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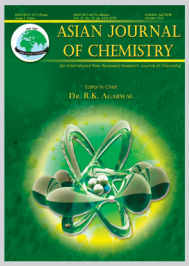

Analysis of Acromatic Acids in River Water by Non-Aqueous Capillary Electrophoresis with Electrokinetic Supercharging

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Electrokinetic supercharging (EKS), a new and powerful on-line preconcentration method for capillary electrophoresis, was utilized in non-aqueous capillary electrophoresis (NACE) to enhance the sensitivity of acromatic acids. The buffer acidity and concentration, leader and terminator length and electrokinetic injection time were optimized, with the optimum conditions being: a background electrolyte of 30 mmol L^{-1} *tris*-acetic acid (pH 7.9), hydrodynamic injecting of 100 mmol L^{-1} ammonium chloride (22 s, 0.5 psi) as leader, electrokinetic injection of the sample (200 s, -10 kV), hydrodynamic injecting of 10 mmol L^{-1} 2-(cyclohexylamino)ethanesulphonic acid (32 s, 0.5 psi) as terminator, before separation (-25 kV). Under these conditions the sensitivity was enhanced between 3868-6480 times when compared to a normal hydrodynamic injection. Detection limits for the five aromatic acids were in the range of 0.08-0.30 ng mL^{-1}. The developed method was further verified by application to river water real sample analysis and with good results.

Key Words: Electrokinetic supercharging, Non-aqueous capillary electrophoresis, Acromatic acids.

INTRODUCTION

Due to its short optical path and small sample volume injected, the sensitivity of capillary electrophoresis is relatively low, which make its limited application in trace analysis. To lower its detection limits, many on-line concentration methods including field amplified sample stacking (FASS)[1,2], field amplified sample injection (FASI)[3], large-volume stacking using the EOF pump (LVSEP)[4,5], field-amplified sample injection with matrix removal *via* an EOF pump (FAEP)[6], dynamic pH junction (DypH)[7], transient-isotachophoresis (tITP)[8], Pseudo-transient isotachophoresis (Pseudo-tITP)[9,10], sweeping[11], micelle collapse (MC)[12], selective exhaustive injection (SEI)[13], selective exhaustive injection-sweeping (SEI-sweeping)[14], dynamic pH junction-sweeping[15], large-volume stacking using the EOF pump-sweeping (LVSEP-sweeping)[16] and electrokinetic surpercharging (EKS)[17-22], have already been developed.

Compared to aqueous capillary electrophoresis, non-aqueous capillary electrophoresis (NACE) have the advantages of good selectivity and extended scope for its various solvent mixture and good solubility of the organic solvent. Among the online concnetration methods, FASS[2], LVSEP[5], pseudo t-ITP[10], LVSEP-ASEI[13] and EKS[21] have been applied to enhance the sensitivity of non-aqueous capillary electrophoresis. Previously, Lu and Breadmore[21] have investigated the on-line concentration of aromatic acids with a acetate electrolye in methanol after EOF reversal and modest sensitivity enhancement from 300-440-fold were achieved. In this paper, we developed a electrokinetic supercharging method to concentrate 5 kinds of aromatic acids in a *tris*-AcOH buffer in methanol. Enhancement factor from 3868-6480 have been achieved. This method was applied to river water real sample analysis with good results.

EXPERIMENTAL

Capillary electrophoresis analysis were carried out in a PACE MDQ capillary electrophoresis system with a photodiode array detector for absorbance measurements at 199 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries purchased from Yongnian Optical Fiber Factory (Hebei, China) were used. The dimensions of the capillary were 60.2 cm × 50 mm i.d. The effective length of the capillary was 50 cm. The temperature of the capillary was kept at 25 ºC. The CE system was interfaced with a computer. 32 karat software (version 7.0) of Beckman was used for data acquisition.

The background electrolyte (BGE) was prepared in methanol and had a concentration of 30 mmol L^{-1} of *tris*-acetic

acid (pH 7.9). The buffer solutions were prepared freshly each day, sonicated for 5 min and filtered through a 0.45 μm membrane filter before use.

Phthalic acid, 2-nitrobenzoic acid, 3-nitrobenzoic acid, 2,5-dinitrobenzoic acid and 2,4-dinitrobenzoic acid were from Aldrich Chemistry Company (Milwakee, USA). Tris acetic acid was from Sigma-Aldrich (St. Louis, MO, USA). 2-(Cyclohexylamino) ethane sulfonic acid (CHES) were from Alfa Aesar (Heysham Lancashire, England). Methanol (HPLC-Grade) was from Tianjin Yongda Chemical Reagent Development Centre(Tianjin China). Glacial acetic acid(G.R.) were from Tianjin Hedong District Hongyan Reagent Factory (Tianjin, China). Acetonitrile was of analytical reagent grade and was from Tianjin Huayue Chemical Reagent Co. (Tianjin, China). Ethyl acetate were of analytical reagent grade and was from Sinopharm Chemical Reagent Co. Water of 18.2 mΩ.cm was treated with a Cascada™ lab water system (Pall Life Science, China).

A stock standard solution of 1 mg mL^{-1} of each analyte was prepared in methanol. A mixed standard solution of the seven analytes was prepared at a concentration of 0.1 mg mL^{-1} in methanol. The working standard solutions were prepared daily by diluting the stock standard solution with methanol. All solutions were stored in dark containers at 4 ºC.

River water, collected from Zhangwei Nan River (Dezhou, China) was filtered though a 0.45 μm membrane syringe filter before analysis. 1 mL of the sample was acidified with 0.1 mL of 1 mol L^{-1} hydrochloric acid, after votex, 0.5 mL of ethyl acetate was added, after shakening, the mixture was centrifuged at 3000 rpm for 3 min. At last, the ethyl acetate layer was removed. The extraction process was repeated three times and the ethyl acetate layer was combined and evaporated to dryness under a N-EVAPTM 111 nitrogen evaporator (Organomation Associates, USA) and the dry residue was solved with 1 mL HPLC grade methanol.

Procedure: Leader (100 mmol L^{-1} ammonium chloride) was introduced into the capillary by hydrodynamic injection at 0.5 psi for 22 s, then the sample was injected electrokinetically by a negative voltage (-10 kV) for 200 s, followed by a small volume of the terminator (10 mM CHES) hydrodynamically injected at 0.5 psi for 32 s. A reverse voltage of -25 kV was applied for both the on-line focusing and the separation of the analytes.

Before use, the capillary was rinsed with 1 mol L^{-1} sodium hydroxide, water, methanol and separation medium for 10 min. Between analysis the capillary was washed with methanol for 2 min and then with the BGE for 4 min. Duplicate injection of the solutions were performed and average peak heights were used for quantification.

RESULTS AND DISCUSSION

Optimization of the separation: Keep the buffer concentration at 30 mmol L^{-1}, the effect of buffer pH on the separation was investigated in the pH 7.1-8.7 range. As shown in Fig. 1a, the migration of the analytes increase with the increase of the buffer pH, which is due to the increased ionization of the analytes. At the same time, separation of the analytes decreased, especially for those between 2-nitrobenzoic acid and 3-nitrobenzoic acid. To keep a compromise between separation and analysis time, pH 7.9 was selected as the optimum.

Keep buffer pH at 7.9, the effects of buffer concentration was investigated in the 10-50 mmol L^{-1} range.The results were shown in Fig. 1b, the migration of the analytes increased with the increase of the buffer concentration, while the resolution decreased with the buffer concentration increase. At the same time, the capillary current increased with the increase of the buffer concentration. The increased capillary current will make the joule heating effects pronounced which will sacrify the detection limits. Keep a compromise between separation, analysis time and detection limit, 30 mmol L^{-1} was adopted. As shown in Fig. 2a, under the optimum conditions, the analytes can be separated in 20 min.

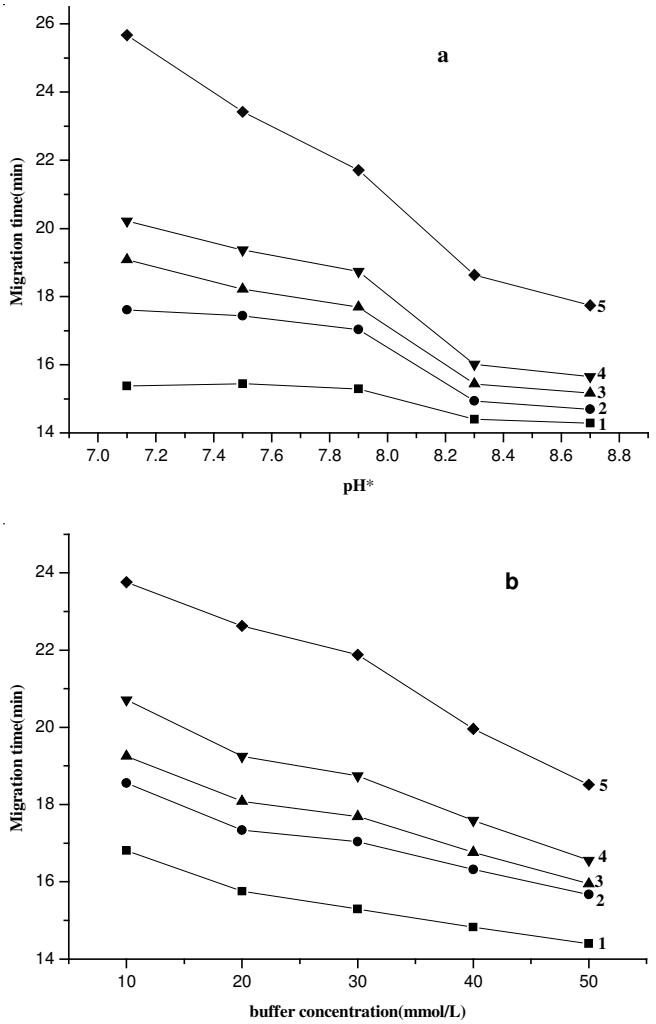

Fig. 1. Effects of pH (a) and buffer concentration (b) on the migration time of the analytes: 1. phthalic acid; 2. 2-nitrobenzoic acid; 3. 3-nitrobenzoic acid; 4. 2,5-dihydroxybenzoic acid; 5. 2,4-dihydroxybenzoic acid. Conditions: 60.2 cm × 50 mm (50.2 cm to detector) fused silica capillary, BGE 30 mmol L^{-1} tris-acetic acid (pH 7.9); voltage, -25 kV; detection was at 199 nm. Sample: hydrodynamic injection of 100 μg mL^{-1} of each acromatic acid for 5 s at 0.5 psi

Sensitivity enhancement by the electrokinetic supercharging system: EKS involves electrokinetically injecting the sample between hydrodynamically introduced leading and terminating ions. When the separation voltage is applied, the

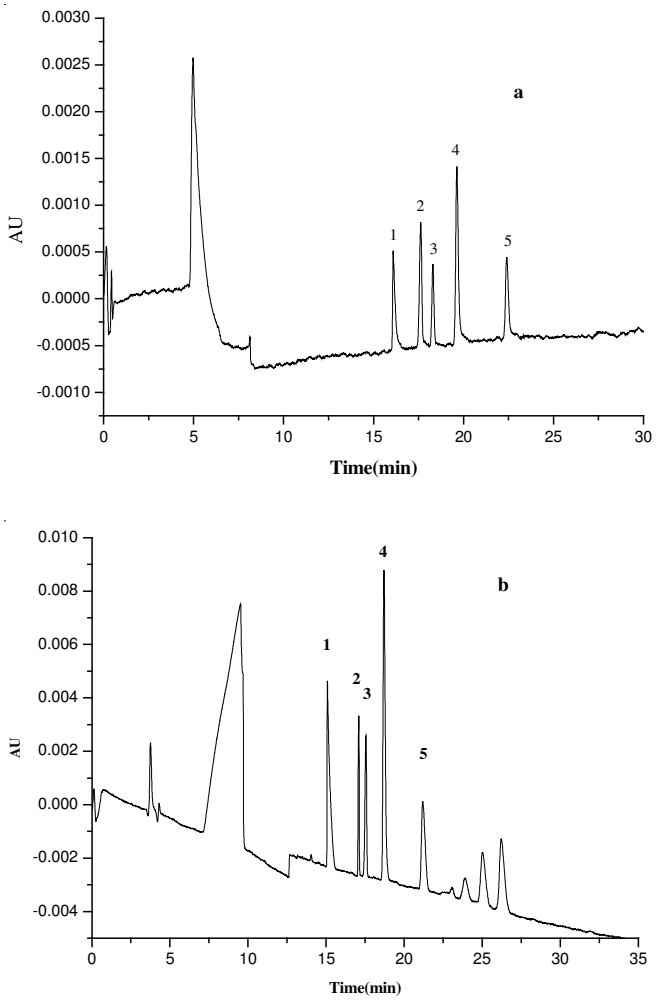

Fig. 2. Electropherograms of normal NACE (10 μg mL^{-1}) (a) and EKS-NACE (10 ng mL^{-1}) (b) conditions: (A) fused silica capillary 60.2 cm × 50 μm id; BGE, 30 mmol L^{-1} *tris*-HoAc (pH 7.9); separation voltage, -25 kV; hydrodynamic injection at 0.5 psi for 5 s; detection, UV at 199 nm. (B) Fused silica capillary 60.2 cm × 50 mm id; BGE, 30 mmol L^{-1} *tris*-AcOH (pH 7.9); separation voltage, -25 kV; hydrodynamic injection of 100 mmol L^{-1} ammonium chloride for 22 s, EKI of sample at -10 kV for 200 s, hydrodynamic injection of 10 mmol L^{-1} CHES at 0.5 psi for 32 s; detection, UV at 199 nm

diffuse band of analytes introduced during electrokinetic injection are restacked between the leader and terminator according to conventional ITP. When the ITP stage destacks, the analytes are separated by conventional CZE. Chloride which has a much larger modibility than the analytes, was used as the leader. 2-(Cyclohexylamino) ethane sulfonic acid has a very low mobility and have been used as the leader. The amount of leader and terminator volume injected into the capillary affects the duration of ITP stacking while the electrokinetic injection time decides the amount of ions injected. Keeping the electrokinetic injection constant at 200 s, the amount of leader and terminator injected into the capillary was varied in 0-5.0 % of the capillary volume. The results showed that when the leader length is shorter than 1.5 % or the terminator length is shorter than 2.3 % of the capillary , the analytes were not fully stacked and peak splitting occurred. So a leader length of 1.9 % and a terminator length of 2.8 % of the capillary was selected. With the selected volume of leader and terminator, the electrokinetic injection time was varied from 80-260 s. As shown in Fig. 3, the peak height increased significantly upto 200 s after which the analytes can't be efficiencty stacked and peak splitting occured. Although this can be solved by increasing the leader and terminator length, which is at the scrifice of the separation. Therefore, 200 s was selected as the optimum electrokinetic injection time.

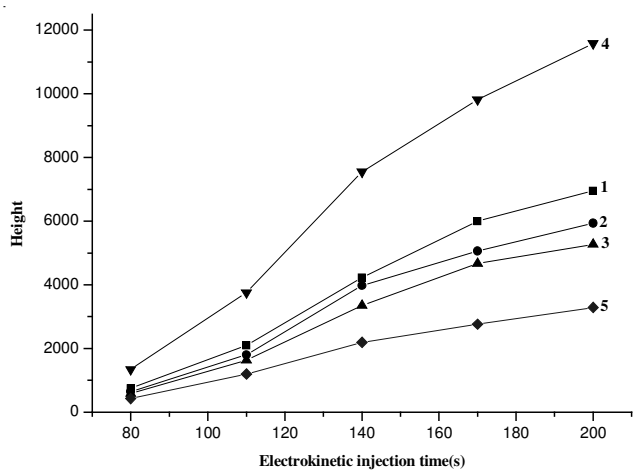

Fig. 3. Effects of electrokinetic injection time on peak height in the EKS system. Sample: hydrodynamic injection of 100 mM NH_4Cl at 0.5 psi for 22 s, EKI of a mixture of 10 ng mL^{-1} of each aromatic acid at -10 kV from 80-200 s hydrodynamic injection of 10 mmol L^{-1} CHES at 0.5 psi for 32 s. All other conditions were the same as Fig. 1.

Analytical performance of electrokinetic supercharging: Under the optimum conditions, an EKS-CZE separation of the 5 analytes is shown in Fig. 3b. The sample-to-sample time was less than 31 min. As shown in Table-1, sensitivity enhancement were from 3868-6480. The relative standard deviation was achieved by five consective injections of a standard mixture, which is also shown in Table-1, were in the range of 0.42-0.93 and 4.7-9.1 % for migration time and peak height respectively. The detection limits and calibration were summerized in Table-2. The detection limits of the five analytes were in the 0.08.0-0.30 ng/mL range, based on three times noise. The calibration graphs were plotted by concentration against peak height and were linear over the range of 0.5-20, 0.5-20, 0.5-20, 0.2-10 and 1.0-40 ng/mL for phthalic acid, 2-nitrobenzoic acid, 3-nitrobenzoic acid, 2,5-dihydroxy benzoic acid and 2,4-dihydroxybenzoic acid, respectively.

Real sample analysis: In chemical industry production, some aromatic acids containing waste water can come into being. If these waste water flow into the river unprecessed, the river water will be polluted and affect the human health. The developed method was further verified by application to real water analysis. Fig. 4 shows results from the direct injection of the extracted river water and spiked with 5 ng mL^{-1} of each of the analytes. No significant peaks were observed in the sample. LLE of spiked water samples with ethyl acetate showed values for recovery of 40.8, 75.5, 47.8, 21.5 and 73.2 % for phthalic acid, 2-nitrobenzoic acid, 3-nitrobenzoic acid, 2,5-dihydroxybenzoic acid and 2,4-dihydroxybenzoic acid, respectively.

TABLE-1
ENHANCEMENT FACTORS (EF) AND REPEATABILITY OF EKS-NACE

Compounds	Normal	EKS		RSD* (%)	
	Height (10 μg mL^{-1})	Height (10 ng mL^{-1})	EF	Time	Height
Phathalic acid				0.91	5.2
2-Nitrobenzoic acid	1323	5936	4487	0.59	7.3
3-Nitrobenzoic acid	860	5273	6131	0.67	6.8
2,5-Dihydroxybenzoic acid	1880	11582	6161	0.75	4.7
2,4-Dihydroxybenzoic acid	850	3288	3868	0.42	9.1

*Based on five determination of the standard mixture of 10 ng mL^{-1}.

TABLE-2
REGRESSION EQUATIONS AND DETECTION LIMITS IN ELECTROKINETIC SUPERCHARGING

Compound	Regression equation*	Correlation coefficient	Linear range (ng mL^{-1})	Detection** limits (ng mL^{-1})
Phathalic acid	Y = 676.82 X + 55.91	0.9998	0.5-20	0.15
2-Nitrobenzoic acid	Y = 570.80X + 58.01	0.9996	0.5-20	0.18
3-Nitrobenzoic acid	Y = 570.70X – 122.31	0.9992	0.5-20	0.18
2,5-Dihydroxybenzoic acid	Y = 1140.89X + 130.89	0.9980	0.2-10	0.08
2,4-Dihydroxybenzoic acid	Y = 324.26X + 42.06	0.9999	1-40	0.30

*In the regression equation, the X value is the concentration of analytes (ng/mL), the y value is the peak height. **Based on three times noise.

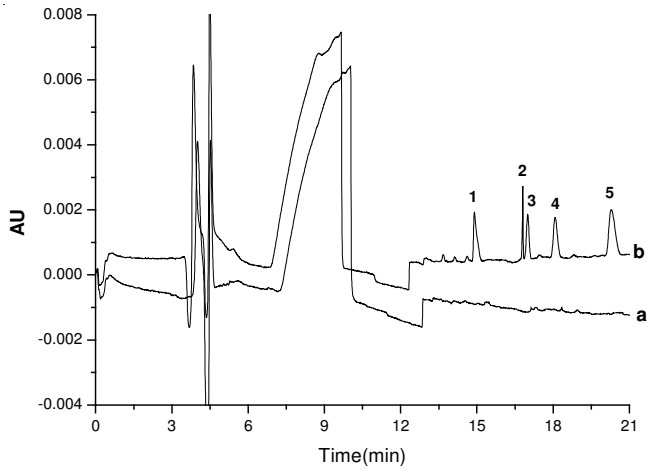

Fig. 4. Electropherogram obtained from EKS-NACE for samples after liquid-liquid extraction (a) blank river water sample after liquid-liquid extraction and (b) river water sample spiked with 5 ng mL^{-1} of the acromatic acids after liquid-liquid extraction. CE conditions is the same as in Fig. 2b

Conclusion

A non-aqueous capillary electrophoresis method with electrokinetic supercharging online preconcentration was developed for the separation of five aromatic acids. Using this method, the enhancment factors ranged from 3,868-6,480. This method was applied to river water sample analysis and recovery experiments were carried out with satisfatory results.

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REFERENCES

1. D.S. Burgi and R.L. Chien, *Anal. Chem*., **63**, 2042 (1991).
2. C.-H. Tsai, C.-C. Tsai, J.-T. Liu and C.-H. Lin, *J. Chromatogr. A*, **1068**, 115 (2005).
3. X. Hou, D. Deng, X. Xu, Y. Lv and J. Zhang, *J. Chromatogr. A*, **1217**, 5622 (2010).
4. Y. He and H.K. Lee, *Anal. Chem*., **71**, 995 (1999).
5. K. Choi, S. J. Kim, Y. G. Jin, Y. O. Jang, J.-S. Kim and D.S. Chung, *J. Chromatogr. A*, **1216**, 6466 (2009).
6. Y.-L. Chen, Y.-J. Jong and S.-M. Wu, *J. Chromatogr. A*, **1119**, 176 (2006).
7. S. Su and Y. Yu, *J. Chromatogr. A*, **1216**, 1490 (2009).
8. X. Wang and Y. Chen, *J. Chromatogr. A*, **1216**, 7324 (2009).
9. Z.K. Shihabi, *Electrophoresis*, **23**, 1612 (2002).
10. M.N. Church, J.D. Spear, R.E. Russo, G.L. Klunder, P.M. Grant and B.D. Anderson, *Anal. Chem*., **70**, 2475 (1998).
11. N. Arroyo-Manzanares, L. Gámiz-Graciá, A.M. Garciá-Campaña, J.J. Soto-Chinchilla and L.E. Garciá-Ayuso, *Electrophoresis*, **31**, 2180 (2010).
12. J.P. Quirino and P.R. Haddad, *Anal. Chem*., **80**, 6824 (2008).
13. B. Kim and D.S. Chung, *Electrophoresis*, **23**, 49 (2002).
14. Y.-H. Lin, J.-F. Chiang, M.-R. Lee, R.-J. Lee, W.-K. Ko and S.-M. Wu, *Electrophoresis*, **29**, 2340 (2008).
15. P. Britz-Mckibbin, K. Otsuka and S. Terabe, *Anal. Chem*., **74**, 3756 (2002).
16. T. Hirokawa, H. Okamoto and B. Gaš, *Electrophoresis*, **24**, 498 (2003).
17. H. Okamoto and T. Hirokawa, *J. Chromatogr. A*, **990**, 335 (2003).
18. J.-M. Busnel, N. Lion and H.H. Girault, *Electrophoresis*, **29**, 1565 (2008).
19. M. Dowod, M.C. Breadmore, R.M. Ruijt and P.R. Haddad, *J. Chromatogr. A*, **1216**, 3380 (2009).
20. M. Urbanek, N. Delaunay, R. Michel, A. Varenne and P. Gareil, *Electrophoresis*, **28**, 3767 (2007).
21. Y. Lu and M.C. Breadmore, *J. Sep. Sci*., **33**, 2140 (2010).
22. I. Botello, F. Borrull, C. Aguilar and M. Calull, *Electrophoresis*, **31**, 2964 (2010).