ELSEVIER

Journal of Chromatography A, 897 (2000) 383–392

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Preparation and evaluation of an imidazole-coated capillary column for the electrophoretic separation of aromatic acids

Chuen-Ying Liu*, Yu-Wan Ho, Yi-Fen Pai

Department of Chemistry, National Taiwan University, Taipei, Taiwan

Received 18 April 2000; received in revised form 13 July 2000; accepted 8 August 2000

Abstract

An imidazole-coated capillary column for electrophoresis has been prepared by means of organosilanization. With mesityl oxide as neutral marker, the results indicated that the electroosmotic flow of the bonded phase displays a dramatic difference in pH dependence in comparison with that of the bare fused-silica column. The presence of positive charges on the coating surface and the anionic exchange property, due to the cationic property of the imidazole group at pH values below 6, allows the separation of geometric isomers that are very similar in ionic mobility. Separation parameters including buffer composition and concentration, pH, applied voltage, and the influence of other additives were investigated. By using acetate buffer (100 m*M*, pH 5.2) and an applied voltage of −15 kV with UV detection at 212 nm, the separation of 11 aromatic acids including mono-, di-, tri- and tetra-carboxylic acids could be achieved in less than 14 min. The average plate number was 3×10^5/m. With acetate buffer (25 m*M*, pH 5.5) and an applied voltage of −25 kV, the addition of silver nitrate or β-cyclodextrin significantly improved the resolution of some more highly charged carboxylic acids. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Imidazole-coating; Aromatic acids

1. Introduction

The importance of capillary electrophoresis (CE) as an analytical tool has increased dramatically over the last 10 years. The technique is no longer of pure academic interest, but can now solve real analytical problems. In many applications analytical determination with ion chromatography may be replaced successfully by CE, advantageously utilizing its higher separation efficiency and speed of analysis [1–3]. However, the separation of fast and slow moving anions in the same run is more complicated than the separation of cations [4]. To solve this problem, various approaches, including the addition of cationic surfactants to the buffer [5–7], dynamic adsorption or permanent coating of the capillary surface with a polymeric cation [8,9] or macrocyclic polyamine [10–12], have been reported. Permanent coatings are being used increasingly, since dynamic coatings are likely to result in poorer detection limits [4,9].

Increasing selectivity is an important goal in all analytical techniques. The selectivity of the bonded phases depends on the nature of the functional group introduced. Imidazole is a good ligand due to the presence of a nitrogen ring atom with a localized pair

*Corresponding author. Tel.: +886-2-2363-0231; fax: +886-2-2363-8543.

E-mail address: cyliu@ccms.ntu.edu.tw (C.-Y. Liu).

0021-9673/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(00)00845-1

of electrons. It is a promising reagent in CE separation, where it has been used as a UV chromophore for the electrophoretic separation and detection of metal ions [1,13–15]. Similar compounds, such as polyvinylimidazole, have been used as an efficient coating agents for high-performance liquid chromatographic supports [16], and a copolymer of vinylpyrrolidone and vinylimidazole has been used for the electrophoretic separation of basic proteins [17]. As far as we know, no imidazole-coated capillary column has been tested in electrophoretic separation.

Organic acids have important applications in pharmaceutics, food and the environment. Several permanent cationic coatings have shown an improved efficiency in controlling electroosmotic flow (EOF) for the separation of organic anions [10–12,18–20]. In this work, modification of the inner wall of fused-silica capillaries with a Si–O–Si–C linkage and then incorporation of imidazole groups for the electrophoretic separation of aromatic acids was investigated. Alteration of the separation efficiency by the addition of cyclodextrin and metal ions, respectively, to the background electrolyte has also been examined.

2. Experimental

2.1. Apparatus

CE separations were performed in a Spectra-Phoresis 1000 capillary system (Thermo Separation, Freemont, CA, USA). All experiments were carried out with 75-μm I.D. fused-silica capillary tubings (J&W Scientific, Folsom, CA, USA) with a total length of 68 cm and a distance between injection and detection of 60 cm. Electropherograms were recorded and processed with a PC 1000 data acquisition system (Thermo Separation products).

A pH meter (Radiometer, PHM 61, Copenhagen, Denmark) was used to measure the pH of the solution.

2.2. Reagents

Most chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). Purified water (18 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions. 3-Chloropropyltrimethoxysilane, 4-methyl-3-penten-2-one (mesityl oxide) and *p*-methoxybenzoic acid (anisic acid) were obtained from Aldrich (Milwaukee, WI, USA). Imidazole was purchased from Acros (Geel, Belgium). Silver nitrate, 1,2,4,5-benzenetetracarboxylic acid (pyromellitic acid), 1,3-benzenedicarboxylic acid (isophthalic acid), α-hydroxybenzeneacetic acid (mandelic acid), benzoic acid and 4-hydroxybenzoic acid were from Merck. 2-Hydroxybenzoic acid (salicylic acid), potassium hydrogen phthalate and 3,4,5-trihydroxybenzoic acid (gallic acid) were from Wako (Tokyo, Japan). 1,2,4-Benzenetricarboxylic acid (trimellitic acid) and 1,4-benzenedicarboxylic acid (terephthalic acid) were obtained from Janssen (Beerse, Belgium). β-Cyclodextrin was from TCI (Tokyo, Japan). Stock solutions (0.01 *M*) of the organic acids were prepared in methanol and diluted appropriately with pure water prior to use. All solvents and solutions for CE analysis were filtered through a 0.45-μm PTFE (Millipore) or cellulose acetate membrane (Whatman).

2.3. Column preparation

The fused-silica capillaries were treated with 1 *M* NaOH (30 min), then with pure water (15 min), 1 *M* HCl (30 min) and pure water (15 min). Before silanization the capillaries were rinsed with methanol (5 min) and then dried in a gas chromatography (GC) oven at 110°C for 1 h under nitrogen flow of 2.5 kg cm^{-2}. The pretreated capillary column was first filled with 3-chloropropyltrimethoxysilane in *m*-xylene (10%, v/v). Both ends of the capillary were sealed with septa, and the capillary was heated at 150°C overnight in a GC oven. Next the column was flushed with *m*-xylene and then reconnected to the GC oven and dried at 110°C under nitrogen flow. For increasing the solubility, the coating solution consisting of imidazole in *m*-xylene (6%, w/v) was preheated at 110°C. The silylated capillary was filled with the coating solution at a temperature of 110°C, then sealed at both ends and heated in the GC oven at 150°C overnight. Finally, the resulting column was flushed successively with acetone and pure water at ambient temperature.

2.4. Electrophoresis conditions

Before analysis the coated capillaries were preconditioned by flushing through the running buffer. They were rinsed with water and buffer between runs. Injection of the samples was done hydrodynamically for 2 s. The samples were detected at 212 nm and the neutral marker, mesityl oxide was detected at 240 nm.

3. Results and discussion

3.1. Characterization of the imidazole-coated capillary column

The nature of the chemical group bound to the column surface was confirmed from the quantity and the direction of EOF. The EOF velocities of both imidazole-coated capillary and bare fused-silica were determined, according to the migration time of mesityl oxide. Fig. 1 shows a dramatic difference in pH dependence between the two capillaries. For the bare capillary the electroosmotic flow-rate increases with pH because of a larger degree of ionization of the silanol groups. However, for the capillary bonded with imidazole groups the EOF is reversed and the migration velocity increases as the pH decreases. At the lower pH employed here, the charge on the column surface of the imidazole-coated capillary is expected to be positive due to protonation of the imidazole group. An increase of pH results in a decrease of the protonated group. This causes the charge reversal of the capillary surface.

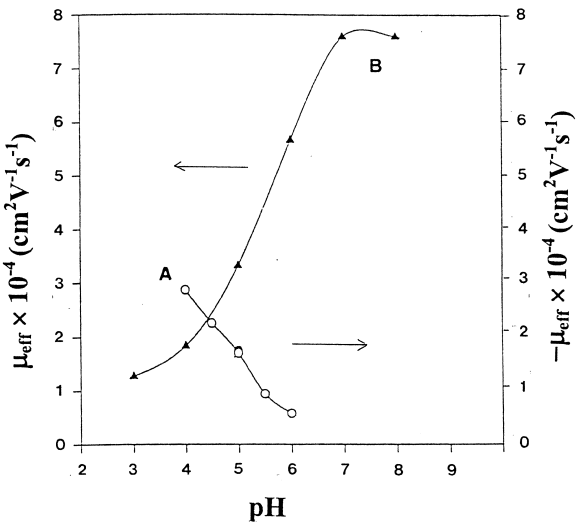

Fig. 1. Effect of pH on electroosmotic flow mobility in various capillary materials. Column, 68 cm (60 cm to detector)×75 μm I.D; EOF marker, mesityl oxide; hydrodynamic injection, 1 s; temp., 25°C; detection at 240 nm. Curve A: imidazole-coated capillary; acetate buffer (25 m*M*); applied voltage, −25 kV. Curve B: bare fused-silica capillary; phosphate buffer (50 m*M*); applied voltage, 25 kV.

3.2. Separation of aromatic acids

The prepared column was evaluated for the separation of aromatic acids. Table 1 lists the physical and chemical properties of the selected compounds.

3.2.1. Effect of pH

The pK_a values of most analytes are below 6 and that of the bonded imidazole group is around 7.0. For having a greater electrostatic interaction between analytes and the bonded imidazole, as well as large variation among the mobilities of the analytes, a pH over the range of 3–6 would be a better choice for the electrophoretic separation. To illustrate this effect, we injected a mixture of 11 aromatic carboxylic acids with phosphate buffer (25 m*M*) as a background electrolyte. At applied voltage of −25 kV, eight peaks at pH 4.0 and nine peaks at pH 4.5 were observed. At pH 5.0, only four sharp peaks and several broad peaks were indicated. On increasing the pH values to greater than 6, more anions comigrated. A less protonated bonded imidazole results in a smaller variation for the mobilities of the analytes. Therefore a lower charge acetate buffer was used as a background electrolyte. The migration for most analytes changed markedly with pH, except for salicylic acid and mandelic acid (Fig. 2). The pairs of pyromellitic acid and trimellitic acid as well as terephthalic acid and isophthalic acid, could be well separated at pH values lower than 5.0. Decreasing acidity of the medium led to a decrease in the variation of mobilities, and hence resolution became poor. At pH values above 5.5, no resolution was found. At pH 4.5, salicylic acid migrated faster than phthalic acid. Moreover, at pH values greater than 4.8, a reverse order was found. In a similar manner, mandelic acid migrated faster than benzoic acid at

Table 1
Chemical and physical properties of the model compounds

Acid	pK_a[a] (25°C, $\mu=0$)	Effective charge						Equiv. conduct.[b]
		pH 4.0	pH 4.5	pH 5.0	pH 5.2	pH 5.5	pH 6.0	
Pyromellitic (254.2)[c]	1.70, 3.12, 4.92, 6.23	1.99	2.25	2.58	2.73	2.94	3.30	68.1
Trimellitic (210.1)	2.48, 4.04, 5.54	1.47	1.82	2.13	2.25	2.45	2.74	62.7
Terephthalic (166.1)	3.61, 4.50	0.95	1.41	1.74	1.82	1.91	1.97	52.6
Isophthalic (166.1)	3.50, 4.50	1	1.43	1.75	1.83	1.91	1.97	54.7
Salicylic (138.1)	2.97, 13.74	0.91	0.97	0.99	0.99	1	1	36.0
Phthalic (166.1)	2.95, 5.40	0.96	1.08	1.28	1.38	1.56	1.80	52.3
Mandelic (152.2)	3.40	0.80	0.93	0.97	0.98	0.99	1	
Benzoic (122.1)	4.20	0.39	0.67	0.86	0.91	0.95	0.98	32.4
Anisic (152.1)	4.08	0.45	0.72	0.89	0.93	0.96	0.99	29.0
4-Hydroxybenzoic (138.1)	4.58, 9.46	0.21	0.45	0.72	0.81	0.89	0.96	31.4
Gallic (170.1)	4.43, 9.11	0.27	0.54	0.79	0.85	0.92	0.97	

[a] Data from Ref. [24,25].
[b] Limiting equivalent ionic conductance (S cm^2 $equivalent^{-1}$): data from Ref. [26].
[c] Molecular mass.

lower pH. But at pH values above 5.0, a reverse order was also observed. The property might result from a greater fraction of salicylic acid and mandelic acid being in the fully ionized form than was the case for the others (Table 1). Therefore the charge-to-mass ratio of salicylic acid is greater than that of phthalic acid, while at pH values higher than 5.0, phthalic acid has a larger charge-to-mass ratio than salicylic acid. This property gives rise to a higher mobility than salicylic acid. A similar effect was exhibited for mandelic acid and benzoic acid. Hence intersection for the plot of electrophoretic mobility versus pH was indicated for both pairs. Due to a lower sensitivity of gallic acid in a medium of pH lower than 5.0, no investigation was carried out under this condition.

3.2.2. Effect of acetate concentration and pH

With acetate buffer (25 m*M*) at a pH above 5.5 and an applied voltage of −25 kV, no resolution was found for the pairs of pyromellitic acid and trimellitic acid as well as terephthalic acid and isophthalic acid. Therefore we increased the buffer concentration from 25 to 75 m*M*. It was found that the electrophoretic mobility of the analytes decreased only slightly. However, baseline resolved separation for the pyromellitic acid and trimellitic acid was demonstrated. We increased the buffer concentration further to 100 m*M*, an improved separation for the terephthalic acid and isophthalic acid was also observed. But a nonlinear current versus voltage relationship was observed.

3.2.3. Organic solvent

Because of organic solvents affecting the solvation, the effect of the addition of methanol (5%, v/v) to the electrolyte was investigated. The results showed that a slower migration for all analytes. Meanwhile, the separation for the mixture of pyromellitic acid, trimellitic acid, terephthalic acid and isophthalic acid became worse. Only the resolution of anisic acid and 4-hydroxybenzoic acid improved significantly. Since the acids have adequate solubility in the aqueous electrolyte, the addition of methanol was not employed.

3.2.4. Influence of applied potential

In a CE system, the working voltage is one parameter to be optimized (Fig. 3). At an applied potential of −25 kV and acetate buffer (pH 5.2, 100 m*M*), the separation for the 11 components of the aromatic acids is less than 17 min. But the resolution for the terephthalic acid and isophthalic acid is only 1.0. Decrease the potential to −10 kV, a better resolution was found. However, the total analysis time was more than 50 min. For maintaining good resolution and short analysis time, a voltage of −15 kV was employed as the optimum condition in further studies.

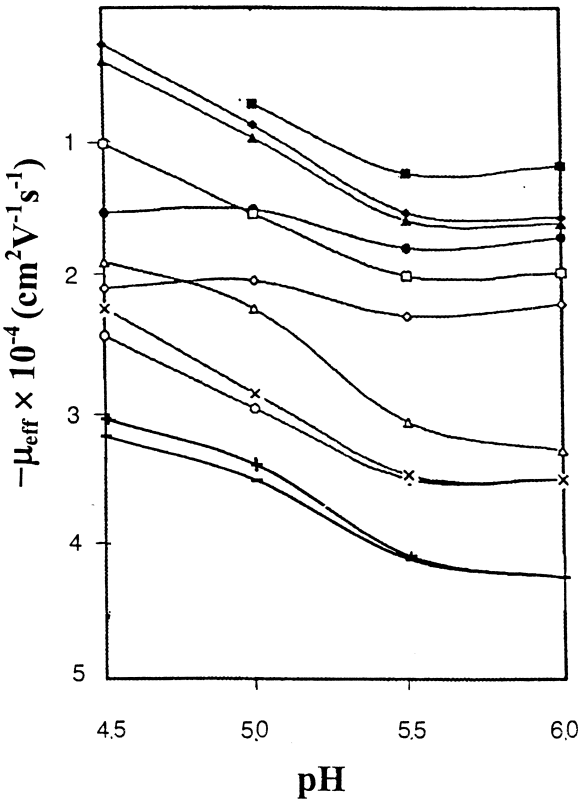

Fig. 2. Influence of pH on the separation of aromatic acids. Column, imidazole-coated capillary (68 cm (60 cm to detector)× 75 μm I.D.); sample concentration, 0.5 m*M* for each; hydrodynamic injection, 2 s; acetate buffer (25 m*M*); applied voltage, −25 kV; temp., 25°C; Detection at 212 nm. Curves: (–) pyromellitic acid; (+) trimellitic acid; (○) terephthalic acid; (×) isophthalic acid; (◇) salicylic acid; (△) phthalic acid; (●) mandelic acid; (□) benzoic acid; (▲) anisic acid; (♦) 4-hydroxybenzoic acid; (■) gallic acid.

3.2.5. Effect of β-cyclodextrin concentration

As the above electrolyte system could not completely resolve terephthalic acid and isophthalic acid, we searched for another system. Buchberger and Winna showed that in the sodium tetraborate buffer containing tetradecyltrimethylammonium bromide with the addition of both barium ion and β-cyclodextrin exerted a significant influence on the EOF for the separation of di- and tri-carboxylic acids [21]. In this work, only acetate buffer and β-cyclodextrin were used to manipulate the separation selectivity of a mixture of mono-, di-, tri- and tetra-carboxylic acids (Fig. 4). The migration order is pyromellitic acid>trimellitic acid>isophthalic acid>phthalic acid>terephthalic acid>mandelic acid>salicylic acid>benzoic acid>gallic acid>4-hydroxybenzoic acid>anisic acid (Fig. 5). In the absence of cyclodextrin the order is pyromellitic acid>trimellitic acid>terephthalic acid>isophthalic acid>phthalic acid>salicylic acid>benzoic acid>mandelic acid> anisic acid>4-hydroxybenzoic acid>gallic acid (Fig. 3). A significant difference is observed between them, except the first two compounds. In this work, an anodic EOF was caused by the positively charged moiety of the imidazole group. The protonated imidazole group could act as the fixed ionic group, and buffer anion as the counter ion. Accordingly anion exchange more or less might be assumed as another way for the analytes to interact with the capillary surface. In view of this fact, the strengths of various analytes interacting with the bonded group are proportional to the charge of analytes, related to the size of the hydrated ions as well as other properties (such as structural shape). Thus the relative strength for the isomers of dicarboxylic acids towards the bonded group would be phthalic acid> isophthalic acid>terephthalic acid. The migration velocity decreased in the order: terephthalic acid> isophthalic acid>phthalic acid (Fig. 3) was consistent with the prediction. With the addition of β-cyclodextrin, the acid with carboxyl groups in the *para*-position would interact most easily with the cyclodextrin host molecule. The *ortho*-isomer is next and the *meta*-isomer is last. Thus the migration velocity changes in the order as isophthalic acid> phthalic acid>terephthalic acid (Fig. 5). The substituents of a monoprotic acid demonstrated a similar phenomenon. The analyte with the most crowding group was eluted in first priority. The addition of β-cyclodextrin to the buffer resulted in complete separation of terephthalic and isophthalic acid. But it took around 35 min for the whole analysis (Fig. 5).

3.2.6. The addition of metal ions

Complexation is a potential means for the manipulation of the selectivity, as the species involved in complexation equilibria with metal ions might undergo a change in charge distribution that may lead to a significant variation in electrophoretic mobility [22]. Lalljie et al. showed that the addition of Ca^{2+} to the buffer induces an acceptable separation of formate

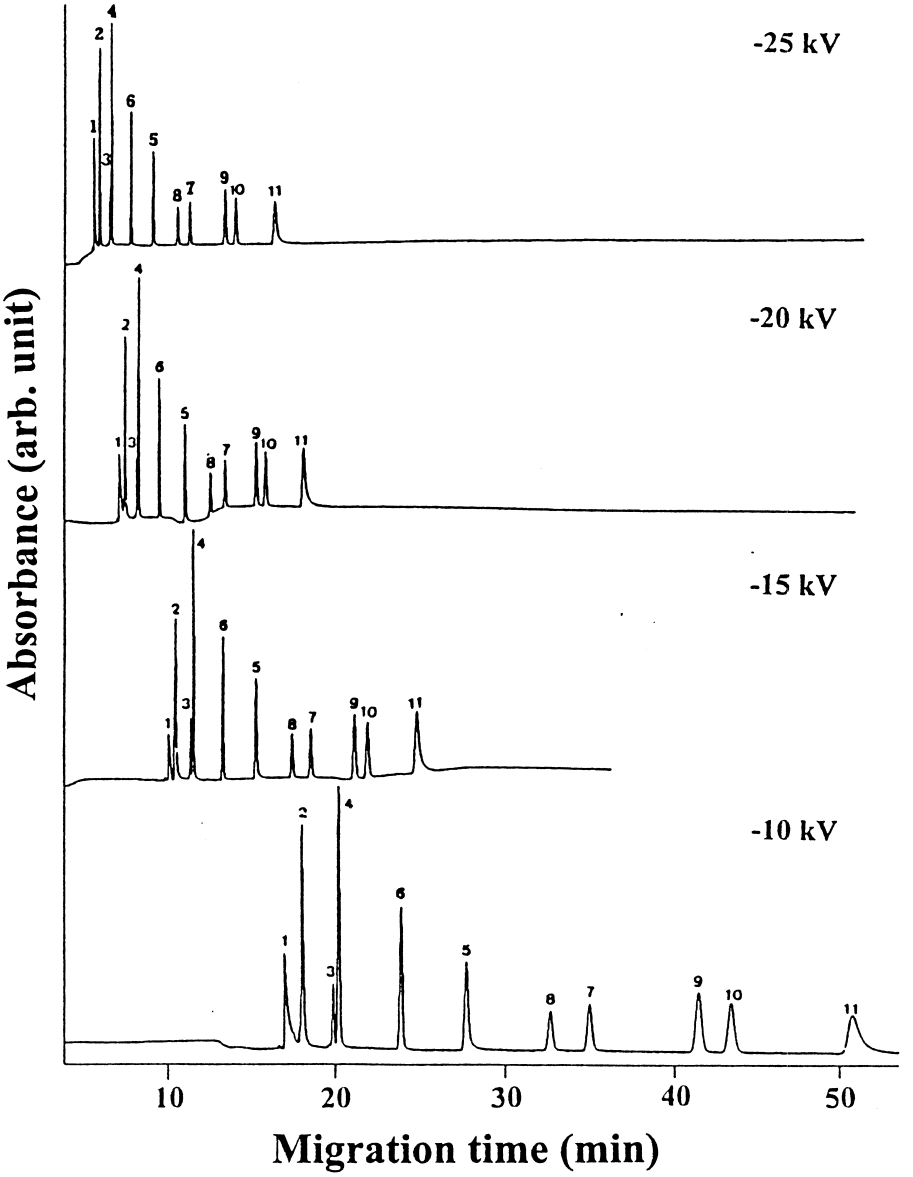

Fig. 3. Influence of the applied voltages on the separation of organic acids. Conditions as in Fig. 2, except acetate buffer (100 m*M*, pH 5.2) and sample concentration: 0.1 m*M* for each, except gallic acid, 0.2 m*M*. Peaks: (1) pyromellitic acid; (2) trimellitic acid; (3) terephthalic acid; (4) isophthalic acid; (5) salicylic acid; (6) phthalic acid; (7) mandelic acid; (8) benzoic acid; (9) anisic acid; (10) 4-hydroxybenzoic acid; (11) gallic acid.

and tartrate via selective retardation of tartrate caused by its complexation with the cation [23]. Buchberger and Winna showed that the presence of barium ions in the carrier electrolyte allowed considerably improved separations of the carboxylic acids [21]. In the present work, the effect of a soft metal ion (silver) on the separation was studied. This process may be expected to cause the formation of metal–analyte complexes in the buffer. With the addition of silver ion (0.1 m*M*) to the buffer, slower migration of the analytes (0.1 m*M* for each) was observed (Fig. 6A), especially for monoprotic acids.

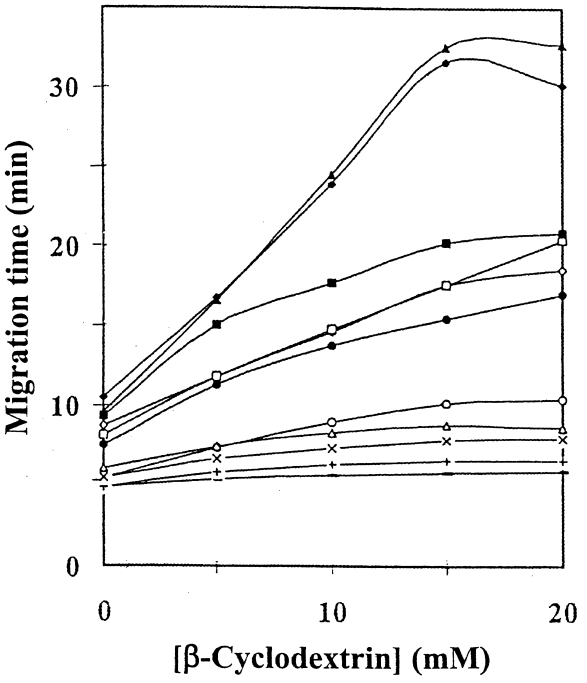

Fig. 4. Dependence of migration time on the concentration of β-cyclodextrin. Conditions as Fig. 3, except acetate buffer (25 m*M*, pH 5.5) with the addition of β-cyclodextrin. Curves: (–) pyromellitic acid; (+) trimellitic acid; (○) terephthalic acid; (×) isophthalic acid; (◇) salicylic acid; (△) phthalic acid; (●) mandelic acid; (□) benzoic acid; (▲) anisic acid; (♦) 4-hydroxybenzoic acid; (■) gallic acid.

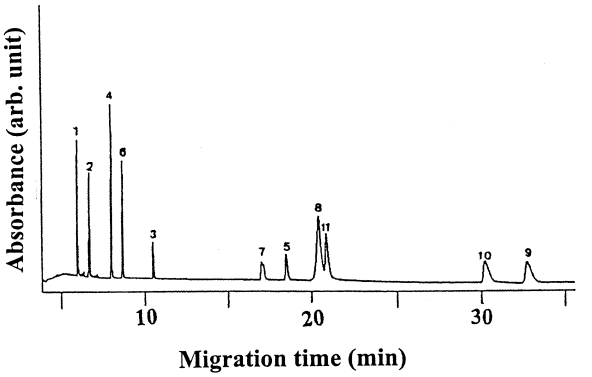

Fig. 5. Electropherogram for the separation of aromatic acids with the addition of β-cyclodextrin. Column, imidazole-coated capillary (68 cm (60 cm to detector)×75 μm I.D.). Sample concentration, 0.1 m*M* for each, except gallic acid, 0.2 m*M*. Hydrodynamic injection, 2 s; acetate buffer (25 m*M*) with β-cyclodextrin (20 m*M*); applied voltage, −25 kV; temp., 25°C; detection at 212 nm. Peaks: (1) pyromellitic acid; (2) trimellitic acid; (3) terephthalic acid; (4) isophthalic acid; (5) salicylic acid; (6) phthalic acid; (7) mandelic acid; (8) benzoic acid; (9) anisic acid; (10) 4-hydroxybenzoic acid; (11) gallic acid.

A lower charge of metal–analyte complex than that of the analyte itself might be the reason. Additionally, the resolution for pyromellitic acid and trimellitic acid improved slightly. We further increased the molar ratio of analyte to metal ion. In comparison with the neat buffer system (Fig. 6B(a)) and the previous results shown in Fig. 6A(b), a significant increase in migration velocity and full resolution for the higher charge polyprotic acids including pyromellitic acid, trimellitic acid, terephthalic acid and isophthalic acid was demonstrated (Fig. 6B(b)). This might be due to the formation of metal complexes with a composition different from those formed in the one to one condition (Fig. 6A(b)).

3.2.7. Reproducibility and stability of the imidazole-coated capillary column

Based on the above results, acetate buffer (100 m*M*, pH 5.2) and a voltage of −15 kV was selected as the optimum condition. In all the work mentioned, the silylation reaction period of the column preparation was 1 day. As the reaction period was further raised to 48 h, a shorter analysis time and a more efficient separation was indicated (Table 2). A greater functionality of the capillary surface results more bonded imidazole groups might be the reason. The electropherogram is shown as Fig. 7.

Under electrophoretic conditions, the column discussed here was usable for a long period of time up to 1 month without noticeable change in the separation properties. The reproducibility of migration time for the column with acetate buffer as well as a mixture of acetate buffer and β-cyclodextrin within a day for the six consecutive analyses has been studied. The results are given in Table 2. The relative standard deviation (RSD) of the migration times was better than ±1.0% for most acids. The day-to-day RSD of migration times obtained at interval of 10 tests among 50 injections ($n=5$) was better than ±2.6% for most acids. After long-term use, the unstable baseline may be seen. In this case, the column washed with a dilute NaOH (1 m*M*) for a while (less than 1 min), then with running buffer is recommended. This process will shorten the conditioning time than that with only running buffer.

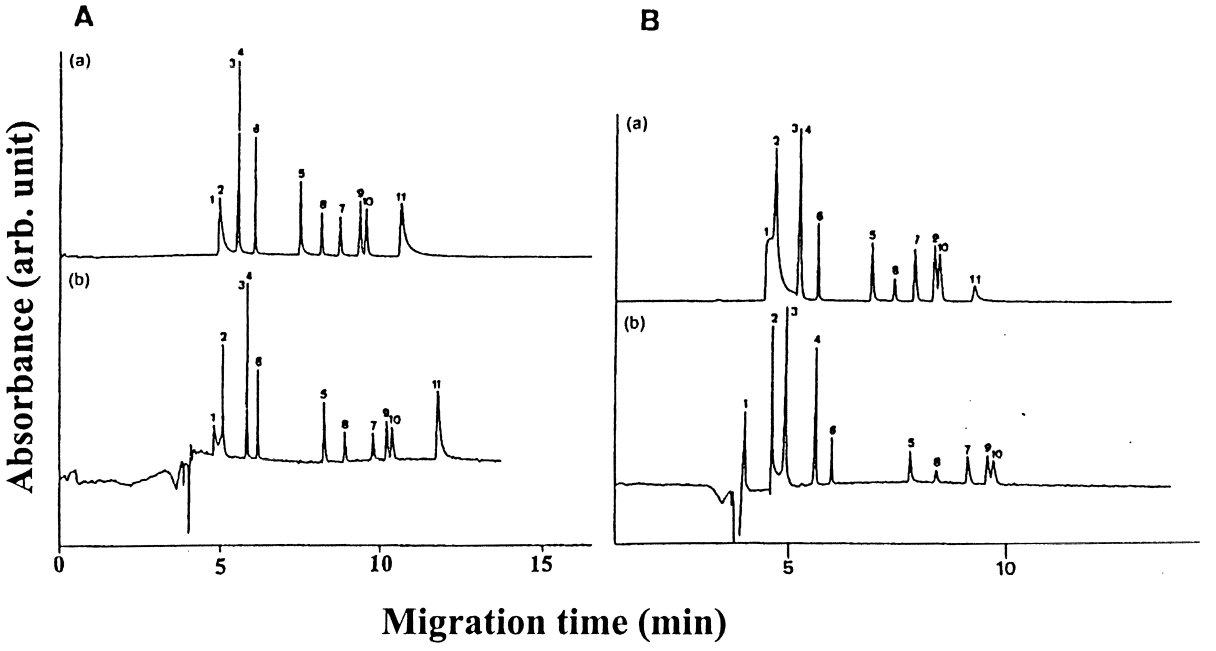

Fig. 6. Effect of Ag(I) on the separation of aromatic acids. Column, imidazole-coated capillary (68 cm (60 cm to detector)×75 μm I.D.). Sample concentration, 0.1 m*M* for each, except gallic acid, 0.2 m*M*. Hydrodynamic injection, 2 s; acetate buffer (25 m*M*, pH 5.5); applied voltage, −25 kV; temp., 25°C; detection at 212 nm. A: (a) without (b) with the addition of Ag(I) (0.1 m*M*). B: conditions as in (A), except sample concentration. Peaks: (1–3) 0.4 m*M*; (4–6) 0.1 m*M*; (7,8,11) 0.2 m*M*; (9,10) 0.15 m*M*. Peak identification as in Fig. 3.

Table 2
Reproducibility and efficiency for the separation of aromatic acids with the imidazole-coated capillary[a]

Acid	Migration time (±RSD, $n=6$) (min)		Theoretical plates (m^{-1})	Plate height (μm)
Pyromellitic	7.37 (0.91%)	5.88[b] (1.00%)	404 000 (111 000)[c]	2.47
Trimellitic	7.72 (0.82%)	6.65 (0.15%)	420 000 (192 000)	2.38
Terephthalic	8.07 (0.58%)	10.55 (0.75%)	244 000 (202 000)	4.09
Isophthalic	8.24 (0.56%)	7.92 (0.68%)	267 000 (208 000)	3.74
Salicylic	9.99 (0.87%)	18.50	398 000 (245 000)	2.51
Phthalic	9.12 (0.32%)	8.74 (0.47%)	213 000 (192 000)	4.69
Mandelic	11.31 (0.49%)	17.00	317 000 (185 000)	3.15
Benzoic	10.87 (1.00%)	20.37	374 000 (178 000)	2.67
Anisic	12.27 (0.63%)	32.50	301 000 (158 000)	3.32
4-Hydroxybenzoic	12.62 (0.77%)	30.25	272 000 (153 000)	3.68
Gallic	13.59 (1.06%)	20.88	115 000 (93 000)	8.70

[a] Column dimensions, 68 cm (effective length, 60 cm)×75 μm I.D.; hydrodynamic injection (2 s); acetate buffer (100 m*M*, pH 5.2); applied potential, −15 kV; detection at 212 nm; silylation reaction period for column preparation is 48 h.

[b] Conditions as (a), except with a mixture of acetate buffer (25 m*M*, pH 5.5) and β-cyclodextrin (20 m*M*); applied potential, −25 kV.

[c] Silylation reaction period for the column preparation is 24 h. Theoretical plate: $5.54(t_m/w_{1/2})^2$; t_m is migration time; $w_{1/2}$ is the width at half peak height.

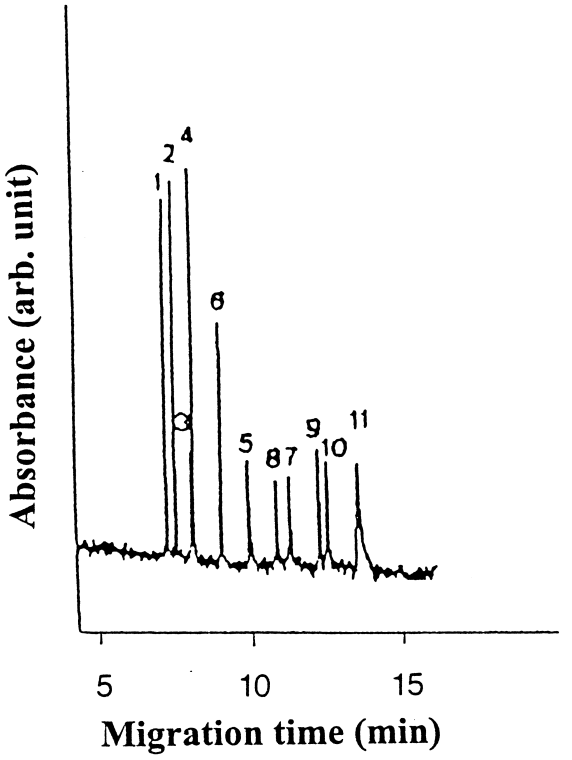

Fig. 7. Electropherogram for the separation of aromatic acids under the optimum conditions. Column, imidazole-coated capillary (68 cm (60 cm to detector)×75 μm I.D.). (Note: silylation reaction period is 2 days.). Sample concentration, 0.1 m*M* for each, except gallic acid, 0.2 m*M*; hydrodynamic injection, 2 s; acetate buffer (100 m*M*, pH 5.2); applied voltage, −15 kV; temp., 25°C; detection at 212 nm.

After long-term use (more than 250 injections), no significant difference for the migration time (RSD< 3.5%) was observed by washing in this manner. A possible explanation of the phenomenon involves some sorptive interaction between analytes and the protonated imidazole groups. The average RSDs for these compounds from capillary to capillary (three different columns, $n=3$) were less than 4.5%. For a bonded capillary, the reproducibility is acceptable. The high precision might be a result of the stable EOF contributed by the bonded column.

4. Conclusions

This work offers an easy way to prepare a bonded capillary for the electrophoretic separation of mono-, di-, tri- and tetra-carboxylates that have a wide variation in the mobility in the same run. By using the negative polarity mode, separation of 11 aromatic acids could be carried out in acetate buffer in less than 14 min, yielding an average plate count of 3×10^5 plates/m. Additionally, the separation that involves the use of complexation reactions, such as host–guest interaction and the addition of metal ions to separate the geometric isomers has also been discussed. Although it applies specifically to the separation of aromatic acids, it can be used for the separation of other anions or even neutral compounds. Further work is in progress.

Acknowledgements

The authors thank the National Science Council of Taiwan for financial support.

References

[1] J. Farre, F. Borrull, M. Calull, Chromatographia 44 (1997) 235.
[2] P.R. Haddad, J. Chromatogr. A 770 (1997) 281.
[3] T. Soga, G.A. Ross, J. Chromatogr. A 834 (1999) 65.
[4] C. Finkler, H. Charrel, H. Engelhardt, J. Chromatogr. A 822 (1998) 101.
[5] X. Huang, J.A. Luckey, M.J. Gordon, R.N. Zare, Anal. Chem. 61 (1989) 766.
[6] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[7] C.M. Shelton, J.T. Koch, N. Desai, J.F. Wheeler, J. Chromatogr. A 792 (1997) 455.
[8] S. Terabe, T. Isemura, Anal. Chem. 62 (1990) 652.
[9] A.H. Harakuwe, P.R. Haddad, J. Chromatogr. A 834 (1999) 213.
[10] J.C. Hsu, W.H. Chen, C.Y. Liu, Analyst 122 (1997) 1393.
[11] C.Y. Liu, W.H. Chen, J. Chromatogr. A 815 (1998) 251.
[12] W.H. Chen, C.Y. Liu, J. Chromatogr. A 848 (1999) 401.
[13] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[14] W. Beck, H. Engelhardt, Fresenius J. Anal. Chem. 346 (1993) 618.
[15] T. Wang, H.K. Lee, S.F.Y. Li, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2485.
[16] R. Lemque, C. Vidal-Madjar, M. Racine, J. Piquin, B. Sebille, J. Chromatogr. 553 (1991) 165.
[17] R.J. Xu, C. Vidal-Madjar, B. Sebille, J.C. Diez-Masa, J. Chromatogr. A 730 (1996) 289.
[18] S. Hjerten, J. Chromatogr. 347 (1985) 191.

[19] M. Chiari, L. Ceriotti, G. Crini, M. Morcellet, J. Chromatogr. A 836 (1999) 81.
[20] V. Dolnik, J. Dolnikova, J. Chromatogr. A 716 (1995) 269.
[21] W. Buchberger, K. Winna, J. Chromatogr. A 739 (1996) 389.
[22] M. Chiari, N. Dell'Orto, L. Casella, J. Chromatogr. A 745 (1996) 93.
[23] S.P.D. Lalljie, J. Vindevogel, P. Sandra, J. Chromatogr. A 652 (1993) 563.
[24] R.M. Smith, A.E. Martell, Critical Stability Constants, Plenum Press, New York, 1977, Vol. 4.
[25] R.M. Smith, A.E. Martell, Critical Stability Constants, Plenum Press, New York, 1989, Vol. 6.
[26] J.A. Dean, Lange's Handbook of Chemistry, McGraw-Hill, New York, 1985.