

Wei-Hsi Chen  
Chun-Chi Lin  
Tse-Shien Chen  
Tarun K. Misra  
Chuen-Ying Liu

Departments of Chemistry,  
National Taiwan University,  
Taipei, Taiwan

## Capillary electrochromatographic analysis of aliphatic mono- and polycarboxylic acids

The parameters influencing the electrochromatographic separation of aliphatic organic acids in a capillary column with a wall-coated macrocyclic polyamine have been studied. Indirect detection using chromate, pyromellitate, trimellitate, *o*-phthalate, benzoate and acetate as background electrolytes has been tested. A complete separation of polyprotic acids could be achieved with pyromellitate buffer (7.5 mM, pH 6.5), and satisfactory results for the simultaneous separation of monoprotic acids and polyprotic acids were found using a capillary column of 70 cm (50 cm effective length)  $\times$  75  $\mu$ m inner diameter, electrokinetic injection ( $-10$  kV, 10 s), benzoate buffer (6 mM, pH 4.6), separation voltage of  $-10$  kV, and detection at 220 nm. For the separation of the geometric isomers fumarate and maleate, acetate buffer was found the best choice among the background electrolytes tested. The method so established has been applied to the determination of organic acids in soy sauce, brandy, lemon juice, spinach juice and cigarette. From the retention behavior, it was found that the separation mechanism on the bonded phase was influenced by the macrocyclic effect, electrostatic attraction, hydrogen bonding, van der Waals forces, and anion exchange, in addition to the differences in electrophoretic mobility.

**Keywords:** Aliphatic carboxylic acids / Capillary electrochromatography / Macrocyclic polyamine / Stationary phase  
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### 1 Introduction

Low molecular weight carboxylic acids in aqueous solutions are important in environmental samples, fermented juice, and biological fluids [1]. Chromatographic methods such as high-performance liquid chromatography (HPLC) [2], ion chromatography (IC) [3], gas chromatography (GC) [4, 5], and ion-exclusion chromatography [6–8] have been used for the determination of organic acids. Among them, HPLC and IC are the most popular methods, since GC analysis requires additional derivatization procedures to enhance sample volatility. The recent advances of capillary electrophoresis (CE) provide a more rapid, economic, and highly efficient separation method that can solve the matrix interference problem as experienced in IC and HPLC separation. The analysis of carboxylic acids in foods and beverages has been described in several reports [9–13]. Other methods such as capillary isotachopheresis (CITP) and micellar electrokinetic chromatography (MEKC) [14] as well as ligand exchange CE have also been reported [15].

Capillary electrochromatography (CEC) is a technique that combines the advantages of capillary zone electrophoresis with those of HPLC. One of the attractive features of CEC is the possibility to vary the selectivity of the chromatographic system by varying the stationary phase. In CEC, the stationary phase not only provides interaction sites for the solutes but also plays the dominant role in the generation of the electroosmotic flow [16].

The binding of anions by organic ligands has been much less investigated than cation complexation, although a multitude of new structures and properties may be expected in view of the role played by anionic species in chemical as well as in biological processes [17]. In a previous paper, we reported the preparation of macrocyclic polyamine-bonded phases for the electrophoretic separation of organic and inorganic anions [18, 19]. Based on the multiple modes separation mechanism, a number of analytes, including aromatic organic acids [20], metal ion speciation [21] and nucleotides [22] could be well separated. This paper presents an experimental study aimed to provide a better understanding of the parameters influencing the separation efficiency of aliphatic organic acids in this bonded phase so that the full potential of the prepared column can be realized.

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**Correspondence:** Professor Chuen-Ying Liu, Department of Chemistry, National Taiwan University, 1, Sec. 4, Roosevelt Road, Taipei, 10617, Taiwan  
**E-mail:** cylieu@ccms.ntu.edu.tw  
**Fax:** +886-2-23638543

## 2 Materials and methods

### 2.1 Apparatus

A high-voltage power supply with a 30 kV capacity (Model 890-CE; Jasco, Tokyo, Japan), a variable-wavelength UV/Vis detector (Jasco 870-CE), and an integrator (Jasco 807-IT) were employed for capillary electrophoresis. The separations were carried out on a fused-silica capillary column with an external coating of polyimide (J&W Scientific, Folsom, CA, USA) and chemically modified with a 28-membered macrocyclic ligand containing oxygen and nitrogen as donor atoms. The modified capillaries were of 75  $\mu\text{m}$  ID and the total length of the capillary was 70 cm with a distance of 50 cm between the injection end and the detection window. Samples were injected electrokinetically from the negative end.

### 2.2 Reagents and chemicals

Most chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). Purified water (18 M $\Omega\text{cm}$ ) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions.  $\gamma$ -Glycidoxypropyltrimethoxysilane was obtained from Aldrich (Milwaukee, WI, USA). All liquid reagents and solvents used in moisture-sensitive reactions were distilled and collected over type 4  $\text{\AA}$  molecular sieves. Acetic acid, ascorbic acid, citric acid, formic acid, fumaric acid, lactic acid, maleic acid, malic acid, malonic acid, sodium acetate, sodium oxalate, succinic acid, tartaric acid, potassium chromate, benzene-1,2,4,5-tetracarboxylic acid (pyromellitate), benzene-1,2,4-tricarboxylic acid (trimellitate), potassium *o*-phthalate, benzoic acid, and potassium hydroxide were purchased from Merck. Benzyl alcohol was obtained from Wako (Tokyo, Japan). Soft drink, brandy and soy sauce were obtained from a supermarket. All solvents and solutions for CEC analysis were filtered through a 0.45  $\mu\text{m}$  PTFE membrane (Millipore).

### 2.3 Column preparation

The capillary column was prepared according to the methods described by Liu *et al.* [19]. Fused-silica capillaries (75  $\mu\text{m}$  ID, 70 cm long) were first flushed with 1 M NaOH (30 min), then pure water (15 min), 1 M HCl (30 min), and pure water (15 min) sequentially. The capillaries were purged with nitrogen for 20 min, then dried at 110°C overnight. For coating the capillary was filled with a 10% w/v solution of  $\gamma$ -glycidoxypropyltrimethoxysilane in toluene.

The capillary was kept for 3 h at 110°C for silylation. After purging with toluene for several minutes to remove unreacted reagent, the capillaries were dried in a vacuum oven. The capillary was then filled with a 1% w/v solution of the macrocyclic compound, 4, 8, 12, 18, 22, 26-hexaaza-1,15-dioxacyclooctaeicosane, [28]ane-N<sub>6</sub>O<sub>2</sub>·6HCl in *N,N*-dimethylformamide. After standing for 10 h at 120°C for functionalization, the dried capillaries were purged with methanol and pure water for several minutes before equilibration with buffer solution. They were then ready for use.

## 3 Results and discussion

Macrocyclic polyammonium molecules have been shown to complex strongly and selectively with a variety of inorganic and organic anions thus laying the basis for the developing field of anion-coordination chemistry. In this work, the prepared macrocyclic polyamine-bonded column was evaluated for the separation of aliphatic carboxylic acids. The electroosmotic flow (EOF) velocity of the bonded phase was determined, according to the migration time of mesityl oxide. For the bare fused-silica capillary the EOF rate increases with pH. However, for the bonded capillary the EOF is reversed and the migration velocity increases as the pH decreases. This has been clearly explained and reported earlier [19].

### 3.1 Composition of the BGE

As most of the aliphatic organic acids exhibit little or no UV absorption, indirect UV detection is normally carried out using BGEs containing chromophores, such as chromate [23–25], phthalate [26, 27], benzoate [28], 2,6-pyridine dicarboxylic acid [29], trimellitate [30], pyromellitate [31] and naphthalene sulfonate [32]. For the separation of oxalate, citrate and tartrate, potassium chromate (pH 8.1, 5 mm) was tested as BGE. With the applied voltage of –20 kV, good separation but with peak broadening for these anions was found. Under similar conditions in untreated capillaries, no peak was found within 40 min. If the polarity of the applied voltage was changed, namely to +20 kV, only one broadened peak with a migration time of 4.5 min was observed. The contribution of the immobilized macrocyclic polyamine to the separation of acidic compounds was obvious.

More analytes including oxalate, citrate, tartrate, malonate, malate, succinate, fumarate and maleate were further separated under this condition. Unfortunately, only oxalate could be separated from the other ana-

**Table 1.** Chemical and physical properties of the model compounds

Organic acid	pK <sub>a</sub> <sup>a)</sup>	Equiv. conductivity (S·cm <sup>2</sup> equiv. <sup>-1</sup> ) <sup>b)</sup>	Effective charge at pH 4.6
Acetic (60) <sup>c)</sup>	4.74	40.9	−0.420
Citric (192.1)	3.13, 4.76, 6.40	70.2	−1.375
Formic (46.0)	3.74	54.6	−0.878
Fumaric (116.1)	3.03, 4.54	61.8	−1.555
Lactic (90.1)	3.86	38.8	−0.846
Maleic (116.1)	1.91, 6.33	61.9	−1.020
Malic (134.1)	3.46, 5.10	58.8	−1.175
Malonic (104.1)	2.84, 5.70	63.5	−1.056
Oxalic (90.0)	1.27, 4.28	74.2	−1.678
Propanoic (74.1)	4.87	35.8	−0.349
Succinic (118.1)	4.20, 5.64	58.8	−0.793
Tartaric (167.1)	2.96, 4.24	64	−1.697
Chromate (115.9) <sup>d)</sup>	−0.2, 6.51	85	
Pyromellitate (254.2) <sup>d)</sup>	1.70, 3.12, 4.92, 6.23	68.1; 38.8; 40.9; 35.8	
Trimellitate (210.1) <sup>d)</sup>	2.48, 4.04, 5.54	62.7	
o-Phthalate (166.1) <sup>d)</sup>	2.95, 5.40	52.3	
Benzoate (122.1) <sup>d)</sup>	4.20	32.4	−0.715

a) [38]

b) [33]

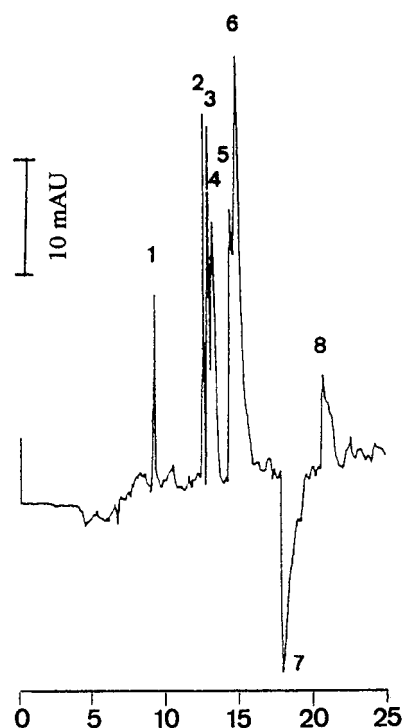
c) Molecular mass

d) BGE

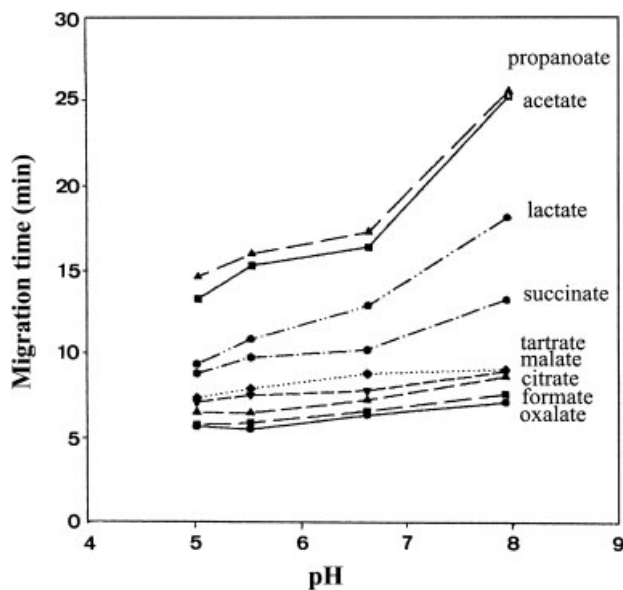
lytes well even by changing the pH over the range from 6.5 to 10.5. The reason might be the background conductivity does not match the analyte conductivity (Table 1). At pH < 6, chromate will convert to the greater extinction coefficient species of dichromate, which is not advantageous for detection. Therefore, no condition of lower pH was tested. Ionic equivalent conductivity is directly related to the electrophoretic mobilities [33]. For improvement of the separation electrolytes with more identical conductivity were investigated.

Trimellitate was first selected as the BGE. As expected, a better efficiency was demonstrated although some peaks were not baseline-resolved and the geometric isomers fumarate and maleate were coeluted (Fig. 1). Here, a negative peak due to the UV absorption of the double bond in the isomer was found. When the pH was increased from 5 to 8, analytes being more ionizable would lead to faster migration. However, slower migration for the analytes and the mobility difference increase with increasing pH were indicated for some of the analytes (Fig. 2). Here, less protonated macrocyclic polyamine would result in a smaller EOF. The other can be explained by the fact that stronger binding occurs usually with the greater charge of the analyte, since electrostatic charge-charge interactions play a dominant role in binding anions on the bonded column.

When pyromellitate (2 mM, pH 7.0) was used instead, six broadened peaks with only one sharp peak (oxalate) for seven compounds injected were indicated (Fig. 3a).



**Figure 1.** Electropherogram of aliphatic acids with trimellitate as BGE. Column: [28]ane-N<sub>6</sub>O<sub>2</sub> bonded phase fused-silica capillary, 70 cm (50 cm to the detector) × 75 μm ID; electrokinetic injection (−10 kV, 10 s); sample concentration, 50 μM for each; trimellitate (5 mM, pH 5.7); separation voltage, −15 kV; detection, 250 nm. Peaks: 1, oxalate; 2, formate; 3, malonate; 4, citrate; 5, malate; 6, tartrate; 7, fumarate and maleate; 8, succinate.

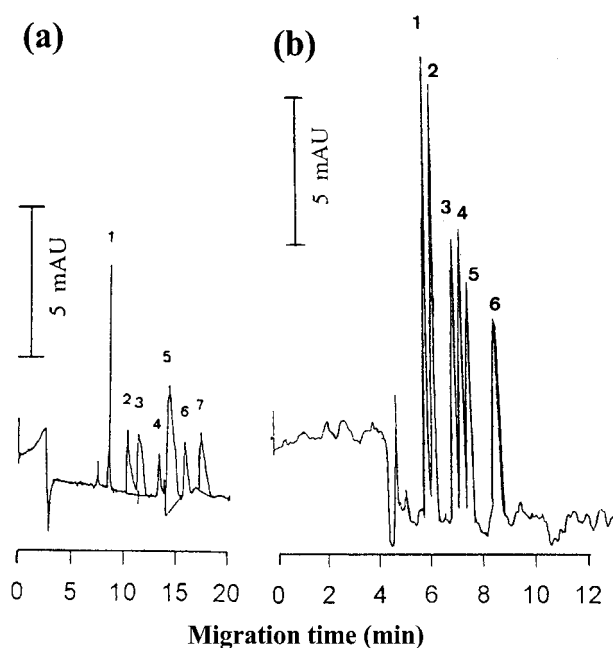


**Figure 2.** Migration time as a function of pH with trimellitate as BGE. Conditions as Fig. 1, except pH.

Increasing the buffer concentration to 7.5 mM a better separation was observed (Fig. 3b). Although a delicate decrease of pH would lead to a slight increase of EOF, increasing the buffer concentration from 2 to 7.5 mM would cause a significant decrease in the EOF. In this case, the opposite seems not to be true. From a more detailed comparison of the two electropherograms, a faster migration and greater intensity for the analytes in latter situation were demonstrated. One possible explanation might be due to the competitive coordination of the BGE and the analytes with the bonded group. Both BGE and analytes have carboxyl groups, and a greater affinity toward the bonded group would be for the BGE in a higher concentration. This made a greater replacement.

The number of theoretical plates calculated is around 40 000–70 000 plates per meter. At this condition, it seems that the mobility of the pyromellitate is more accessible to the analytes. Moreover, attempts to separate the mixture of poly- and monoprotic carboxylic acids were unsuccessful, even by changing the pH to adjust the mobility of pyromellitate to match that of the analytes. With respect to the coordination property of the macrocyclic polyamine, Dietrich *et al.* [17] have reported that no complex formation for the singly charged anions like acetate occurred. Pyromellitate has four carboxyl groups. Much stronger affinity of the BGE to the bonded phase than the analyte may provide the answer.

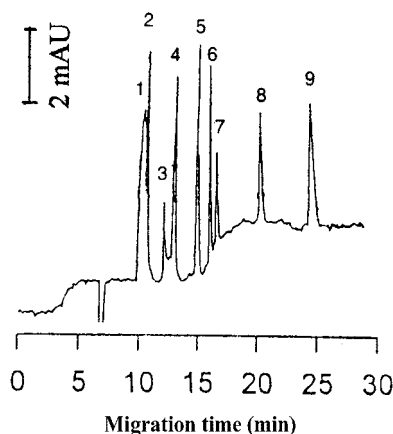
*o*-Phthalate as well as benzoate which has less carboxyl groups with smaller conductivity (Table 1) was therefore tested for suitability. *o*-Phthalate still can not reflect the



**Figure 3.** Electropherogram of aliphatic acids with pyromellitate as BGE. Column and electrokinetic injection as in Fig. 1; sample concentration, 20  $\mu$ M for each; separation voltage,  $-20$  kV; detection, 250 nm. (a) Pyromellitate (2 mM, pH 7.0); (b) pyromellitate (7.5 mM, pH 6.5). Peaks: (a) 1, oxalate; 2, formate; 3, malonate; 4, citrate; 5, malate; 6, tartrate; 7, succinate. (b) 1, Oxalate; 2, malonate; 3, citrate; 4, malate; 5, tartrate; 6, succinate.

advantages for the separation of the mixture of polyprotic- and monoprotic acids. Meanwhile, significant longer migration times than those with pyromellitate buffer for the slower eluted tartrate and succinate was indicated. However, significant improvement in resolution for the mixture of poly- and monoprotic acids with benzoate as BGE was found. An even more significant effect on the selectivities for the resolution of oxalate and formate, as well as tartrate and succinate can be seen by altering the applied voltage from  $-20$  kV to  $-10$  kV, while maintaining the benzoate concentration of 6 mM and pH at 4.6.

A comparison of the separation data showed that the applied voltage of  $-10$  kV was the best. At this condition, good separation of the mixture including tri-, di- and monoprotic carboxylic acids, except the pair of oxalate and formate was indicated (Fig. 4). The migration order is oxalate (earliest) > formate > malonate > citrate > malate > tartrate > succinate > lactate > acetate. In the work of Shamsi and Danielson [32], the elution order was oxalate (earliest) > malonate > formate > fumarate > maleate > succinate > malate > citrate > tartrate. They used naphthalene sulfonates (4 mM) in  $H_3BO_3$  (100 mM)/



**Figure 4.** Electropherogram of aliphatic acids with benzoate as BGE. Column and electrokinetic injection as in Fig. 1; sample concentration, 20  $\mu\text{M}$  for each; benzoate (6 mM, pH 4.6); separation voltage,  $-10$  kV; detection, 220 nm. Peaks: 1, oxalate; 2, formate; 3, malonate; 4, citrate; 5, malate; 6, tartrate; 7, succinate; 8, lactate; 9, acetate.

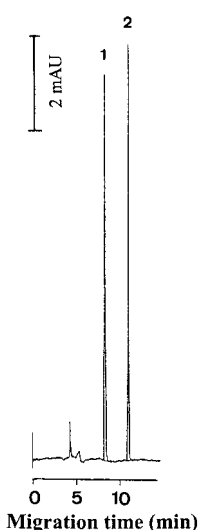
$\text{Na}_2\text{B}_4\text{O}_7$  (5 mM)/diethylenetriamine (2 mM), pH 8 buffer as BGE. All analytes were fully ionized and electrophoretic separation was the major separation mechanism in their work. While the separation may be more complex in our case, not only electrophoretic separation but also anion coordination and even anion exchange might play important roles for the separation on the macrocyclic polyamine-bonded column. As a result of this, a longer chain length between the neighboring carboxyl groups would stabilize the anion-polyamine complex as it reduces the steric hindrance and increases the hydrophobic force for the complexation. Hence, oxalate is eluted first, malonate next, and succinate is last.

Malate, tartrate, and citrate are hydroxy acids. In general, they might have more hydrogen bonding interaction than the above-mentioned compounds with the bonded group. Moreover, compounds with too many OH groups between the neighboring carboxyl groups would not favor the complexation. Therefore, tartrate was eluted earlier than succinate. As suggested by Yatsunami *et al.* [34], only two end carboxylate groups of citric acid could coordinate with the macrocyclic polyamine. In other words, the third carboxyl group between them would hinder the complexation, so citrate migrated faster than malate and tartrate. There is little affinity and probably only anion exchange for the singly charged carboxylate anion with the protonated bonded group. Formate has the smallest mass and greatest equivalent conductance among the monocarboxylates (Table 1). Lactate has a bulkier group than acetate. Therefore, the elution order is formate > lactate > acetate.

### 3.2 The separation of geometric isomers

As mentioned in the section above, fumarate and maleate were coeluted with trimellitate buffer. Meanwhile, a poor sensitivity was obtained with indirect detection due to the high extinction coefficient of the analytes. Fumarate has also been reported that it was not detected using benzoate as the indirect photometric detection electrolyte for CE [35]. That is why we use acetate buffer (20 mM, pH 4.5) as BGE with applied voltage of  $-20$  kV and direct UV detection at 218 nm. This makes possible the separation of fumarate and maleate with almost identical equivalent conductance (Fig. 5). However, the effective charge of fumarate is much larger than that of maleate at pH 4.6 (Table 1). The separation should be mainly based on the difference in electrophoretic mobility. However, it is believed more or less that maleate would have greater interaction with the bonded phase than fumarate. Since it has two carboxyl groups on the same side of the double bond, a slower migration would be expected. In this system a rather low detection limit, 5–10 nM and a wider linear range, 0.05–2  $\mu\text{M}$  were indicated. By comparison with Hsu's work [18], a more satisfactory result was obtained. It was also clear that 28ane- $\text{N}_6\text{O}_2$  had a greater affinity and higher selectivity than 24ane- $\text{N}_6\text{O}_2$  for the polycarboxylate coordination.

Table 2 gives the analytical performance of the bonded phase under optimized conditions for the separation of a standard mixture containing seven carboxylic acids. The calibration plots for all test solutes were linear in the concentration range of 0.5–30  $\mu\text{M}$  with correlation coefficients better than 0.9956. Detection limits ( $S/N = 3$ ) ranging from 0.1 to 0.4  $\mu\text{M}$  were achieved. A good detection limit was shown by the comparison with those of Wu *et al.* [36], Soga and Ross [37] as well as Shamsi and Danielson [32] (Table 2).



**Figure 5.** Separation of geometric isomers. Column and electrokinetic injection as in Fig. 1; sample concentration, 1  $\mu\text{M}$  for each; acetate (20 mM, pH 4.5); separation voltage,  $-20$  kV; detection, 218 nm. Peaks: 1, fumarate; 2, maleate.



**Table 2.** Analytical performance for the separation of aliphatic acids<sup>a)</sup>

Analyte	Migration time (min)	Separation efficiency		Linear equation			Detection limit			
	$t_m \pm SD$ (RSD%)	$N$ ( $m^{-1}$ ) <sup>c)</sup>	$R_s$ <sup>d)</sup>	Slope	Intercept	$R^2$	This work $\mu M$ (ppb)	Wu [36] ( $\mu M$ )	Soga [37] (ppm)	Shamsi [32] (ppb)
Oxalate	6.9 $\pm$ 0.3 (4.3)	14 300	–	2 733	4428	0.9978	0.1 (9)	~2	1.8	40
Formate	7.1 $\pm$ 0.3 (4.1)	43 250	0.78	1 281	1303	0.9963	0.4 (16)	–	1.0	20
Citrate	8.7 $\pm$ 0.4 (4.6)	49 200	3.55	3 015	2486	0.9978	0.1 (20)	–	2.2	45
Malate	10.3 $\pm$ 0.5 (4.5)	48 400	3.10	2 326	2088	0.9956	0.1 (14)	–	–	40
Tartrate	11.0 $\pm$ 0.5 (4.6)	49 000	2.50	1 985	3525	0.9977	0.1 (17)	–	2.0	45
Succinate	11.3 $\pm$ 0.5 (4.9)	48 500	1.26	1 660	988	0.9989	0.2 (24)	–	1.2	50
Actate	14.0 $\pm$ 0.7 (5.0)	26 900	7.81	1 180	2180	0.9968	0.4 (24)	–	0.9	–
Fumarate <sup>b)</sup>	8.5 $\pm$ 0.2 (1.9)	45 100	–	30 231	1561	0.9970	0.005 (0.6)	–	–	30
Maleate <sup>b)</sup>	11.0 $\pm$ 0.3 (2.7)	44 300	9.67	23 340	2086	0.9938	0.01 (1.2)	–	–	35

a) Confidence level, 95% ( $n = 3$ ); electrokinetic injection,  $-10$  kV, 10 s; benzoate buffer (6 mM, pH 4.6); applied voltage,  $-15$  kV; indirect detection at 220 nm

b) Acetate buffer (20 mM, pH 4.5); applied voltage,  $-20$  kV; detection at 218 nm

c)  $N = 5.54 (t_m/w_{1/2})^2$

d)  $R_s = 2(t_{m2} - t_{m1})/(w_1 + w_2)$

### 3.3 Analysis of organic acids in real samples

The potential of the discussed method for manipulating peak selectivity during analysis of aliphatic acids in complex matrices such as foods and beverages was demonstrated. Figure 6 shows the electropherogram obtained from soy sauce and brandy. Due to the dark color of the soy sauce, 1/50 000-fold dilution of the sample was injected without filtration. Spiking with known standards and migration time identified peaks in real samples. The applied voltage over the range of  $-20$  kV to  $-10$  kV could be used. Since the ionic radius of the chloride ion is smaller than the radii of organic anions, a lower affinity toward the bonded group would expect. Another fact is that the conductivity of chloride ion ( $110 \text{ S}\cdot\text{cm}^2 \text{ equiv.}^{-1}$ ) is greater than that of any other analytes shown in Table 1. All of these led to the earliest elution of the chloride ion. The proposed method demonstrated that simultaneous determination of inorganic and organic anions in a single run is possible (Fig. 6). Seven acids and three acids were present in soy sauce and brandy, respectively. The quantitative results were summarized as in Table 3.

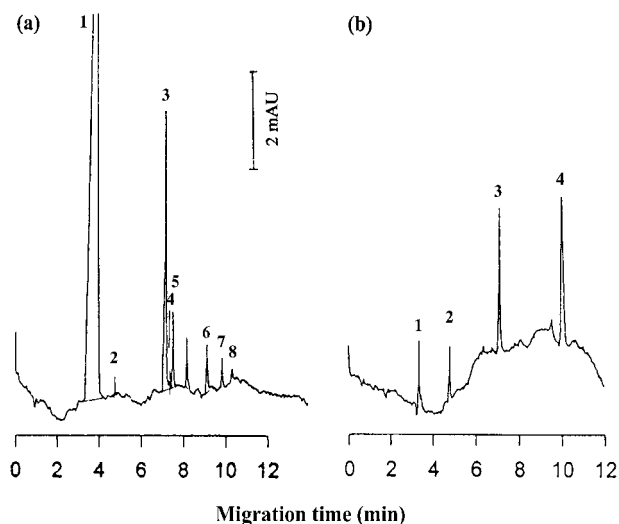
The organic anions were also identified in lemon juice. Citrate, succinate, lactate, and ascorbate were detected at 220 nm. Only a small peak of ascorbate was shown. While 245 nm as analytical wavelength was used instead, a large positive absorbance peak was observed (Fig. 7). Figure 8 shows the separation of acids in spinach juice. Oxalate is evident in the electropherogram. Also evident

**Table 3.** Results for the determination of aliphatic acids in soy sauce and brandy<sup>a)</sup>

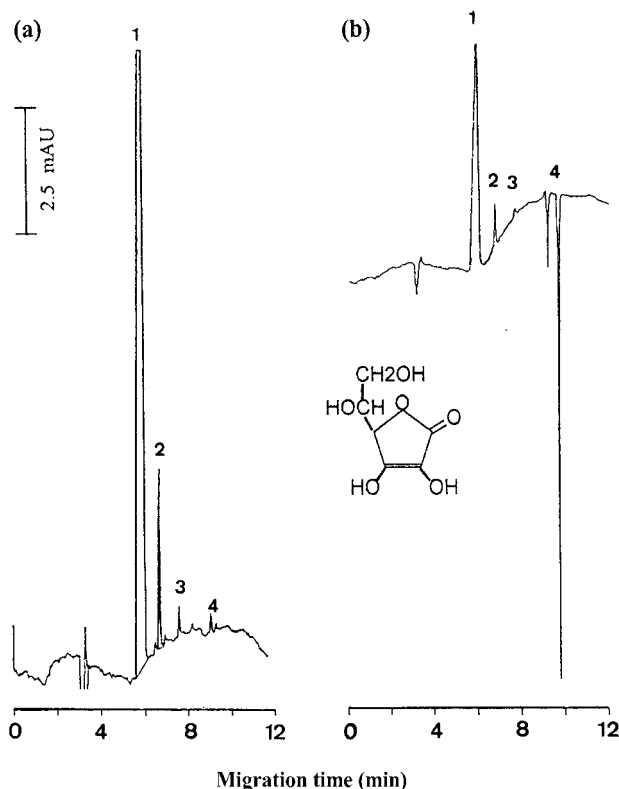
Analyte	Sample ( $\mu M$ )	
	Soy sauce	Richard Hennessy X.O. brandy
Formate	–	1.7 $\pm$ 0.2
Acetate	9.6 $\pm$ 0.3	9.9 $\pm$ 0.2
Lactate	34.5 $\pm$ 0.7	–
Malate	83 $\pm$ 2	–
Tartrate	15 $\pm$ 0.5	5.1 $\pm$ 0.3

a) Separation conditions as in Fig. 6. Equations of the graphical standard addition method for soy sauce: malate,  $y = 1877x + 15706$  ( $R^2 = 0.9965$ ); tartrate,  $y = 1590x + 2465$  ( $R^2 = 0.9981$ ); lactate,  $y = 588x + 2026$  ( $R^2 = 0.9887$ ); acetate,  $y = 1135x + 1030$  ( $R^2 = 0.9964$ ). Brandy: formate,  $y = 1504x + 2570$  ( $R^2 = 0.9987$ ); tartrate,  $y = 1630x + 8343$  ( $R^2 = 0.9946$ ); acetate,  $y = 1284x + 12746$  ( $R^2 = 0.9981$ ), where  $y$  is the peak area ( $\mu V\cdot s$ ) and  $x$  is the spiked concentration ( $\mu M$ ). Confidence level, 95%; data obtained from three measurements

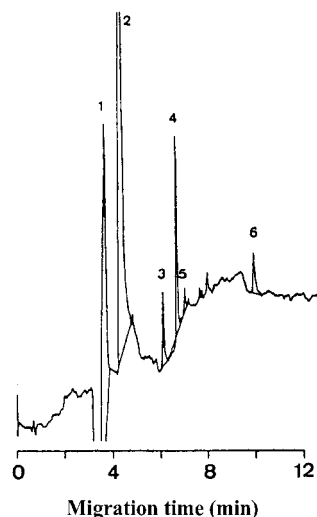
is the huge response for chloride and malate. A shag of cigarette was immersed in an ethanol-water mixture (1:3 v/v) for 17 h. Then the filtrate was diluted 2000-fold with pure water. The electropherogram indicated that chloride, formate, citrate, malate, tartrate, succinate, lactate, and acetate were present in the cigarette (Fig. 9).



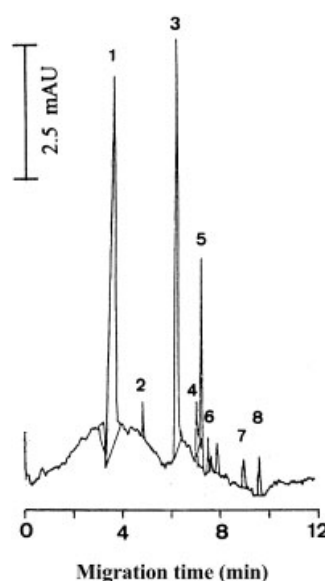
**Figure 6.** Electropherograms of organic acids in (a) soy sauce (1/50 000-fold dilution) (b) Richard Hennessy X.O. brandy (1/2000-fold dilution). Column and electrokinetic injection as in Fig. 1; benzoate (6 mM, pH 4.6); separation voltage;  $-20$  kV; detection, 220 nm. Peaks: (a) 1, chloride; 2, formate; 3, malate; 4, tartrate; 5, succinate; 6, lactate; 7, acetate; 8, propanoate. (b) 1, Chloride; 2, formate; 3, tartrate; 4, acetate.



**Figure 7.** Electropherograms of organic acids in lemon juice. Conditions as in Fig. 6, except sample: 1/10 000-fold dilution and detection at (a) 220 nm. (b) 245 nm. Peaks: 1, citrate; 2, succinate; 3, lactate; 4, ascorbate.



**Figure 8.** Electropherograms of organic acids in spinach juice. Conditions as in Fig. 6. Peaks: 1, chloride; 2, oxalate; 3, citrate; 4, malate; 5, succinate; 6, acetate.



**Figure 9.** Electropherograms of organic acids in cigarettes. Conditions as in Fig. 6; sample, ethanol-water extract of shag and 1/2000-fold dilution. Peaks: 1, chloride; 2, formate; 3, citrate; 4, malate; 5, tartrate; 6, succinate; 7, lactate; 8, acetate.

#### 4 Concluding remarks

For the separation of aliphatic acids in CE or CEC, EOF modifier and UV absorber were needed in addition to the BGE. In this work, no modifier was needed for the EOF reversal, therefore, a rather low detection limit (Table 2) could be obtained. Polycarboxylate recognition on this home-made macrocyclic polyamine-bonded phase is probably the sum of the total interactions arising from the macrocyclic effect, electrostatic attraction, hydrogen bonding, van der Waals forces and anion exchange, in addition to the electrophoresis. To establish the optimum conditions for a complete separation of the poly- and monocarboxylates, benzoate is the most suitable and highly effective electrolyte for the indirect detection. Applying the proposed method to the determination of

aliphatic acids in foods and beverages, no significant interference from sample matrices was shown. The citric acid cycle (Krebs cycle) is central to energy-yielding metabolism. Four- and five-carbon intermediates of the cycle serve as biosynthetic precursors for a wide variety of products. Therefore, we predict that the established method would be suitable to determine the organic acids in other fermented products or biological materials. Additionally, the bonded phase was found to be excellent for the determination of the geometric isomers fumaric acid and maleic acid, but only with acetate buffer as BGE.

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