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pH-Mediated Field Amplification On-Column Preconcentration of Anions in Physiological Samples for Capillary Electrophoresis

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Abstract

Two limitations of capillary electrophoresis (CE) are the low sample loadability of the capillary and an incompatibility with high ionic strength samples. Several strategies have been described to preconcentrate and lower the ionic strength of physiological samples prior to CE analysis. These have included both off-capillary and on-capillary approaches. We have previously described a version of on-column field-amplification stacking termed pH-mediated stacking. pH-mediated stacking was initially developed for the separation of cations. In this report, we describe the application of pH-mediated sample stacking to anions. In this method, an electrokinetic injection is used to introduce analyte anions into the CE system and simultaneously replace the sample matrix cations with ammonia from the background electrolyte. Base is then electrokinetically injected to neutralize the sample zone and create a low conductivity region across which the analyte anions will stack. Using this method, a sensitivity enhancement of more than 66-fold was achieved without loss in separation efficiency relative to normal electrokinetic injection. Detection limits of 0.3 μM for four phenolic acids in a physiological sample were achieved using simple UV absorbance detection. The limit to the amount of sample that could be loaded using this technique was the length of the separation capillary. To further increase the amount of sample that could be loaded, a double-capillary system was developed. Using the double-capillary system the sensitivity was increased more than 300-fold and detection limits of 0.06 μM were achieved.

Capillary electrophoresis (CE) has become a powerful analytical technique.[1,2] A major limitation of CE analysis is the small sample volume that can be loaded into the separation capillary without compromising the separation. CE is also generally incompatible with high ionic strength samples because of band broadening induced by the weaker electric field across the sample zone relative to the separation electric field. In most cases, physiological samples are subjected to pretreatment, usually involving solid-phase or liquid–liquid extraction, prior to CE analysis. The need for such pretreatment steps increases the difficulty of the analysis and decreases the sample throughput.

Several approaches have been described to preconcentrate samples in the CE capillary prior to separation. The most common approach is termed field amplification[3-9] and involves injecting the analytes in a low ionic strength matrix. In this case, the electric field across the sample is stronger than that across the separation capillary. Analyte ions of the proper charge will rapidly migrate across the sample zone and stack at the interface of the sample zone and background electrolyte (BGE). Up to a 100-fold sample preconcentration has been reported using field-amplification stacking.[7] This approach has been modified using polarity reversal to remove the low conductivity sample zone after stacking but prior to CE separation.[10-12] The limitation of this method is the need to have the sample matrix be of lower ionic strength

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than the BGE. This condition is generally not met for physiological samples without pretreatment prior to analysis because a BGE of more than 100 mM salt is not typically used. The use of very high concentration BGEs (>1 M) to achieve field-amplification stacking of high ionic strength samples has recently been reported.[13]

We have recently demonstrated the field-amplification on-column stacking of cations from physiological samples without the need for pretreatment steps.[14,15] In this method, termed pH-mediated field-amplification stacking, the BGE is made from the salt of a weak acid, such as acetate. The high ionic strength sample is electrokinetically injected into the separation capillary resulting in the displacement of sample anions, such as chloride, with acetate ions. The sample injection is immediately followed by electrokinetic injection of strong acid resulting in neutralization of the acetate in the sample zone and creation of a low conductivity region across which analyte cations stack. This method has recently been modified for the on-column preconcentration of anionic DNA fragments.[16]

This report describes the application of pH-mediated field-amplification to anionic analytes. This requires reversal of the electroosmotic flow (EOF) and the separation voltage polarity in order that anions electromigrate in the same direction as the EOF and toward the detector end of the separation capillary. The BGE is made from the salt of a weak base, such as ammonium, and neutralization is achieved by electrokinetic injection of hydroxide ion. A limitation of pH-mediated field amplification stacking is that a significant portion of the separation capillary is used for the stacking process, leaving little capillary for the separation. This limits the amount of sample that can be injected while maintaining a sufficient separation. This limitation is overcome by using a double-capillary system in which one capillary is used for stacking and the other capillary is used for separation.

EXPERIMENTAL SECTION

Materials

Tetradecyltrimethylammonium bromide (TTAB), *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, syringic acid, imidazole hydrochloride, methylamine hydrochloride, and Tris hydrochloride (Tris(hydroxymethyl)aminomethane) were purchased from Sigma Chemical (St. Louis, MO). All chemicals were analytical grade or better and used as received. Nano-pure water (Sybron-Barnstead, Boston, MA) was used for preparation of all solutions.

Apparatus

An ISCO model 3850 capillary electrophoresis system (Lincoln, NE) was used for these studies. A 70-cm length (50 cm to the detector) of 75-μm i.d. (360-μm o.d.) fused-silica capillary (Polymicro Technology Inc., Phoenix, AZ) was used for the separation. The on-column UV detector was operated at 275 nm. A Datajet integrator (model SP4600, Spectra-Physics, San Jose, CA) connected to a PC workstation was used for data acquisition.

For double-capillary stacking a zero dead volume “T” junction (MicroQuartz Sciences, Phoenix, AZ) was used to connect three lengths of 75-μm-i.d. fused-silica capillary. Capillary A of the “T” was 50 cm, capillary B was 70 cm, and capillary C was 20 cm in length. The polyimide coating was removed from the fused silica to create a detection window 20 cm from the end of arm B.

Solutions

Ringer’s solution consisted of 155 mM NaCl, 5.5 mM KCl, and 2.3 mM $CaCl_2$ at pH 7.4. Stock solutions of the phenolic acids were prepared at a concentration of 10 mM in Ringer’s solution. Standard mixtures of the phenolic acids were then prepared by dilution of the stock solutions

in Ringer's solutions. Ammonium hydroxide buffers were prepared by dissolving the amount of ammonium chloride to the give the desired concentration and adjusting the pH with 1 M NaOH solution. Alkaline-EDTA solution was prepared by titrating a 0.5 mM disodium EDTA solution with NaOH to pH 13. Other buffers were prepared by making solutions of the appropriate hydrochloride salt at the desired concentration and titrating to the desired pH with 1 M NaOH. All solutions were filtered through an Acrodisc 0.22-μm syringe filter (Fisher Scientific, Pittsburgh, PA) prior to use.

CE Procedures

The CE system was operated in the negative polarity mode with a reversed electroosmotic flow (EOF). The CE background electrolyte (BGE) solution consisted of 100 mM ammonium hydroxide buffer, pH 9.3, containing 0.5 mM TTAB. TTAB was used to reverse the electroosmotic flow.[5,16] The CE anode was placed at the detection end of the separation capillary. A separation voltage of 12 kV was used except as noted in the text. Under these conditions, the electromigration of anions and the EOF are both directed toward the detection end of the separation capillary.

Each new capillary was sequentially rinsed hydrodynamically with alkaline-EDTA solution, pH 13, for 30 min, water for 10 min, and then BGE for 10 min, all at 40 psi. After each separation, the system was flushed hydrodynamically with BGE for 40 s at 40 psi. For pH-mediated stacking, samples and 0.1 M NaOH were sequentially injected electrokinetically. Electrokinetic injections were made at 15 kV except as noted in the text. The injection times for both the sample and NaOH are listed in the text.

RESULTS AND DISCUSSION

Effect of Injection Volume

CE systems have low sample volume capacity, easily becoming overloaded. The effect of electrokinetic injection time and voltage on the CE separation is shown in Figure 1. To a point longer injection times and higher injection voltages result in greater peak heights. But the volume injected quickly overloads the capacity of the capillary and a loss of separation efficiency results. Longer injection times and higher injection voltages then produce broader, less-well-resolved peaks.

The ionic strength of the sample relative to that of the BGE also determines the maximum volume that can be injected. The higher the ionic strength of the sample the smaller the maximum volume that can be injected without loss in separation efficiency. This is due to "destacking" of the sample when the sample zone is of higher conductivity than the BGE. In this case, the electric field across the sample zone is weaker than that across the BGE. Analyte ions moving from the sample zone into the BGE accelerate resulting in band broadening. The maximum sample injection before loss in separation efficiency occurs is summarized in Table 1 for both electrokinetic and hydrodynamic injection for samples of various ionic strengths. An unacceptable loss of separation efficiency was defined as a decrease in N by more than 25% relative to N for a hydrodynamic injection of 2 s with the sample in BGE. These results indicate that only extremely small injection amounts are allowed without losing separation efficiency when the sample matrix has a high salt concentration. Consequently, a much lower concentration sensitivity will result for physiological samples using typical injection methods for CE.

pH-Mediated Field Amplification Sample Stacking

We have recently reported a pH-mediated field amplification method for the on-column preconcentration of cations in high ionic strength samples.[15] This stacking procedure could

be extended to the determination of anions by reversing the EOF and polarity of the separation voltage. This causes the electrophoretic migration of anions and the EOF to be in the same direction and toward the detector. The BGE must be made from the salt of a weak base (e.g., an ammonium salt), and the electrokinetic injection of the sample must be immediately followed by electrokinetic injection of strong base.[18] In this case, sample cations (Na^+) are displaced by the ammonium ion during electrokinetic injection of the sample. The ammonium in the sample zone is then titrated to ammonia by the electrokinetic injection of hydroxide ions. Although the sample zone is migrating away from the injection end during the base injection, hydroxide ions will quickly overtake the front of the sample zone due to the severalfold faster migration rate of hydroxide relative to other anions. After the base injection is stopped, the titrated zone ceases to grow and the analyte anions stacked at the front of this zone separate electrophoretically in the remaining BGE zone.

Evidence for this mechanism is provided by observing the electrophoretic current during the injection and stacking process (Figure 2). When the electrokinetic injection of the sample is initiated, the electrophoretic current quickly rises to a relative high value due to introduction of the high conductivity zone. This electrophoretic current is considerably higher than that observed with BGE alone in the system. When the electrokinetic injection of hydroxide is initiated, the electrophoretic current decreases dramatically, reaching a value much lower than when BGE alone is in the system. This is because the hydroxide has titrated the ammonium salt in the sample zone, creating a very low conductivity zone. Finally, during the separation phase, the electrophoretic current slowly rises until it returns to the value of when only BGE is in the system. This reflects the movement of the titrated zone through the capillary and ultimate elution from the capillary driven by the EOF.

Optimization of Base-Mediated Field Amplification Stacking

The amount of sample injected relative to the amount of hydroxide injected is critical to the extent of preconcentration that can be achieved. For electrokinetic injection, the relative amount injected is a function of the injection times of the sample and hydroxide if the injection voltage is the same. If too little hydroxide is injected, the titrated zone will not reach the front of the sample zone. This portion of the sample will not be stacked and a broad zone of unstacked analyte in front of the sharp peaks of the stacked portion will be observed. This is the case seen in the electropherograms in the upper left corner of Figure 3, in which the amount of sample injected is large relative to the amount of hydroxide injected. On the other hand, if too much hydroxide is injected, the separation efficiency degrades as seen in the lower electropherograms of Figure 3. This is because too large a portion of the capillary is being used for stacking and an insufficient length remains for the separation. In addition, most of the electric field will be across the large titrated zone, leaving a very weak electric field for separation. For these same reasons, the absolute amount of sample that can be injected is limited by the length of the capillary used for stacking.

The optimal results were obtained when the ratio of hydroxide (0.1 M NaOH) injection time to sample injection time was 65/30 using the same injection voltage. This ratio was the same independent of the absolute sample injection time. In other words, a 18-s sample injection required a 39-s base injection and a 30-s sample injection required a 65-s base injection. Using a 70-cm capillary, the longest sample injection time that maintained the separation efficiency was 30 s. The optimal ratio of hydroxide injection time to sample injection time depends on the hydroxide concentration. Lower concentrations of NaOH require longer relative injections for complete stacking. This results in more of the capillary being used for stacking and thus poorer separation efficiencies. Higher concentrations of NaOH need shorter relative injection times for stacking but result in Joule heating and bubble formation during the electrokinetic injection. A concentration of 0.1 M NaOH was found to be the best compromise.

The optimal base injection time to sample injection time is also dependent upon the properties of the BGE. Longer base injections are required for BGEs of higher ionic strength or more slowly migrating cations. Therefore, the stacking conditions must be optimized for each BGE. However, this process typically only requires a few injections for which the base injection time is varied with the sample injection time held constant. The longest acceptable sample injection time can then be determined using a fixed ratio for the base injection time. It should be noted that, for the best performance in terms of separation efficiency and sensitivity, optimization of the detection conditions should be performed whether a stacking procedure is used or not.

Effect of Sample Ionic Strength on pH-Mediated Field Amplification Stacking

The pH-mediated field amplification stacking method was developed using Ringer's solution as a typical physiological matrix. The effect of the sample matrix ionic strength on the stacking efficiency was evaluated using various dilutions of Ringer's solution as the sample matrix. As seen in Table 2, the ionic strength of the sample had little effect on the separation. The higher ionic strength samples actually exhibited somewhat higher separation efficiencies. This is likely because at the higher sample ionic strength the BGE ammonium ions more effectively displace the sample cations during electrokinetic injection. More complete titration of the sample zone during hydroxide injection results, producing a greater difference in the electric fields across the sample zone and BGE. This is borne out by the slower migration times at higher sample ionic strengths. As the injection times were the same for all samples, that the migration times increase as the sample ionic strength increases is an indication of a weaker separation electric field for these samples. That higher sample ionic strength actually increases the efficiency of the pH-mediated field-amplification stacking technique is fortuitous for physiological samples. However, the sensitivity gain by pH-mediated field-amplification stacking decreased as the sample ionic strength increased. This is because the high ionic strength of the sample results in a weaker electric field for electrokinetic injection and less efficient injection of the sample.

Stacking Performance

Comparison of electropherograms using normal electrokinetic injection relative to pH-mediated field-amplification stacking are shown in Figure 4. A detection limit of 0.3 μM (S/N) = 3) was achieved for the phenolic acids in Ringer's solution using simple UV-absorbance detection by pH-mediated stacking. This was a 66-fold improvement relative to normal electrokinetic injection and a 100-fold improvement relative to hydrodynamic injection. The maximum sample injection was determined as the point at which the separation efficiency had deteriorated by 25% relative to a 2-s hydrodynamic injection with the sample in BGE.

The reproducibility and sensitivity of the pH-mediated field amplification stacking procedure are listed in Table 3. For all four phenolic acids tested, the linear range was from 0.3 μM to at least 30 μM. In addition, the reproducibility of the migration time was good at a relative standard deviation of less than 2% for all four compounds.

Alternative Background Electrolyte Systems

Other buffer systems were investigated as the BGE electrolyte in order to determine if pH-mediated stacking could be used over a wide range of pH and with other buffer systems. Buffers ranging in pH from 7.0 (150 mM imidazole) to 10.7 (100 mM methylamine) were tested as the BGE. Significant pH-mediated stacking was observed with each BGE buffer (Figure 5). Injections made without stacking were similar in appearance to that of Figure 4A in all of the buffer systems. In all cases 100 mM NaOH was used as the stacking titrant. Because the BGE buffers varied in concentration and electrophoretic mobility, the relative sample injection time to base injection time had to be determined for each BGE. As can be seen in Figure 5, the pH of the BGE significantly changed the CE separation. Only at pH 9.3 are the baselines of the

four phenolic acids resolved. However, that pH-mediated stacking occurred in all of the buffer systems indicates that this technique can be used over a wide pH range and that the pH of the BGE can be selected for optimal separation without being limited by the requirements of pH-mediated stacking.

Effect of Separation Capillary Length

As described above, the limit to the amount of sample that can be injected and stacked is determined by the length of the CE capillary. The longer the separation capillary, the more sample that can be injected and still stacked and separated. This is because a significant portion of the capillary is being used for the stacking process and is not available for separation. As seen in Figure 6, longer injections can be made into longer separation capillaries without significant loss in resolution. The longer injection time results in a higher sensitivity. The upper limit to the length of the separation capillary is the applied voltage necessary to achieve a good separation. Longer capillaries require higher applied voltage to achieve the same electric field provided by lower applied voltages with shorter capillaries. While the use of longer separation capillaries provided more capillary length for the separation, they did not eliminate the problem of the large low conductivity zone created at the injection end of the capillary. In fact, this situation is more pronounced with the longer capillaries because more sample is injected and the titrated zone is proportionately larger. The resulting weak separation electric field is the reason for the decrease in resolution seen with longer capillaries. For the separation of the phenolic acids, a capillary between 70 and 80 cm provided the greatest sensitivity while maintaining resolution.

Double-Capillary Stacking System

To overcome this limitation to the length of capillary that can be used for pH-mediated sample stacking, a double-capillary system was developed. In this system, one capillary is used for stacking and the other capillary is used for separation. The system is shown schematically in Figure 7. Three pieces of 75-μm-i.d. fused silica capillary of lengths 50 cm (capillary A), 70 cm with 50 cm to the detection window (capillary B), and 20 cm (capillary C) were connected using a fused-silica zero dead volume “T” union. For the pH-mediated stacking, the electrophoretic voltage was applied across capillaries A and C. This in essence provides a capillary of 70 cm with 50 cm for stacking. When the stacked analyte zone arrived in the “T”, the separation voltage was switched to be across capillaries C and B. The separation then occurred in capillary B, which has a 50 cm usable length for the separation. The timing of the separation voltage switch was determined by using a single capillary of 70 cm with 50 cm to the detection window, which is equivalent to capillary A–C with the detection window located at the “T”. Using injection conditions identical with those of the double-capillary system, the time for the analyte zone to arrive at the detector of the single capillary was used as the time to switch the separation voltage. It is important that the switch time not be early as this leaves the sample in capillary A, in which case it is lost upon switching the voltage to capillary B–C. On the other hand, if the voltage switch is made too late, some of the titrated zone will have migrated into capillary C along with the sample zone. This can result in a loss in separation efficiency. Switching the voltage slightly after the optimal time is preferable to switching the voltage early.

The double-capillary system provides significant improvements for pH-mediated sample stacking relative to a single-capillary system. First, the overall effective length of the double-capillary system is much longer than a single capillary without production of Joule heating. This is because only a portion of the overall length (roughly half) is used at any given time. For the system described above, the stacking capillary is 70 cm long with 50 cm available for stacking and the separation capillary is 90 cm long with 50 cm available for separation. The equivalent single capillary would have to be 120 cm long with 100 cm to the detection window.

The second advantage is that the double-capillary system removes the titrated sample zone from the separation capillary. In the double-capillary system, the titrated zone remains in capillary A and is not part of the electrophoretic system during the separation. Therefore, the entire electric field is available for separation and not lost across the titrated zone. Figure 8 shows an electropherogram of the phenolic acids using the double-capillary system. The sensitivity was increased 5-fold relative to the single-capillary system without loss in resolution. In addition, because of the stronger separation electric field the separation time was half that of the single-capillary system.

CONCLUSIONS

A pH-mediated sample stacking procedure for anions has been developed. This technique resulted in a 66-fold increase in sensitivity without loss in separation efficiency relative to normal electrokinetic injection. A detection limit of 0.3 μM was achieved for several phenolic acids in a Ringer's solution matrix. Using the double-capillary stacking system a detection limit of 0.06 μM was achieved, or a 300-fold increase in sensitivity relative to normal electrokinetic injection.

Acknowledgements

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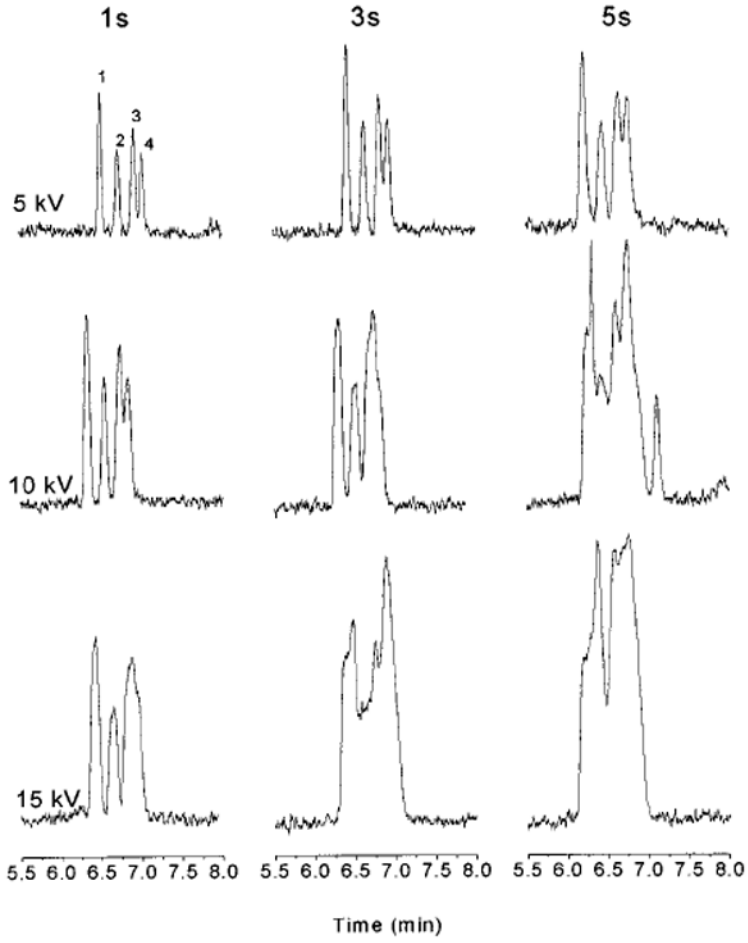

Figure 1.

Effect of sample loading on separation efficiency. Sample: 50 μM each analyte in 50% (v/v) Ringer's solution. Peak identities: 1, p-hydroxybenzoic acid; 2, vanillic acid; 3, *p*-coumaric acid; 4, syringic acid. The BGE was 100 mM ammonium hydroxide buffered at pH 9.3 and containing 0.5 mM TTAB. CE capillary: 75 μm i.d. × 70 cm long (50 cm to detection window). The electrokinetic injection time and voltage are shown on the figure. The separation voltage was 12 kV.

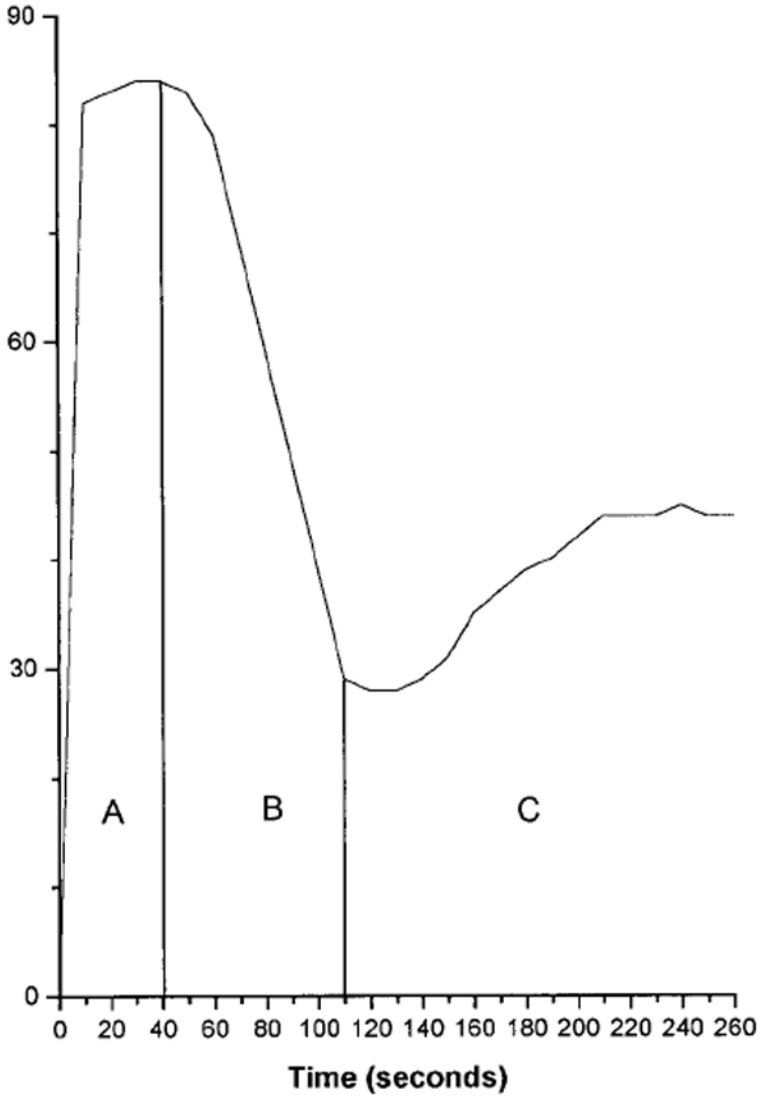

Figure 2.

Change in electrophoretic current during the pH-mediated field amplification stacking procedure. Zone A, sample injection at 15 kV; zone B, hydroxide injection at 15 kV; zone C, separation at 12 kV. Sample: 10 μM analytes in 90% Ringer's solution. The electrophoretic current for the system with just BGE was 44 μA at 12 kV and 56 μA at 15 kV. All other conditions were as in Figure 1.

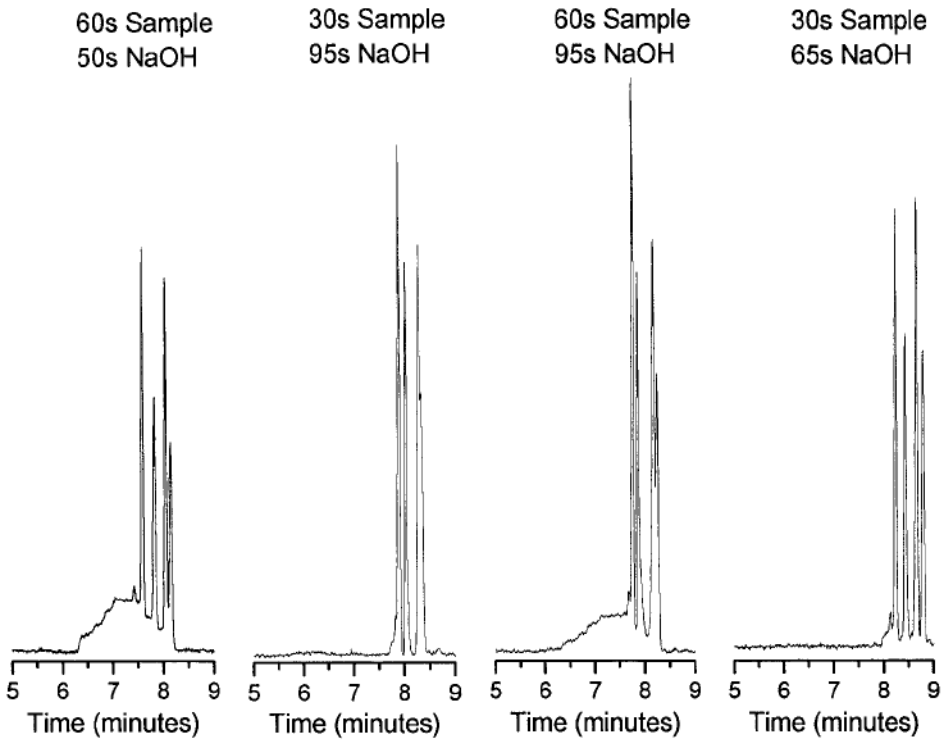

Figure 3.

Effect of sample injection time and hydroxide injection time on CE separation. Sample injection time and hydroxide (0.1 M NaOH) injection time as listed on figure; all at 15 kV. Sample: 10 μM analytes in 90% Ringer's solution. All other conditions were as in Figure 1.

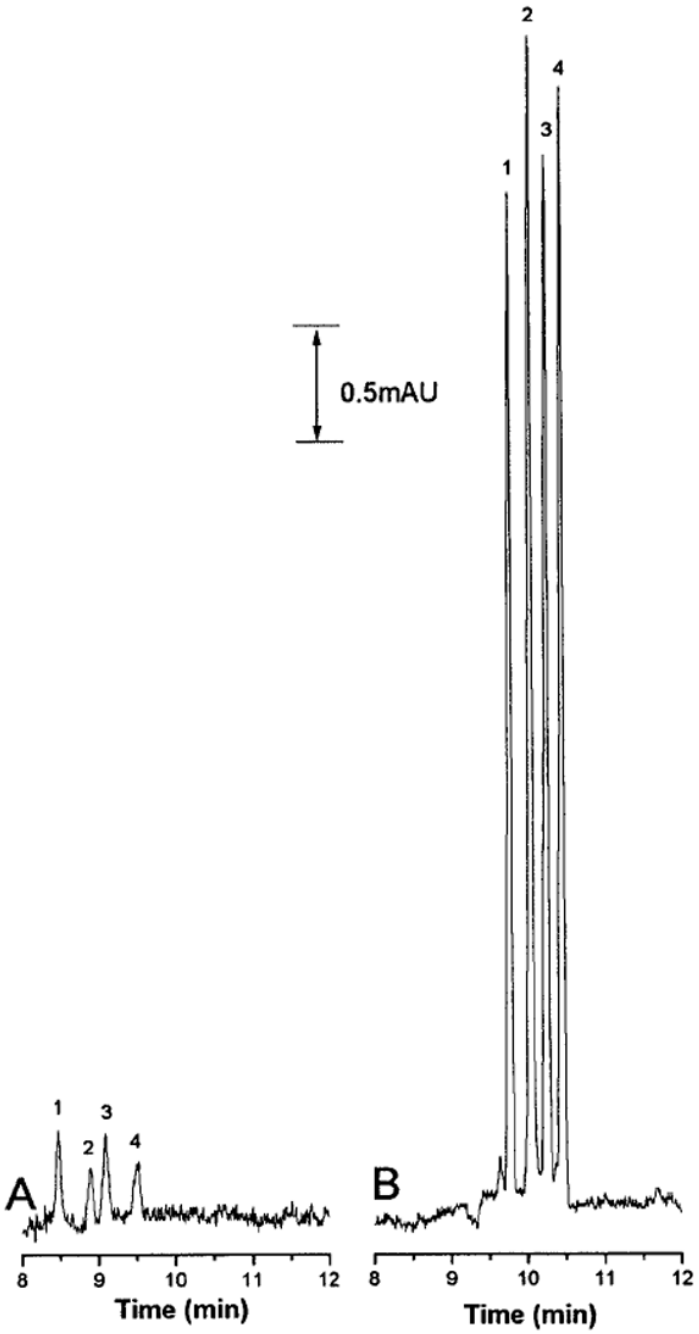

Figure 4.

Comparison of normal electrokinetic injection (A) and pH-mediated field amplification stacking (B). (A) Without stacking, sample was injected for 2 s at 0.5 kV with 15 kV for separation. (B) With stacking, sample was injected for 30 s and hydroxide was injected for 65 s, both at 15 kV. Sample: 10 μM analytes in 90% Ringer's solution. All other conditions were as in Figure 1.

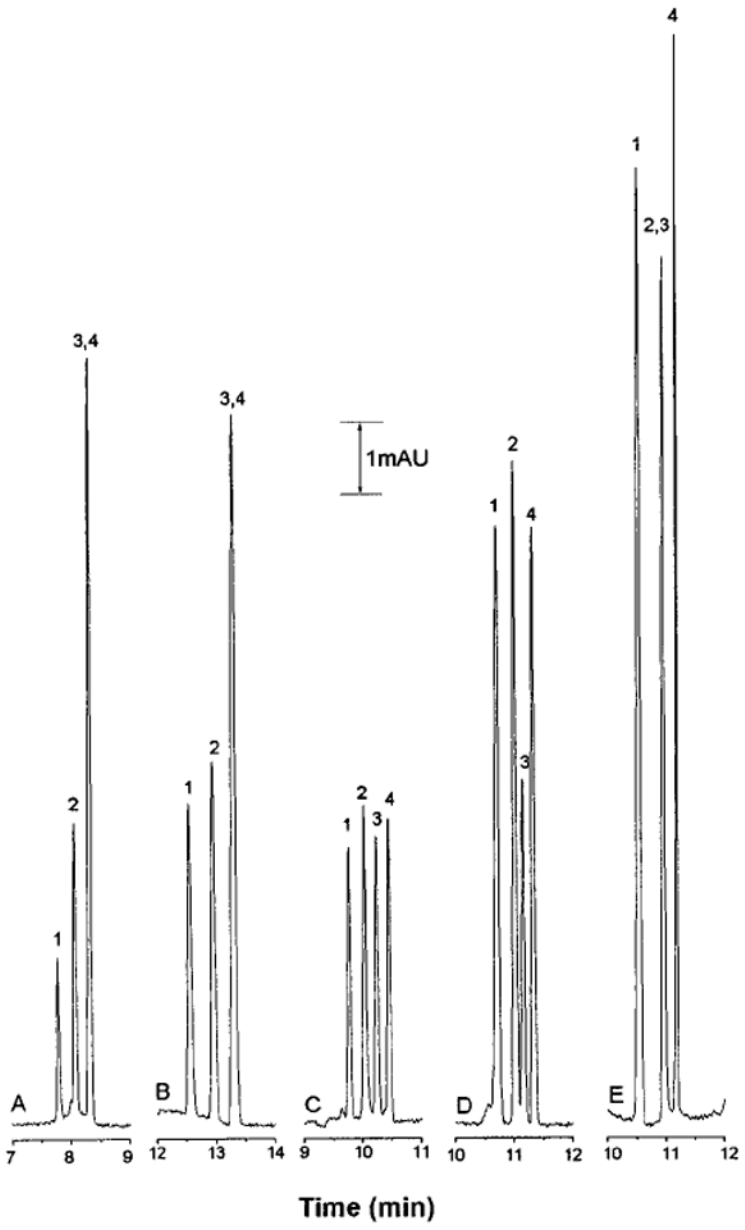

Figure 5.

pH-mediated field amplification stacking using various BGEs. (A) 150 mM imidazole at pH 7.0, 80 s sample injection and 300 s hydroxide injection at 15 kV. Separation voltage was 13 kV. (B) 150 mM Tris at pH 8.1, 50 s sample injection and 240 s hydroxide injection at 16 kV. Separation voltage was 12 kV. (C) 100 mM ammonium hydroxide at pH 9.3, 30 s sample injection and 65 s hydroxide injection at 15 kV. Separation voltage was 12 kV. (D) 300 mM ammonium hydroxide at pH 10.0, 20 s sample injection and 70 s hydroxide injection at 15 kV. Separation voltage was 9 kV. (E) 100 mM methylamine at pH 10.7, 25 s sample injection and 45 s hydroxide injection at 13 kV. Separation voltage was 8 kV. Sample: 10 μM analytes in 90% Ringer's solution. All other conditions were as in Figure 1.

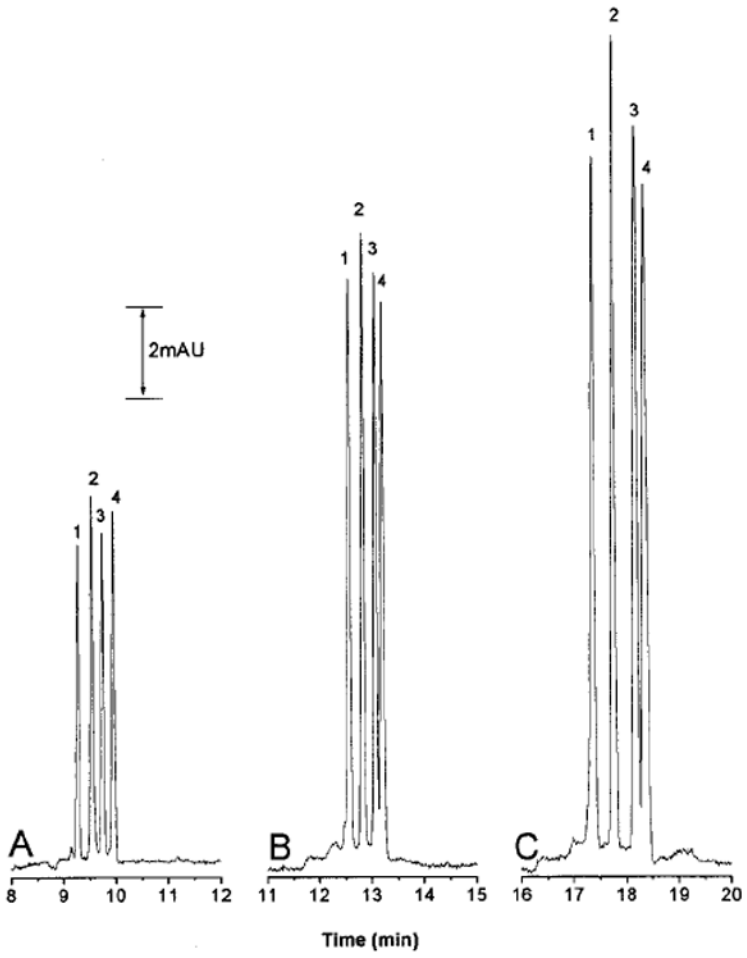

Figure 6.

Effect of capillary length on sensitivity and resolution. (A) 70 cm (50 cm to detection window), 30 s sample injection and 65 s hydroxide injection at 15 kV. (B) 85 cm (65 cm to detection window), 40 s sample injection and 80 s hydroxide injection at 18 kV. (C) 100 cm (80 cm to detection window), 40 s sample injection and 78 s hydroxide injection at 24 kV. The separation voltage was 12 kV in all cases. Sample: 10 μM analytes in 90% Ringer's solution. All other conditions were as in Figure 1.

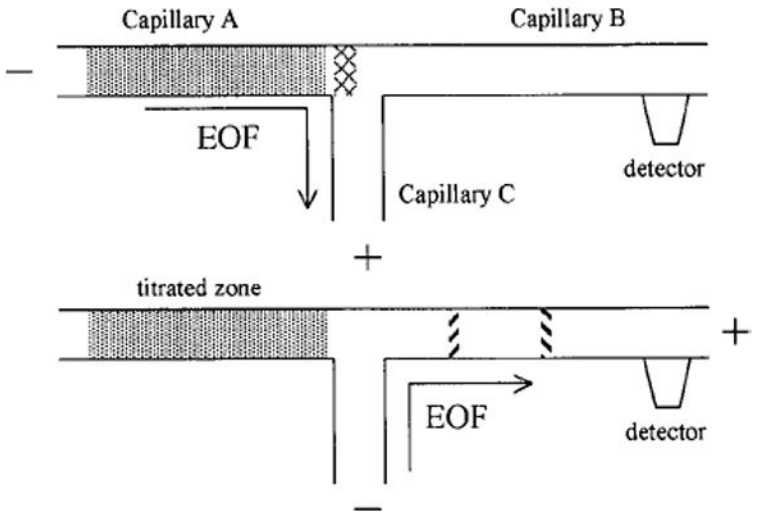

Figure 7.
Schematic diagram of the double-capillary system. Capillary A, 50 cm capillary; capillary B, 70 cm capillary (50 cm to detection window); capillary C, 20 cm capillary. All capillaries were 75 μm i.d.

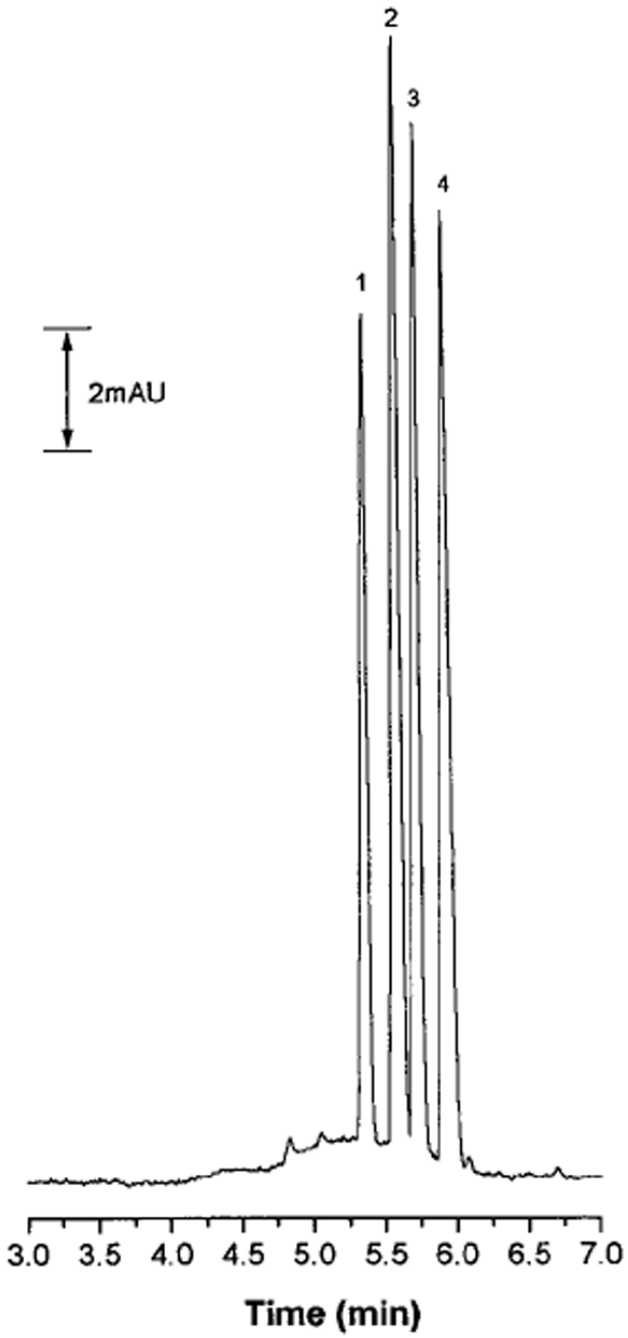

Figure 8.

Electropherogram obtained from the double-capillary system. Sample as in Figure 5. For injection and stacking the CE voltage was across capillaries A and C. Sample was injected for 3.5 min and hydroxide was injected for 10 min at 16 kV, stacking was run for 7 min at 17.5 kV. The CE voltage was then switched to be across capillaries C and B. A separation voltage of 22 kV was used. Sample: 10 μM analytes in 90% Ringer's solution. All other conditions were as in Figure 1.

Table 1

Effect of Sample Matrix on Sample Loadability

sample matrix	hydrodynamic injection (nL)	LOD (μM)	electrokinetic injection (s)	LOD (μM)
water	120	1	4 (16 kV)	0.2
25% Ringer's solution	15	5	10 (5 kV)	0.6
50% Ringer's solution	5.5	10	1 (5 kV)	12
90% Ringer's solution	<5.5	>25	2 (0.5 kV)	20

Table 2

Effect of Sample Matrix On Sensitivity and Efficiency[a]

compound	Ringer's solution (%)	Sensitivity (mAU/mM)	Migration time (min)	efficiency (*N*)
p-hydroxybenzoic acid	25	864.5 ± 80.0	7.83 ± 0.27	212 282 ± 252
	50	708.5 ± 28.3	7.91 ± 0.21	216 095 ± 153
	90	497.0 ± 25.7	8.33 ± 0.19	240 259 ± 125
vanillic acid	25	535.5 ± 9.0	8.00 ± 0.31	141 824 ± 213
	50	500.7 ± 12.6	8.05 ± 0.21	142 178 ± 98
	90	342.8 ± 19.8	8.53 ± 0.19	161 238 ± 80
p-coumaric acid	25	636.9 ± 26.3	8.25 ± 0.33	104 740 ± 168
	50	447.5 ± 18.8	8.30 ± 0.23	106 014 ± 81
	90	468.1 ± 10.6	8.73 ± 0.21	117 283 ± 68
syringic acid	25	505.2 ± 18.6	8.37 ± 0.30	155 246 ± 199
	50	394.0 ± 19.7	8.46 ± 0.23	158 603 ± 117
	90	347.2 ± 17.4	8.89 ± 0.22	175 135 ± 107

[a]Conditions: sample injection for 30 s and 0.1 M NaOH injection for 65 s, both at 15 kV. $n = 4$.

Table 3

Reproducibility and Sensitivity[a]

compound	Sensitivity (mAU/mM)	migration time (min)	RSD (%)	detection limit (μM)	linear range (μM)	*r*
p-hydroxybenzoic acid	497.0 ± 25.7	8.35 ± 0.13	1.6	0.30 ± 0.03	0.3–30.0	0.9985
vanillic acid	342.8 ± 19.8	8.53 ± 0.12	1.4	0.30 ± 0.02	0.3–30.0	0.9997
p-coumaric acid	468.1 ± 10.6	8.74 ± 0.15	1.7	0.30 ± 0.07	0.3–30.0	0.9981
syringic acid	347.2 ± 17.4	8.91 ± 0.15	1.7	0.30 ± 0.09	0.3–30.0	0.9991

[a] n = 20, S/N = 3.