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Dynamic control and indirect absorption detection for high-speed capillary electrophoretic separation of organic acids

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Abstract

Dynamic control and indirect absorption detection have been combined for the separation of eight small aliphatic organic acids in less than 4 min. Electroosmotic flow (EOF) coefficients in 5 m*M* 8-hydroxyquinoline-5-sulfonic acid (8-HQSA) (pH 3.00) and 3 m*M* 1,2,4,5-benzenetetracarboxylic acid (BTA) solutions are 4.35 and $1.65 \cdot 10^{-4}$ $cm^2/V\,s$, respectively. In the BTA system, relatively large amounts of sodium ions adsorbed into the capillary wall are the most probable reason for the small EOF, in turn causing problems for the separation of all acids. In contrast to BTA, 8-HQSA could be used for the separation of all eight organic acids. Limits of detection of analytes are at the level of several tens of μ*M* at pH 3.00 in the 8-HQSA system. This new technique provides several features such as high speed, reasonable resolution and sensitivity, and ease of operation. © 1998 Elsevier Science B.V.

Keywords: Detection; Electrophoresis; Buffer composition; Organic acids; Benzenetetracarboxylic acid; Hydroxyquinolinesulfonic acid

1. Introduction

Aliphatic organic acids are difficult to separate and detect due to their diverse dissociation constants (K_a) and the lack of suitable detection modes. The development of suitable analytical methods for these compounds is important because of their functions in biological system and their presence in foods, beverages and medicines. For example, the increase in the level of some organic acids, so called acidaemia, can lead to acidotic coma and even to death [1].

Reversal of electroosmotic flow (EOF) by adding cetyltrimethylammonium bromide (CTAB) to the running buffer has been used to separate six aliphatic acids in weaker acidic conditions with conductivity detection [2]. Alternatively, dynamic modification of the electrolyte pH at the inlet of the capillary by steady addition of a modifying electrolyte was useful for better separation of anions [3]. Changes in effective mobilities of analytes by formation of complexes with divalent metal ions have also been employed to improve the separation performance of organic acids [4]. Simply controlling system temperature [5] and performing voltage programming [6] have been applied to generating significant changes in buffer viscosity and pH for better separations of anions. Recently, we developed a simple method using dynamic control to improve the separation performance of organic acids [7]. High-speed and high-resolution capillary electrophoretic separations of organic acids were achieved resulting from high EOF and pH changes in weak acidic conditions.

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A sensitive detection method with wide applicability in CZE is lacking for the separation of aliphatic acids and amino acids. Many different approaches, such as indirect absorption, have been developed to overcome this. Several useful and inexpensive chromophores, including chromate [8] phthalate [9] benzoate [10] naphthalene monosulfonate [11] and 1-naphthylacetic acid [12] have been used for the analyses of anions from diverse samples. However, it is generally not easy to choose suitable chromophores for the analysis of small aliphatic acids with wide ranges of effective mobilities. One easy way to match effective mobilities of analytes for better separation results is the use of binary buffers [12].

In this study, we further investigated dynamic control for the separation of aliphatic acids in indirect absorption detection. The possibility to simultaneously perform indirect absorption detection and dynamic control in CZE was tested with two different chromophores for the separation of eight aliphatic acids. The effects of EOF and chromophores on the separation performance of organic acids were compared. High-speed separation and sensitive indirect absorption detection for acids were also emphasized.

2. Experimental

2.1. Instrument

A commercial electrophoretic instrument from Bio-Rad (BioFocus CE 2000, Hercules, CA, USA) was used. The fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was 35 cm×75 μm I.D. At 30 cm from the injection end, the polyimide coating was burned off to form the detection window. The detection wavelength was set at 290 nm.

2.2. Chemicals

All chemicals were of reagent grade and were obtained from Sigma (St. Louis, MO, USA), except sodium hydroxide, 8-HQSA and BTA which were from Fisher (Fair Lawn, NJ, USA). Buffer solutions were 5 m*M* 8-HQSA or 3 m*M* BTA and slightly adjusted by 0.1 *M* H_3PO_4 or NaOH to pH 3.00. The injected concentration of each analyte was $3\cdot10^{-4}$ *M* or $3\cdot10^{-5}$ *M*.

2.3. Capillary equilibrium and separation

The capillary was pre-equilibrated with 0.1 *M* NaOH for 1 day before use. Between each run, the capillary was equilibrated with 0.1 *M* NaOH via a high pressure (100 p.s.i.; 1 p.s.i.=6894.76 Pa) for 3 min, then the remaining base inside the capillary was washed out with running buffer via a high pressure for 10 s. Analytes were introduced into the capillary by applying a high voltage at 1 kV for 2 s. The separation was performed at 20 kV.

3. Results and discussion

Since the molar absorptivities of the aliphatic organic acids are very small ($\epsilon<10^3$) in low-UV range (around 210 nm), UV measurement is not suitable for the detection of diluted acids. Thus, the use of indirect absorbance for universal and relatively sensitive detection of acids is essential. 8-HQSA has been widely used for sensitive detection of metal ions. Maximal absorption of 8-HQSA is found at 380 nm (ϵ is close to $1.5\cdot10^4$ cm^{-1} mol^{-1} l) in weaker acidic solutions. In addition, good solubility in water and strong acidic characteristics make it a suitable candidate for the analysis of organic acids. In this study, we chose a detection wavelength of 290 nm for two reasons: better base line and the lack of visible light in our system. Fig. 1 shows the separation of eight organic acids in 5 m*M* 8-HQSA at pH 3.00. All eight organic acids were separated in less than 4 min because of the very high EOF and relatively small electrophoretic mobilities (EPM) of the analytes. The migration order agrees with the dissociation constants of eight organic acids shown in Table 1. For citric acid, malic acid and tartaric acid better separation was achieved at pH 3.00 than at pH 3.35 (not shown here) in the 8-HQSA system. On the other hand, worse results were obtained for acetic acid and propionic acid at pH 3.00. In addition to better control of dissociation of acids in weak acidic conditions, the reversed direction of EOF and

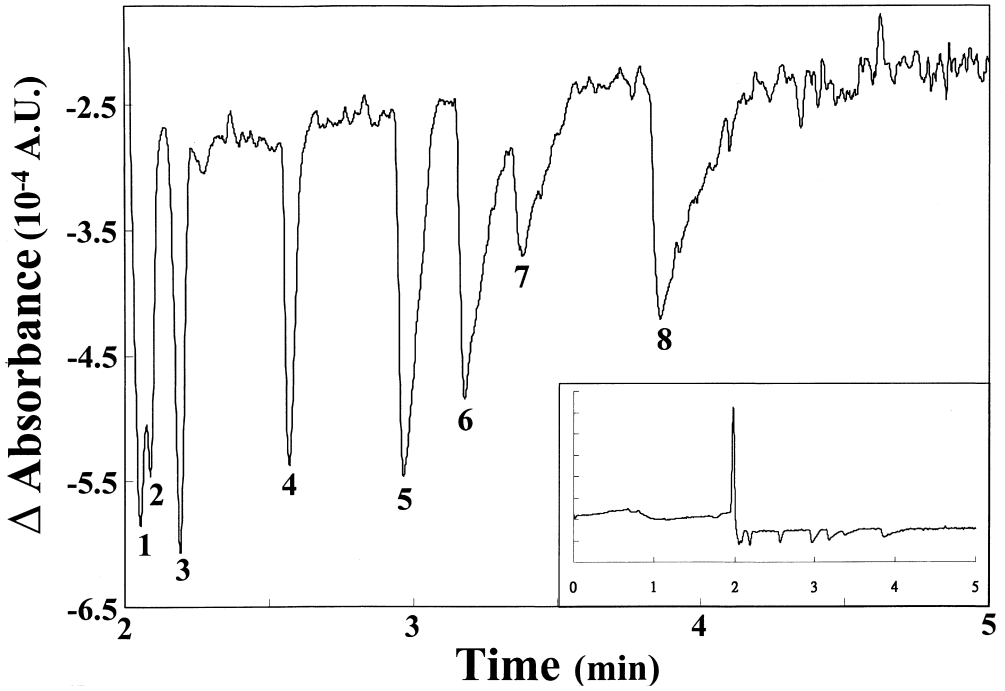

Fig. 1. Separation of eight analytes in 5 m*M* 8-hydroxy-quinoline-5-sulfonic acid (pH 3.00) buffer solution, with indirect absorption detection and dynamic control in CZE. Column: 35 cm (30 cm effective length) 75 μm I.D.×365 μm O.D. Detection wavelength was set at 290 nm. Peak identities: 1, propionic acid; 2, acetic acid; 3, ascorbic acid; 4, lactic acid; 5, formic acid; 6, citric acid; 7, malic acid and 8, tartaric acid. The inset shows the whole scale.

EPM of analytes is also credited for the high resolution.

Another chromophore, 1,2,4,5-benzenetetracarboxylic acid (BTA) was used for the evaluation of the effects of electrolytes on the separation results in our new method. Fig. 2 shows that only four weaker acids could be detected. Table 2 shows the EOF and EPM of analytes obtained from the 8-HQSA and BTA systems. EOF coefficients were 4.35 and

Table 1
Dissociation constants (pK_a) and molecular masses (M_r) of organic acids used in this study

Acid	M_r	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
Propionic acid	74.08	4.87			
Acetic acid	60.05	4.75			
Ascorbic acid	176.13	4.10	11.79		
Formic acid	46.03	3.75			
Malic acid	134.09	3.40	5.11		
Citric acid	192.12	3.14	4.77	6.39	
Lactic acid	90.08	3.08			
Tartaric acid	150.09	2.98	4.34		
BTA	254.15	1.87	2.72	4.30	5.52
8-HQSA[a]	243.24	1.60	8.76		

[a] Protonation constant is 4.10.

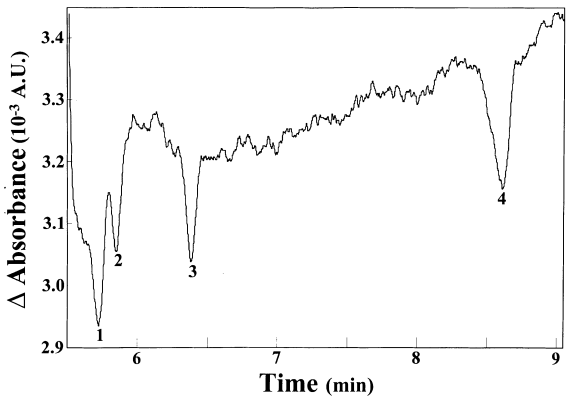

Fig. 2. Separation of eight analytes in 3 m*M* 1,2,4,5-benzenetetracarboxylic acid (pH 3.00) buffer solution with indirect absorption detection and dynamic control in CZE. Conditions are the same as those in Fig. 1.

Table 2
Electrophoretic mobilities (m_{ep}) of analytes in 5 m*M* 8-HQSA and 3 m*M* BTA at pH 3.00 via dynamic control in CZE

Acid	$-m_{ep}$ ($\cdot 10^{-5}$ cm^2/V s)	
	8-HQSA	BTA
Propionic acid	1.63	1.10
Acetic acid	2.31	1.41
Ascorbic acid	4.24	2.71
Lactic acid	10.02	6.33
Formic acid	14.51	
Citric acid	16.41	
Malic acid	18.06	
Tartaric acid	21.27	

EOF coefficients were $4.35 \cdot 10^{-4}$ cm^2/V s in 5 m*M* 8-HQSA and $1.65 \cdot 10^{-4}$ cm^2/V s in 3 m*M* BTA. Positive value means flow is toward cathode end.

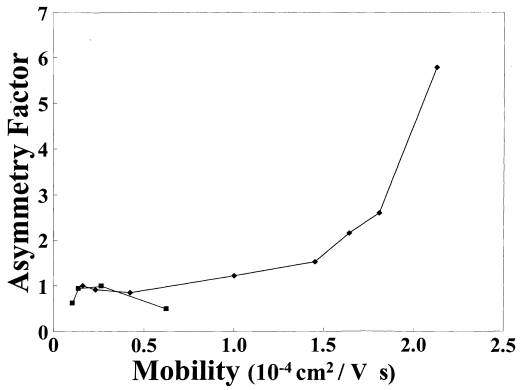

Fig. 3. Asymmetry factors of acids from the results shown in Figs. 1 and 2. Symbol identities: ♦ from Fig. 1 and ■ from Fig. 2.

$1.65 \cdot 10^{-4}$ cm^2/V s in the 8-HQSA and BTA solutions, respectively. One of the possible reasons for the smaller EPM of all analytes obtained in the BTA system is the smaller ζ potential in the BTA system because of the addition of larger amounts of sodium ions [13]. It is surprising that two electrolytes, 8-HQSA (zwitterion) and BTA (polyprotic acid), had such significantly different effects on the change of EOF at pH 3.00. In BTA, adsorption of positively charged sodium ions (≈3 m*M* in solution) into the capillary wall causes the reduction of the ζ potential, in turn decreasing EOF [14]. It agrees with the result shown by Salmon et al. that EOF is proportional to the reciprocal of the square root of the concentrations of NaOH [15]. In addition, competitive adsorption into capillary wall between positively charged species, such as sodium ions and zwitterionic 8-HQSA in solution (pH 3.00) is possible for higher EOF in the 8-HQSA system. The adsorption of 8-HQSA onto the capillary wall through Coulombic attractions by its cationic amine, or through hydrophobic interactions by its heterogeneous ring, may leave its negatively charged sulfonate group outwards the capillary wall. Hence, EOF remains higher. On the other hand, in BTA solution, sodium ions are dominant in the capillary wall because BTA is relatively hydrophilic and carries more negative charges. In other words, it is more difficult for BTA to be adsorbed into capillary wall than 8-HQSA via Coulombic interactions or Van der Waals forces.

8-HQSA is a zwitterion, while BTA is a strong tetraprotic acid. It is predictable that separation performance should be different if analytes with a wide range of mobilities are separated in these two electrolytes. Fig. 3 shows the asymmetry factors (B/A) of analytes in two different electrolytes [16]. Among these eight analytes, B/A ratios of citric acid, malic acid and tartaric acid are higher than 1 at pH 3.00, while for weaker monoprotic acids, the ratios are slightly smaller than 1. The mobility of 8-HQSA at pH 3.00 ranges between those of lactic acid and formic acid from B/A ratios. The result shows that 8-HQSA is a zwitterion at pH 3.00. It is noted that BTA carries more than one negative charge at pH 3.00, its mobility is much higher than those of 8-HQSA and weak acids. The deviations of B/A ratios from 1 are reasonably different from those obtained in 8-HQSA.

Fig. 4 shows the separation efficiencies of the organic acids in 8-HQSA and BTA systems. For weak monoprotic acids, better efficiencies, as high as one hundred twenty thousand theoretical plates (N), were obtained by using BTA. Comparable efficiencies were also available for weak acids in 8-HQSA. Worse efficiencies were observed for polyprotic acids in 8-HQSA system. Since migration times of all analytes are close, the irregular and significant decreases in efficiency are abnormal as migration times increase. The trend of the changes in separation efficiencies relating to migration times for all acids in 8-HQSA can not be predicted from theory. Several possible factors may account for this observation. First, mobilities of analytes and carrier electrolytes are significantly different. This results in

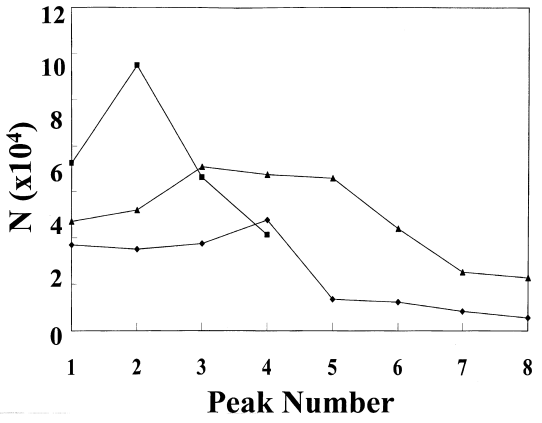

Fig. 4. Efficiencies of acids from the results shown in Figs. 1 and 2,5. Symbol identities as in Fig. 3.

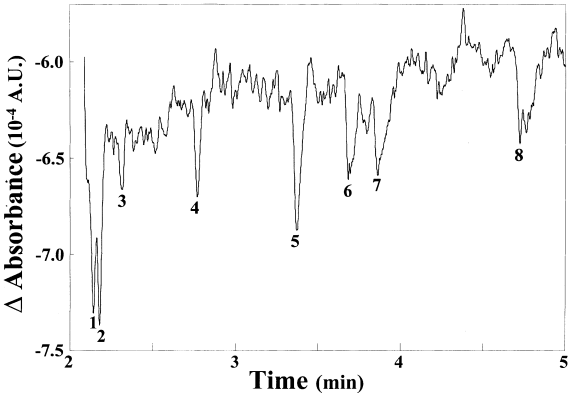

Fig. 5. Separation of eight analytes in 5 m*M* 8-hydroxyquinoline-5-sulfonic acid (pH 3.00) buffer solution with indirect absorption detection and dynamic control in CZE. Conditions as in Fig. 1 except that the concentrations of analytes injected are $3 \cdot 10^{-5}$ *M*.

significant peak tailing and fronting for some analytes. Second, EOF decreases gradually because of dissociation of negatively charged species of capillary wall. At longer migration time, retardation occurs because the EPM of these acids are higher than small local EOF. This causes irregularly large band broadening on strong polyprotic acids in the 8-HQSA system. The significant decreases in efficiencies of ascorbic acid and lactic acid in BTA reflected the existence of dynamic flow. No detection of polyprotic acids in BTA system also supports the existence of dynamic flow. The agreements rule out that the efficiency is dominated by the differences in mobilities. In other words, they strongly support the view that dynamic flow is an important factor on the determination of efficiency in this study. Third, the changes in the composition of the anodic inlet vial resulting from electrolysis [17,18]. Changes in dissociation of acids and electrolytes at higher pH may generate larger differences in mobilities between analytes and carrier electrolytes. The occurrence of a significant increase in band broadening for tartaric acid supports this point. Fourth, disturbance of the peak of propionic acid from system peak is one possible explanation for slightly smaller efficiency of propionic acid than that of acetic acid. Finally, irregular fluctuation in absorbance, possibly, because of dynamic flow and generation of pH gradient, should be considered.

At first glance, the high sensitivity is not predicted by the use of our new dynamic technique, since more serious fluctuation on the signal and shift of the baseline was expected. The other possible problem on sensitivity is low displacement ratio when low pH buffer solution is used. To overcome these shortcomings the use of low concentration of a chromophore with pK_a slightly lower than pH for optimum separation conditions is suggested. It is acceptable in our system since carrier electrolytes with low buffer capacity are better for dynamic control. The merits of the use of carrier electrolyte with very low ionic strength on sensitivity are reflected in the limit of detection ($S/N=3$) for all analytes estimated from Fig. 5 are in several tens of (μM ranges. It is especially sensitive to citric acid because of its charge capacity and similarity of mobility to that of the chromophore.

4. Conclusion

To our knowledge, this is the first paper demonstrated the use of indirect absorption detection and dynamic flow for the analysis of organic acids in weaker acidic conditions. This new technique is very useful for the analysis of small analytes, which have wide ranges of dissociation constants and a lack of strong optical characteristics. Aliphatic organic acids, amino acids, peptides and amines are most suitable candidates to be analyzed by this new technique. Overall, it is important to point out some features of

this new approach, including high speed, reasonable sensitivity, good resolution and ease of operation. High sensitive detection of organic acids by indirect fluorescence detection with this new approach is also on intensive study in our group now.

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