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On-Line Preconcentration in Capillary Electrochromatography Using a Porous Monolith Together with Solvent Gradient and Sample Stacking

Joselito P. Quirino,† Maria T. Dulay, and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, California 94305-5080

Preconcentration effects of solvent gradient and sample stacking are investigated on a photopolymerized sol−gel (PSG) in capillary electrochromatography. The porous PSG monolith has a high mass-transfer rate. This characteristic promotes preconcentration of dilute samples. Plugs of samples more than 2 cm in length prepared in the separation solution (nongradient condition) are injected onto the PSG column. The extent of preconcentration is quite significant, showing up to a 100-fold increase in peak heights of the separated analytes. Even larger preconcentrations are achieved under gradient conditions by dissolving the sample in a matrix with a higher concentration of noneluting solvent (water). For eight alkyl phenyl ketones and four polycyclic aromatic hydrocarbons that serve as neutral test analytes, improvements in peak heights obtained under gradient conditions can be more than a 1000-fold. Indeed, injection of a 91.2-cm plug, which is more than 3 times the total length of the capillary, was possible with only a minor loss in resolution. Five peptides serve as charged test analytes. Nongradient conditions in which the sample is hydrodynamically injected onto the PSG column show sizable preconcentration because of sample stacking. The use of a solvent gradient with the same ionic strength, however, does not appear to have practical value because of destacking caused by the changing organic composition that affects the conductivity. As an alternative preconcentration method, we demonstrate that electric field-enhanced sample injection on the PSG yielded up to a 1000-fold improvement in detection sensitivity for the test peptides.

A major challenge in capillary electrophoresis (CE) techniques including capillary electrochromatography (CEC) is the detection of samples containing analytes at low concentrations. The lack of sensitivity at low concentration stems from the small sample volume and short optical path length for on-line detection. Dedicated sample preparation schemes that enrich the target analytes before sample injection are often necessary in order to obtain the necessary sensitivity for many real-world analyses. Schemes such as solvent−solvent extraction and solid-phase extraction are often very tedious and time-consuming.

* Corresponding author: (e-mail) zare@stanford.edu.

† Present address: Pharmacyclics, Inc., 995 E. Arques Ave., Sunnyvale, CA 94086-4521.

An alternative to these schemes is on-line preconcentration. In CE, these include isotachophoresis, sample stacking, sweeping, and the use of a dynamic pH junction.[1] In CEC using particle (e.g., octadecyl silica) packed columns, focusing effects similar to that in gradient high-performance liquid chromatography have been reported.[2−7] These focusing effects were achieved using (1) step-gradient elution,[2,5,6] (2) preparation of the sample in a noneluting solvent,[3,5,6] or (3) injection of a water plug after sample injection.[4] Taylor and Teale[2] were the first to report the use of a step-gradient for the preconcentration of drug mixtures. Stead et al.[3] achieved a 17-fold increase in the detection sensitivity of a mixture of steroids by preconcentration using a noneluting sample matrix. Zhang et al.[5] also used a noneluting solvent for the preconcentration of benzoin and mephenytoin by a factor of 134 and 219, respectively. Yang and El Rassi[4] reported on the preconcentration of a dilute sample of pesticides using a short plug of water injected after a long plug of sample. Hilhorst et al.[6] reported on the preconcentration of structurally related steroids using a noneluting matrix and step-gradient elution. A gain in sensitivity of 7−9 times was reported. Similarly, Tegeler and El Rassi[7] preconcentrated a mixture of carbamate insecticides using a combination of a noneluting matrix and step-gradient elution. The maximum allowable sample plug length was ~20 cm, and a 500-fold sensitivity increase is achieved for carbofuran. Zhang and co-workers.[5] achieved a further increase in detection sensitivity by combining field-enhanced sample injection with solvent gradient elution. A 17 000-fold increase in peak height for a positively charged analyte, propatenene, was demonstrated.

Recently, we reported that photopolymerized sol−gel (PSG) used as a monolithic stationary phase in CEC[8] preconcentrates a

(1) Quirino, J. P.; Terabe, S. *J. Chromatogr., A* **2000**, *902*, 119−135.
(2) Taylor, M. R.; Teale, P. *J. Chromatogr., A* **1997**, *768*, 89−95.
(3) Stead, D. A.; Reid, R. G.; Taylor, R. B. *J. Chromatogr., A* **1998**, *798*, 259−267.
(4) Yang, C. M.; El Rassi, Z. *Electrophoresis* **1999**, *20*, 2337−2342.
(5) Zhang, Y.; Zhu, J.; Zhang, L.; Zhang, W. *Anal. Chem.* **2000**, *72*, 5744−5747.
(6) Hilhorst, M. J.; Somsen, G. W.; de Jong, G. J. *Chromatographia* **2001**, *53*, 190−196.
(7) Tegeler, T.; El Rassi, Z. *Anal. Chem.* **2001**, *73*, 3365−3372.
(8) Dulay, M. T.; Quirino, J. P.; Bennett, B. D.; Kato, M.; Zare, R. N. *Anal. Chem.* **2001**, *73*, 3921−3926.

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variety of neutral and charged analytes even without the use of a solvent gradient or sample stacking.[9] Preconcentration takes place because of the high mass-transfer rates possible in the porous structure. The extent of preconcentration follows the retention factor k for a given analyte.[9] In this regard, the PSG monolith acts simultaneously as an extractor and as the stationary phase of a chromatographic separator.

Here, we further expand the studies on preconcentration in CEC on a PSG column by showing the effects of solvent gradient and sample stacking. The advantages and disadvantages of using a higher percentage of noneluting solvent in the sample matrix for neutral and charged analytes are discussed using eight alkyl phenyl ketones (APKs), four polycyclic aromatic hydrocarbons (PAHs), and five peptides as test analytes. Electric field-enhanced sample injection of cationic peptides is then applied to the PSG system.

METHODOLOGY

All electrophoresis experiments were performed with a Beckman P/ACE 2000 (Beckman Instruments, Fullerton, CA) equipped with a UV absorbance detector. Fused-silica capillaries (75-μm inside diameter $\times$ 365-μm outside diameter) were purchased from Polymicro Technologies (Pheonix, AZ). The capillaries were thermostated at 20 °C. Detection was done at 214 or 254 nm. Injections were done using pressure (0.5 or 20 psi) or voltage (1−22 kV) and varied in duration from 4 to 720 s. Data analysis was performed with GRAMS/32 version 4.02 (Galactic Industries Corp., Salem, NH). All electrochromatograms presented here were drawn to the same scale using GRAMS/32.

Unless stated, all reagents were purchased from Sigma-Aldrich (Milwaukee, WI) in the highest grade available. The sol−gel solution was prepared in a manner similar to that given in refs 8 and 9. For the APK mixture and peptide mixture experiments, a pentafluorophenyl-bonded PSG column was prepared in the same way as in ref 9. The polymerization length of the monolithic structure was controlled by removing a 15-cm stripe of the polyimide coating of the capillary[8] prior to irradiation for 5 min at 365 nm. The trifluoropropyl (TFP)-bonded PSG column used for the PAH mixture is prepared the same as that in the APK mixture except the PSG material is 10 cm in length. The same TFP-bonded PSG 10-cm column is used for the experiments shown in Figure 6. Unreacted reagents were flushed from the column with ethanol. The total length of the capillary was 25.6 cm (18.8 cm from inlet to the detector window). The detector window is positioned after the PSG material. The resulting PSG column is conditioned with the separation solution prior to use.

Sample plug lengths at different injection conditions were determined using thiourea prepared in the separation solution. Thiourea was injected into the column by pressure or applied voltage. The sample plug lengths were calculated using the following expression, $(L_d/t_R)t_{inj}$, where L_d is the length of the capillary from the inlet to the detector, t_R is the elution time of thiourea, and t_{inj} is the injection time of sample.

The samples were thiourea, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, decanophenone, naphthalene, phenanthrene, pyrene, benz[*e*]acephenanthylene, bradykinin, angiotensin II, tripeptide I (Gly-Gly-Gly), tripeptide II (Val-Tyr-Val), and methionine enkephalin. Stock solutions of thiourea (1 mg/mL), APKs (1 μg/mL), and PAHs (0.1−1 mg/mL) were prepared in acetonitrile or aqueous acetonitrile. Stock solutions of test peptides (1 mg/mL) were prepared in water. Stock solutions were mixed and then diluted with various sample matrixes prior to injection. The separation solution consisted of various portions of 50 mM ammonium acetate (pH ~6) or 50 mM phosphoric acid (pH ~2), water, and acetonitrile, and was degassed by sonication for 2 min. A new sample solution was used for every injection to maintain the same concentration of acetonitrile and analyte in the sample solution. Other conditions can be found in the text or figure captions. As a precautionary note, proper care should be taken when handling the analytes, sol−gel reagents, and organic solvents as they are suspected to be health-threatening chemicals.

RESULTS AND DISCUSSION

In chromatographic separations for which no on-line preconcentration occurs, an increase in injected volume of sample leads to loss of column efficiency (resolution) because of peak broadening of the analyte bands. When on-line preconcentration is possible, the volume of sample injected can be increased without sacrificing column efficiency. A major advantage of on-line preconcentration is that it lowers the detection limit for a given analyte. Another advantage is that preconcentration may be used to clean up the analytes from possible interfering species found in the sample matrix. In what follows, we show the power of the PSG column for on-line preconcentration in CEC.

A. Use of Solvent Gradients: Neutral Analytes. *Effect of Increasing the Percentage of Noneluting Solvent in the Sample Matrix.* Figure 1 shows the effect of increasing the concentration of water in the sample matrix on the separation of thiourea and eight APKs. The sample matrixes consist of different ratios of 50 mM ammonium acetate, water, and acetonitrile. The retention factors, k, obtained using the separation condition in Figure 1 for acetophenone (peak 2), propiophenone (peak 3), butyrophenone (peak 4), valerophenone (peak 5), hexanophenone (peak 6), heptanophenone (peak 7), octanophenone (peak 8), and decanophenone (peak 9) are 0.18, 0.25, 0.32, 0.41, 0.53, 0.67, 0.85, and 1.33, respectively. In this study, thiourea (peak 1) is used as the essentially unretained neutral solute for the determination of k. The value of k and migration time follow the increase in alkyl chain length. The sample plug is kept at 1.1 cm for all experiments. Figure 1a depicts a condition in which the sample and the separation solutions contain the same concentration of water (40%). Peak shapes and resolution are better when the water concentration in the sample matrix is greater than 40% (see Figure 1b−d), except in the case where the sample matrix contains 80% water. Peak widths are narrowed by at least 50% in Figure 1b−d as compared to Figure 1a. For the closely eluting peaks 2−5, resolution is less than 1 in Figure 1a whereas resolution is greater than 1 in Figure 1b−d. The decrease in migration time in Figure 1e is quite noticeable and may be caused by the electric field increase in the separation solution, which results from the lower conductivity of the separation solution compared to the sample solution. The higher local electroosmotic flow in the separation solution produced by the increase in electric field increases the bulk electroosmotic flow of the entire liquid inside the column.

(9) Quirino, J. P.; Dulay, M. T.; Bennett, B. D.; Zare, R. N. *Anal. Chem.*, *73*, 5539−5543.

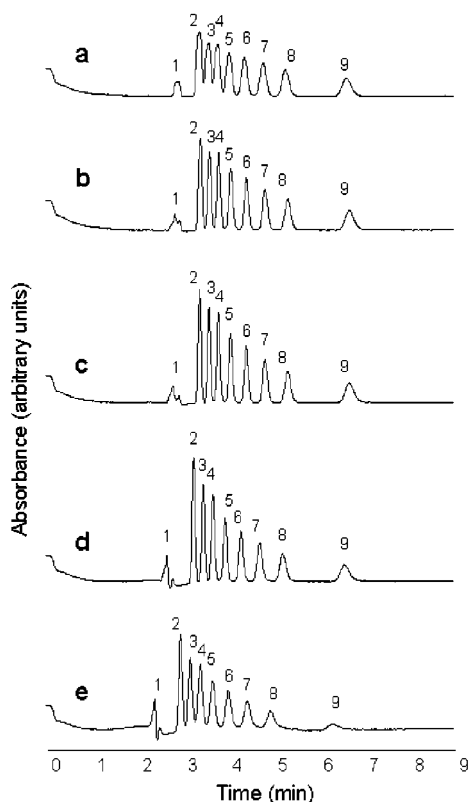

Figure 1. Electrochromatograms of the separation of thiourea (peak 1), acetophenone (peak 2), propiophenone (peak 3), butyrophenone (peak 4), valerophenone (peak 5), hexanophenone (peak 6), heptanophenone (peak 7), octanophenone (peak 8), and decanophenone (peak 9). Conditions: plug lengths, 1.1 cm; separation solution, 5 mM ammonium acetate in 60% acetonitrile; sample matrix, 5 mM ammonium acetate in 60% acetonitrile (a), 5 mM ammonium acetate in 50% acetonitrile (b), 5 mM ammonium acetate in 40% acetonitrile (c), 5 mM ammonium acetate in 30% acetonitrile (d), and 5 mM ammonium acetate in 20% acetonitrile (e); sample concentration, 2 nL/mL each; applied voltage, 15 kV; detection, 254 nm.

The decrease in resolution in Figure 1e may be caused by a mixing effect at the boundary between the sample and separation solutions. Mixing is caused by the mismatch of local velocities that occurs because of the differences in conductivities.[10]

Figure 2 presents a plot of the percentage of water in the sample matrix and the logarithm of the peak height ratio (peak height obtained with lower concentrations of acetonitrile in the sample matrix divided by the peak height obtained with the separation solution used as sample matrix). The data indicate that a limit exists to which the peak heights can be improved by increasing the concentration of the noneluting solvent in the sample matrix. Preconcentration is improved owing to the increased attraction of the analytes to the PSG phase. Note from Figure 2 that when the value for the logarithm of the peak height ratio is less than 1, about 1, or greater than 1 there is a decrease, no change, or increase, respectively, in peak height compared to a similar injection using the separation solution as the sample matrix. For all test APKs, peak heights improved when the water concentration was increased from 40 to 50% and from 50 to 60%. Peak heights did not improve when the percentage of water was increased from 60 to 70% or more, except for the two lowest k analytes (acetophenone, propiophenone) when the percentage of water was increased from 60 to 70%. Peak heights worsened for the higher k analytes (heptanophenone, octanophenone, decanophenone) in the 80% water matrix. The reason for the decrease in peak heights is the decrease in the solubility of the high k analytes in the highly aqueous sample matrix. The corrected peak areas, which is a measure of the amount of sample loaded for octanophenone and decanophenone, are 10−60% lower in the 80% water matrix compared to the other sample matrixes used. To avoid solubility problems, the test APKs in succeeding experiments were prepared in matrixes having at least 30% acetonitrile.

(10) Quirino, J. P.; Terabe, S. *J. Chromatogr., A* **1997**, *781*, 119−128.

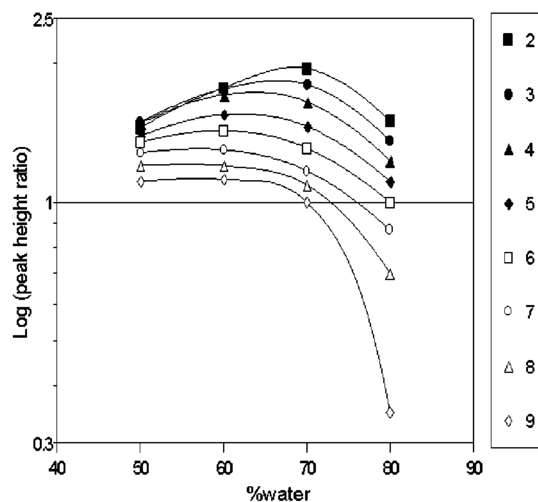

Figure 2. Effect of the percentage of noneluting solvent (water) on the peak height ratio (peak height obtained from a higher concentration of water in the sample matrix/peak height obtained from a sample matrix similar to that of the separation solution). Conditions and identification of peaks are the same as in Figure 1.

Parts b and c of Figure 3 each show a 2.74-cm injection of sample prepared in 40% water (separation solution) and 70% water, respectively. Notice in Figure 3c the improved resolution and peak shapes of the sample components that are analyzed under gradient conditions in which the sample solution has higher water content than the separation solution. Figure 3a is a typical injection (0.2 mm) for comparison. Improvements in peak heights under the gradient condition in Figure 3c are 36, 35, 38, 41, 42, 38, 32, and 24 times for acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, and decanophenone, respectively. The RSDs ($n = 5$) of measured peak heights ranged from 0.9 to 2.5%. RSDs ($n = 5$) of migration time ranged from 0.3 to 0.5%. The procedure is therefore reproducible in a single column.

Effect of Retention Factor. Figure 4 shows the effect of higher k values on preconcentration ($n = 3$). The k values are higher in Figure 4b than in Figure 4a because of the high percentage of water in the separation solution. The analyte molecules are more attracted to the PSG phase at high percentages of water. The distribution constant K (molar concentration of solute in the PSG phase divided by the molar concentration of solute in the separation solution), which is directly proportional to k, increases with increasing concentration of water in the separation solution. In Figure 4b, the k values for acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, and decanophenone are 0.29, 0.47, 0.65, 0.92, 1.28,

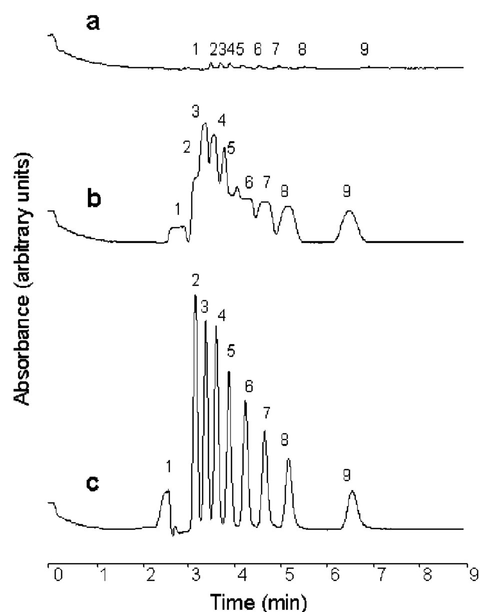

Figure 3. Electrochromatograms of the separation of thiourea and eight alkyl phenyl ketones. Conditions: plug lengths, 0.2 (a) and 2.74 cm (b, c); separation solution, 5 mM ammonium acetate in 60% acetonitrile; sample matrix, 5 mM ammonium acetate in 60% acetonitrile (a, b), and 5 mM ammonium acetate in 30% acetonitrile (c); other conditions and identification of peaks are the same as in Figure 1.

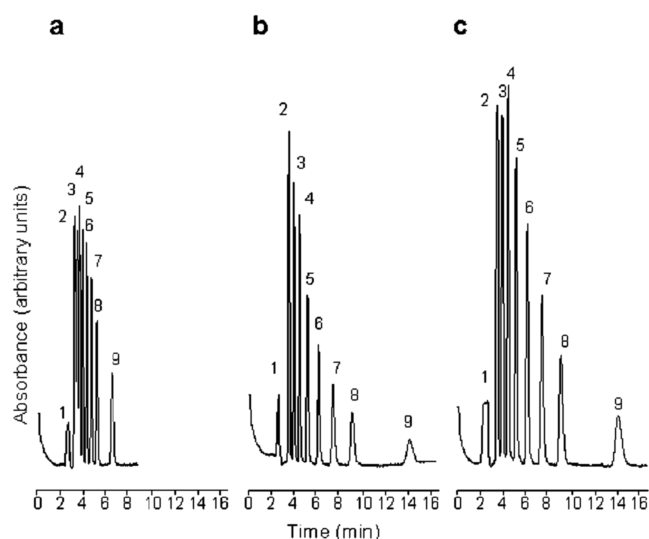

Figure 4. Electrochromatograms of the separation of thiourea and eight alkyl phenyl ketones. Conditions: plug lengths, 2.74 cm (a, b), 5.48 (c); separation solution, 5 mM ammonium acetate in 60% acetonitrile (a), 50% acetonitrile (b, c); sample matrix, 5 mM ammonium acetate in 40% acetonitrile (a) and 5 mM ammonium acetate in 30% acetonitrile (b, c); other conditions and identification of peaks are the same as in Figure 1.

1.76, 2.37, and 4.25, respectively. To maintain the gradient effect constant, the organic solvent ratio between the separation solution and sample matrix is kept at the same value for Figure 4a and b. For reasons still unknown, the result in Figure 4b shows that for analytes with lower k values (acetophenone, propiophenone) there are slight increases in peak heights compared to Figure 4a. For the other test solutes, there are some decreases in peak heights.

Figure 4c illustrates what happens for a longer injection plug of 5.48 cm and a higher percentage of water in the separation

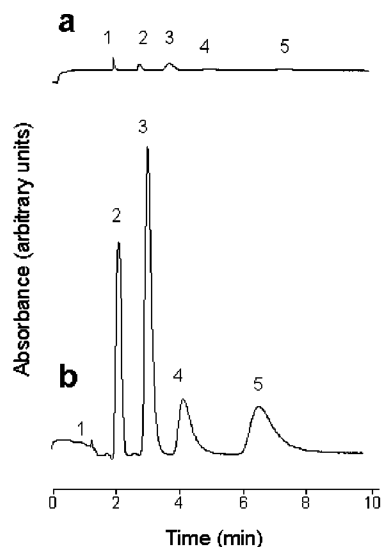

Figure 5. Electrochromatograms of the separation of thiourea (peak 1), naphthalene (peak 2), phenanthrene (peak 3), pyrene (peak 4), and benz[*e*]acephenanthylene (peak 5). Conditions: plug length, 0.22 mm (a) and 19.5 cm (b); separation solution, 5 mM ammonium acetate in 60% acetonitrile; sample matrix, 5 mM ammonium acetate in 60% acetonitrile (a) and 5 mM ammonium acetate in 36% acetonitrile (b); sample concentrations, 11−53 (a) and 1.1−5.3 μg/mL (b); applied voltage, 30 kV; detection 214 nm.

solution. Improvements in peak heights are 31, 33, 55, 44, 44, 37, 29, and 19 times for acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, and decanophenone, respectively. As in Figure 3c, the improvements in peak heights do not follow k, unlike in nongradient conditions.[8] The improvements in peak heights are comparable to those obtained with a higher percentage of organic solvent between the separation solution and the sample matrix (Figure 3c). Note that the injection plug is 2 times shorter in Figure 3c than in Figure 4c. Therefore, for neutral analytes, two approaches exist for using gradients on the PSG. The first is to increase the organic solvent ratio between the separation solution and the sample matrix. The second is to increase the retention factor k in the separation by increasing the percentage of water in the separation solution while maintaining a reasonable percentage of organic solvent between the separation solution and the sample matrix. Analysis is faster with the first approach, whereas the resolution is better with the second one.

Figure 5b shows the application of a solvent gradient for improved detection of four PAHs. A high percentage of acetonitrile (60%) in the separation solution, a shorter PSG length (10 cm), and a high electric field strength (781.3 V/cm) are used for faster analysis times. Figure 5a is a 0.22-mm typical injection of sample prepared in the separation solution. Figure 5b is a 19.5-cm injection using a gradient where the sample is in a 36% acetonitrile matrix, which provides a large difference in the percentage of organic solvent between the sample matrix and the separation solution. Plugs longer than 19.5 cm cause broadening of the naphthalene peak. It is interesting to note that the injection length is longer than the length from the inlet to the detector window (18.8 cm). The faster eluting thiourea zone is actually observed during the sample injection. The thiourea zone is therefore at the detection window at the start of the separation voltage.

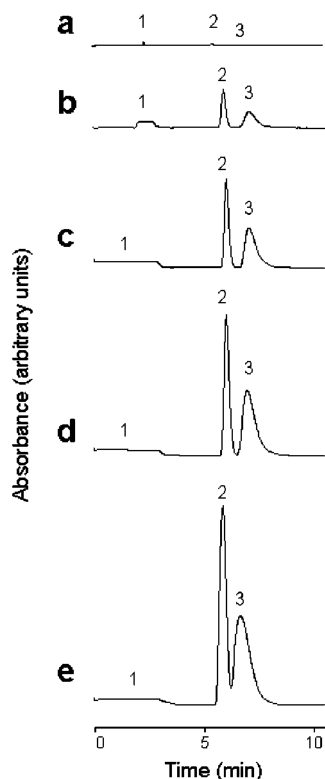

Figure 6. Electrochromatograms of the separation of thiourea (peak 1), decanophenone (peak 2), and pyrene (peak 3). Conditions: plug length, 0.23 mm (a) and 7.6 (b), 22.8 (c), 45.6 (d), and 91.2 cm (e); separation solution, 5 mM ammonium acetate in 60% acetonitrile; sample matrix, 5 mM ammonium acetate in 60% acetonitrile (a) and 5 mM ammonium acetate in 40% acetonitrile (b−e); sample concentrations, 9−50 (a) and 0.9−5 μg/mL (b−e); applied voltage, 22 kV; detection 254 nm.

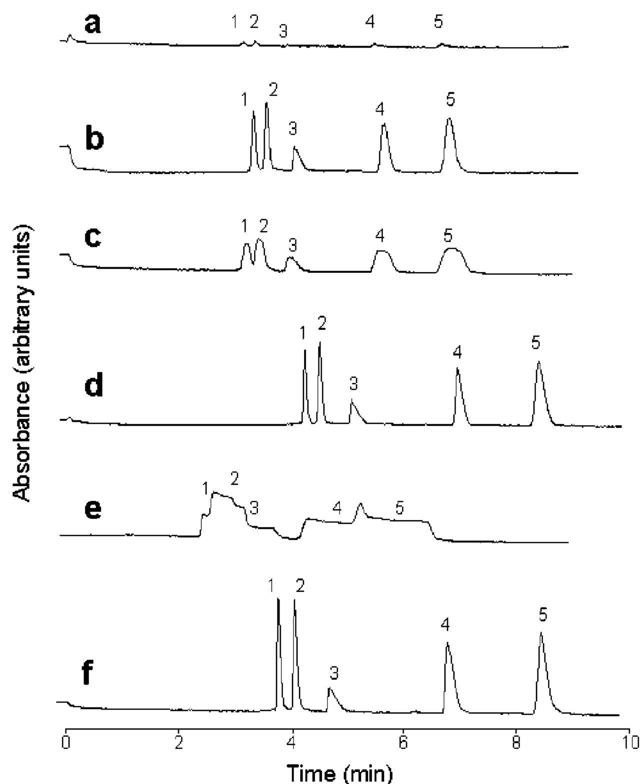

Figure 7. Electrochromatograms of the separation of bradykinin (peak 1), angiotensin II (peak 2), tripeptide I (peak 3), tripeptide II (peak 4), and methionine enkephalin (peak 5). Conditions: plug lengths, 0.1 mm (a) and 1.8 cm (b−f); separation solution; 10 mM phosphoric acid in 40% acetonitrile; sample matrix, same as separation solution (a, b), 10 mM phosphoric acid in 20% acetonitrile (c), 10 mM phosphoric acid in 70% acetonitrile (d), 50 mM phosphoric acid in 40% acetonitrile (e), and 0.05 mM phosphoric acid in 40% acetonitrile (f); peptide concentrations, 16.7 μg/mL each; applied voltage, 12 kV; detection, 214 nm, 20 °C.

Improvements in peak heights for naphthalene (peak 2), phenanthrene (peak 3), pyrene (peak 4), and benz[*e*]acephenanthylene (peak 5) are 346, 437, 409, and 315 times, respectively. Note that the sample concentrations in Figure 5b are 10-fold lower than in Figure 5a. For naphthalene, phenanthrene, and pyrene, the values stated above are 6.9, 3.5, and 3.2 times better than that previously reported under nongradient conditions, respectively.[9]

Pushing the Limits of Injection. Figure 6 illustrates the increases in peak heights of decanophenone (peak 2) and pyrene (peak 3) with increasing plug lengths. The injection is increased from 0.23 mm (Figure 6a) to 7.6 (Figure 6b), 22.8 (Figure 6c), 45.6 (Figure 6d), and 91.2 cm (Figure 6e), which corresponds to 0.1, 30, 89, 178, and 356% of the total capillary length. The high porosity or the low resistance to flow of the PSG material makes it possible to introduce increasing lengths of the sample solution in a rather effortless manner. Although longer than 91.2-cm injection is still possible, it is not performed, however, owing to loss of resolution as observed in Figure 6e. Figure 6a is a typical injection of analytes having concentrations 10 times higher than those are in the other figures. The electrochromatogram in Figure 6d or e is perhaps the first demonstration in CEC showing sample injections longer than the total capillary length. The volume of sample injected is also greater than 1 μL. A comparison of the peak heights obtained in Figure 6a and e suggests improvements in peak heights of 1118 and 1104 times for decanophenone and pyrene, respectively. These values are the highest reported sensitivity improvements for neutral analytes using a simple on-line preconcentration technique in CEC. The strong interaction of the analytes to the PSG in the gradient matrix and the inherent rapid mass-transfer characteristics of the PSG allowed for the observation of such marked preconcentration effects.

Successful separations have been done with PSG in 250-μm-i.d. capillaries (data not shown). The use of this large-diameter capillary opens the possibility of performing semipreparative separations involving long plug injections.

B. Use of Solvent Gradient: Charged Analytes. *Effect of Increasing the Percentage of Noneluting Solvent in the Sample Matrix.* Parts b−d of Figures 7 show the effect of different percentages of water in the sample matrix on the CEC separation of the peptide test mixture. Injections are done using pressure wherein the injection length is fixed at 1.8 cm. Figure 7a is a typical injection (0.1 mm). The sample matrix in Figure 7b is the same as the separation solution (10 mM phosphoric acid in 40% acetonitrile). The sample matrix in Figure 7c and d contains 20% acetonitrile and 70% acetonitrile, respectively. Although it is expected that the peak shapes would be better under a gradient condition (Figure 7c) as compared to a nongradient one (Figure 7b), the resulting peak shapes are better using a higher concentration of acetonitrile in the sample matrix (Figure 7d).

Sample Stacking and Destacking on a PSG Column. Better peak shapes are observed in Figure 7d resulting from sample stacking. Sample stacking is the focusing of charged analytes when analytes pass the concentration boundary that separates regions of high and low electric field strengths.[1] The high electric field zone is the lower conductivity sample matrix containing more acetonitrile whereas the low electric field region is the higher conductivity separation solution. Note that acetonitrile has a lower conductivity than water; thus, a higher concentration of acetonitrile results in lowering the sample matrix conductivity. The broadening effect of using a higher concentration of acetonitrile in the sample matrix (reverse gradient effect due to higher concentration of eluting solvent) is not observed because the cationic peptides immediately migrate to the separation buffer once voltage is applied; thus, the peptide zones are already in the separation solution before it reaches the PSG material. Note also that the injection plug (1.8 cm) is shorter than the open section length (3.5 cm) of the PSG column. Sample stacking is also shown in Figure 7f where the sample is prepared in a matrix having a lower concentration of buffer component and a similar percentage of acetonitrile compared to the separation solution.

There are therefore two approaches to perform sample stacking on a PSG. The first is to increase the percentage of organic solvent, such as acetonitrile. The second is to decrease the concentration of the buffer component in the sample matrix. Increasing the percentage of acetonitrile or other suitable organic solvent is especially useful for real samples containing a high concentration of salts. Desalting, for example, by dialysis, is therefore not necessary to make a lower conductivity solution for injection. Use of organic solvents is also useful for biological samples when deproteination is part of the sample preparation.

Undesirable peak shapes are observed in Figure 7c resulting from destacking. Destacking is the broadening of charged analytes when analytes pass the concentration boundary that separates regions of low and high electric field strengths. The low electric field zone is the high conductivity sample matrix containing more water. Destacking is also shown in Figure 7e where the sample is prepared in a matrix having a higher concentration of buffer component and a similar percentage of acetonitrile compared to the separation solution.

Sample stacking and destacking are basically caused by the change in electrophoretic velocity at the concentration boundary. Electrophoretic velocity is the product of electrophoretic mobility and electric field strength. Focusing occurs (sample stacking) when the electrophoretic velocity decreases at the concentration boundary whereas broadening occurs (destacking) when the electrophoretic velocity increases at the concentration boundary. Sample stacking and destacking are also explained using the fundamentals of isotachophoresis and Kohlrausch rules.[11]

C. Field-Enhanced Sample Injection. Figure 8b shows the successful application of field-enhanced sample injection on the PSG column. Figure 8a is a typical injection of sample prepared in the separation solution. The analytes in Figure 8b are 100 times less concentrated than those in Figure 8a. Improvements in peak heights for bradykinin (peak 1), angiotensin II (peak 2), tripeptide I (peak 3), tripeptide II (peak 4), and methionine enkephalin (peak 5) are 1040, 820, 810, 950, and 711 times, respectively. For the

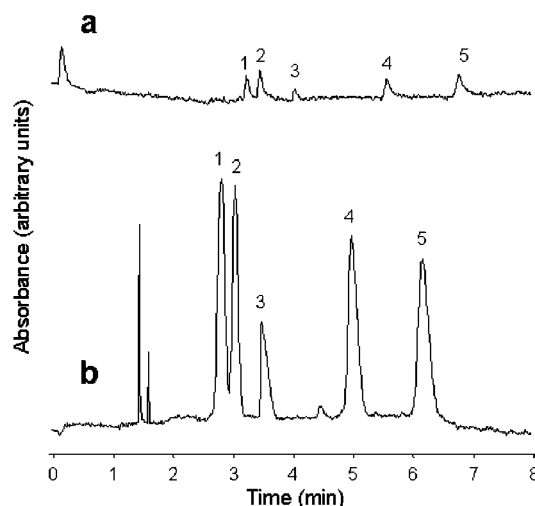

Figure 8. Electrochromatograms of the separation of five test peptides. Conditions: injection, 0.1 mm using 0.5 psi pressure (a), 15 s at 5 kV (b); sample matrix, 10 mM phosphoric acid in 40% acetonitrile (a), 0.5 mM phosphoric acid in 40% acetonitrile (b); peptide concentrations, 16.7 mg/mL each (a), 167 ng/mL each (b); separation voltage, 12 kV; other conditions are the same as in Figure 7.

preconcentration procedure, RSDs ($n = 5$) of peak heights ranged from 6.2 to 16.2% while RSDs ($n = 5$) of migration time ranged from 0.7 to 1.5%. Reproducibility of peak heights should be improved with the use of an internal standard.

Field-enhanced sample injection is performed by dissolving the sample in a low-conductivity matrix (0.5 μM phosphoric acid in 40% acetonitrile), followed by injection using voltage with the negative electrode at the detector end. As the voltage is applied, the low-conductivity sample matrix enters the capillary by virtue of electroosmotic flow (EOF) while the cationic peptides enter the column by virtue of both EOF and electrophoretic flow. Only a very small plug of sample matrix is introduced because the low pH of the separation solution markedly decreases the EOF, which prevents the dissociation of silanol groups at the capillary walls. An unretained neutral solute (thiourea) was actually detected after 30 min.

The electric field in the sample matrix zone introduced into the column is much higher than the separation zone. This effect causes the high electrophoretic velocity of the cationic peptides entering the capillary. The high analyte electrophoretic velocity causes a large amount of peptides to be introduced; unlike in hydrodynamic injection, the volume of sample loaded limits the amount of sample introduced. The high analyte electrophoretic velocity also causes focusing or preconcentration of peptides at the concentration boundary between the sample matrix and separation solution (sample stacking). Introduction of a water plug before electrokinetic injection, which is suggested to be useful in sample stacking with electrokinetic injection,[12] did not improve the peak heights because of the similar direction of the EOF and analyte electrophoretic velocities. The low-conductivity sample matrix that enters the capillary also maintains the enhancement of the electric field at the inlet end of the capillary during injection.

With the conditions in Figure 8, optimum electrokinetic injection time at 5 kV is found to be 15 s. Longer injections lead

(11) Quirino, J. P.; Terabe, S.; Bocek, P. *Anal. Chem.* **2000**, *72*, 1934−1940.

(12) Chien, R. L.; Burgi, D. S. *Anal. Chem.* **1992**, *64*, 489A−496A.

to broadening of the peaks. After the injection, the separation voltage is applied with the same polarity as in the injection (negative electrode at the detector side). The analytes move to the cathode and are subsequently preconcentrated again on the basis of their retention on the PSG column. The method is considered selective for cations because cations are mostly introduced into the capillary. The injected neutrals will migrate after the unretained neutral marker and the cations because the EOF is very slow. Note that, at the pH used, all the analytes are either positively charged or neutral. Applicability of the technique to other cationic samples must also be possible.

CONCLUSION

The preconcentration effect of a PSG for neutral analytes is improved by using a solvent gradient. The limiting factor for using gradients is the decrease in solubility of hydrophobic analytes at higher concentrations of the noneluting solvent (water). Application of gradients to charged analytes is hindered by the effect of eluting or organic solvent on the conductivity of the sample matrix. Electrokinetic preconcentration in the PSG using sample stacking by hydrodynamic injection or electrokinetic injection is useful for positively charged analytes. On-line preconcentration methodologies using the PSG as shown here will further broaden the applicability of CEC in solving many chemical analysis problems.

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