s injection of 50 μ M HBA, 100 mM NH₄OH BGE with 0.5 mM TTAB at pH 9, 50 μ m ID capillary $\times$ 80 (65) cm, separation and injection at -12 kV, $n = 3$

Table 2
Effect of BGE ionic strength on injection ratio, S/N, and peak efficiency for HBA with base-stacking

BGE ionic strength	Min. NaOH (s)	S/N	$W_{1/2}$ (min)	Migration time (min)	Efficiency (N/1000)
25 mM	35	29	4.87 $\pm$ 0.47	11.51 $\pm$ 0.89	112 $\pm$ 6
50 mM	35	38	4.32 $\pm$ 0.02	10.75 $\pm$ 0.05	123 $\pm$ 2
100 mM	40	40	4.45 $\pm$ 0.11	13.36 $\pm$ 0.02	180 $\pm$ 8

Conditions: 30 s of 50 μ M HBA in 90% Ringer's, NH₄OH BGE with 0.5 mM TTAB at pH 9, 50 μ m ID capillary $\times$ 80 (65) cm, separation and injection at -12 kV, $n = 3$

Table 3
Effect of BGE pH/type on base-stacking injection ratio and peak efficiency for HBA and 8-OHdG compared to normal electrokinetic injection

BGE/Analyte	Sample in Ringer's solution, with base-stacking				Sample in BGE, without base-stacking		
	Min. NaOH (s)	Peak height (mAU)	$W_{1/2}$ (s)	Efficiency (N/1000)	Peak height (mAU)	$W_{1/2}$ (s)	Efficiency (N/1000)
Methylamine, pH 10.5 HBA 8-OHdG	40	2.73 ± 0.01	3.24 ± 0.00	201 ± 3	0.435 ± 0.005	10.92 ± 0.18	24 ± 1
	15	2.28 ± 0.11	4.44 ± 0.00	231 ± 5	0.477 ± 0.006	11.94 ± 0.66	45 ± 5
N ₄ OH, pH 9.0 HBA 8-OHdG	40	2.22 ± 0.05	4.56 ± 0.12	180 ± 8	0.602 ± 0.011	11.04 ± 0.12	31 ± 1
	15	1.69 ± 0.05	6.52 ± 0.16	173 ± 6	0.530 ± 0.014	13.02 ± 0.48	70 ± 5
Tris, pH 8.0 HBA 8-OHdG	15	2.93 ± 0.02	4.92 ± 0.06	194 ± 10	0.625 ± 0.009	11.16 ± 0.30	34 ± 1
	5	2.05 ± 0.02	7.68 ± 0.29	166 ± 16	0.435 ± 0.025	14.46 ± 2.04	23 ± 7
Imidazole, pH 7.0 HBA 8-OHdG	20	3.13 ± 0.10	4.14 ± 0.06	155 ± 4	0.487 ± 0.010	10.92 ± 0.12	28 ± 1
	8	9.39 ± 0.27	1.44 ± 0.06	4498 ± 15	0.542 ± 0.011	10.74 ± 0.42	66 ± 6

Conditions: 30 s injection of 50 μM HBA and 8-OHdG standards in 90% Ringer's for base-stacking or 10 s injection in BGE for normal electrokinetic injection; 100 mM BGE (pH ≈ pK_a) with 0.5 mM TTAB; 50 μm ID capillary × 80 (65) cm; separation and injections as −12 kV, *n* = 3