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Hydrophobic-phase-modified fused-silica columns for capillary electrophoresis

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Abstract

Coated capillaries modified with a hydrophobic layer were developed. Linear hydrocarbons and ethylbenzene modified surfaces greatly improved the electrophoretic performance of the capillaries. The column efficiency for organic compounds reached as high as 327 000 theoretical plate numbers per meter on a 50 μm I.D. linear hydrocarbon (C_6) surface treated fused-silica capillary column. This value did not change during 50 repeated analyses and the columns showed strong stability against 0.1 *M* NaOH and 0.1 *M* HCl. The relative standard deviation of the run-to-run, day-to-day, and capillary-to-capillary coating with hydrophobic layer showed values of ≤2.5%, and good reproducibility. The separations of four aromatic amines and six pharmacological amines at pH 2.5 is reported.

Keywords: Capillary columns; Coated columns; Efficiency; Hydrocarbons; Ethylbenzene; Amines

1. Introduction

Capillary electrophoresis (CE) can be accepted as a major separation and analytical technique, if the column surface is reliably and consistently deactivated. The active groups on the surface of untreated fused-silica tubing adsorb some analytes, making separations difficult and inconsistent. Electroosmotic flow (EOF), to which the resolution is closely related, can vary because of the adsorption at the tubing surface, and this also makes it difficult to obtain reproducible results. In addition, the EOF desired for a separation may not be achievable with the required separation medium when bare fused-silica tubing is used. The surface coating becomes a crucial issue when the sample matrix is relatively dirty and high recovery of the analyte is required. An ideal coating completely deactivates the surface, is hydrolytically stable over a wide pH range, and ensures reproducible separations. Furthermore, the ideal coating allows one to decrease or remove, reverse or stabilize, the EOF by changing pH. Different methods have been studied to overcome these problems [1–12]. Recently some attention has been paid to the use of polymeric hollow fibers [13–17] as columns in CE.

In this paper, we report results obtained by surface modification of fused-silica columns with stable hydrophobic surface coating. The EOF varied significantly with pH (pH>5) for the coated columns with respect to the untreated columns. The presence of the EOF in the coated columns may be explained by an incomplete *n*-hydrocarbons and *n*-hydrocarbons–ethylbenzene coverage. The separations of

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low-molecular-mass samples were easily achieved on the coated columns at pH 2.5. The separation of four aromatic amines and six pharmacological amines recommend the use of these modified columns for basic compound analysis. A simple, but successful, method is reported.

2. Experimental

2.1. Chemicals

1-Iodoethane (C_2), 1-iodohexane (C_6), 1-iodooctane (C_8), 1-iododecane (C_{10}), 1-iododododecane (C_{12}) and 2-bromoethylbenzene (EB) were purchased from Fluka (Buchs, Switzerland). Chemical reagent grade potassium phosphate monobasic, potassium phosphate dibasic, sodium tetraborate, sodium carbonate, 1 *M* hydrochloric acid (standard solution), methanol, methylene chloride, toluene, acetone, *n*-hexane, benzyl alcohol and potassium chloride were purchased from Carlo Erba (Milan, Italy).

Amine standards: *o*-tolidine (o-T), α-naphthylamine (α-N), 4-biphenylamine (4-B) and benzidine (B) were purchased from Riedel de Haen (Seelze, Germany), while 2-diphenylmethoxy-N,N-dimethylethanamine (diphenhydramine), 4-methoxyphenyl-2-ethylpropylamine, chetocaine, 4-(2-aminoethyl)-1,2-benzenediol (dopamine), 1-diphenylmethyl-4-methylpiperezine (cyclizine) and N-methyl-3,3-diphenylpropylamine) were purchased from Recordati (Rome, Italy).

Deionized water for the preparation of buffer solutions, as well as for rinsing capillaries, was obtained from a Milli-Q water system (Millipore, Milford, MA, USA). Fused-silica capillary tube (10 m×50 μm I.D.×180 μm O.D.) was obtained from SGE (Austin, TX, USA).

2.2. Equipment

A BioFocus 3000 capillary electrophoresis system (Bio-Rad, Richmond, CA, USA), controlled by a HP Vectra 4/50 computer (Hewlett-Packard, Rome, Italy) with BIOFOCUS and SPECTRA v. 5.00 software (Bio-Rad) was used. Organic compounds and benzyl alcohol (marker) were detected with on-column UV absorbance at 200 nm, while a phosphate buffer was used as electrolyte, containing 30 and 40 m*M* potassium phosphate monobasic; the pH value was adjusted to pH 2.50 with 1 *M* hydrochloric acid and controlled with a pH meter basic 20 (Crison Instruments, Alella, Barcellona, Spain). Buffer solutions were filtered with a 0.2-μm filter (Alltech, Deerfield, IL, USA). Sample injection was performed by pressure (34 474 Pa×3 s).

2.3. Coating procedure

The fused-silica capillary columns were first sequentially washed using 2 ml of methylene chloride, 2 ml of methanol and 3 ml of acetone. After drying the capillary column by a stream of nitrogen, it was coated with a pure 1-iodoethane (C_2), 1-iodohexane (C_6), 1-iodooctane (C_8), 1-iododecane (C_{10}) and 1-iododododecane (C_{12}) using the following procedure:

(a) The pure C_2, C_6, C_8, C_{10} or C_{12} (25–30 μl) were passed through the capillary columns (0.8–1 m) at a rate of about 2.5–3 cm s^{-1}. Excess pure compound was removed by a stream of nitrogen for 10 min. The capillary columns were then sealed at both ends and heated in an oven at 50°C overnight. After the reaction, all columns were successively washed with 3 ml of toluene, 3 ml of methylene chloride, 3 ml of methanol, 3 ml of deionized water and 5 ml of running buffer (total time: 50–60 min). The obtained capillary columns were tested to determine the EOF and the efficiency (N m^{-1}, the number of theoretical plates per meter);

(b) The pure 2-bromoethylbenzene (EB), 25–30 μl, was passed through the capillary columns previously coated with the *n*-hydrocarbons at a rate of about 2.5–3 cm s^{-1}. Excess pure compound was removed with a stream of nitrogen for 10 min. Capillary columns were then sealed at both ends and heated in an oven at 50°C overnight. After the reaction, all columns were washed sequentially with 3 ml of toluene, 3 ml methylene chloride, 3 ml of methanol, 3 ml of deionized water and 5 ml of running buffer (total time: 50–60 min). These capillary columns were tested to determine the EOF and the efficiency (N m^{-1}).

3. Results and discussion

3.1. Characterization and stability of the hydrocarbon layers

The EOF is the most important parameter in CZE separations. Its stability is important for the reproducibility of the migration times. The absence of EOF is required for isoelectric focusing and capillary gel electrophoresis. The dependence of the EOF on pH shows a typical sigmoidal curve, caused by the dissociation of the surface silanol groups.

The EOF measured for the capillary columns coated with *n*-hydrocarbons is compared in Fig. 1 to the EOF obtained with the uncoated capillary column of the same batch and identical surface pretreatment. It is important that the capillaries are taken from the same batch, because the EOF characteristics are also a function of the storage age of the capillary. A capillary stored for 1 year in the laboratory showed only half of the initial EOF measured immediately after delivery and opening. As can be seen in Fig. 1, the columns coated with C_{10} and C_{12} show only a slight decrease of the EOF at high pH (≈3% reduction compared to that of untreated columns). The columns coated with C_2 and C_6 show a high decrease of the EOF at high pH (≈45–50% reduction compared to that of untreated one), while the

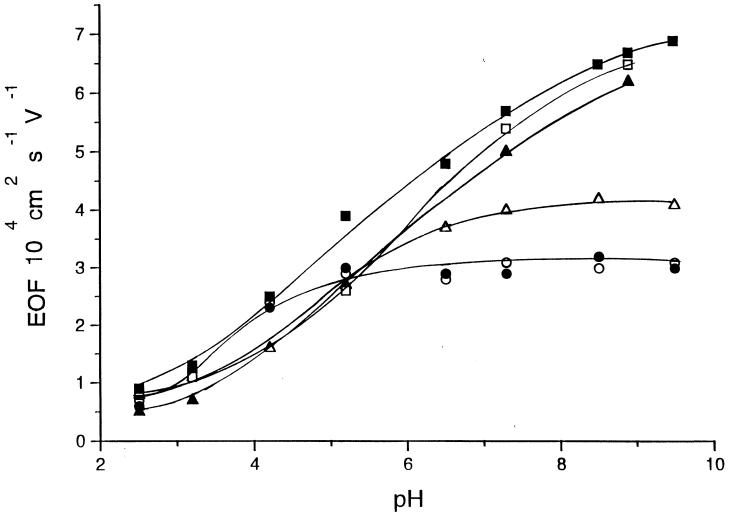

Fig. 1. Influence of the pH on the EOF of uncoated and hydrocarbon coated capillary columns. Conditions: detection, 200 nm; applied voltage, 10 kV; temperature, 25°C; electrolyte systems, 30 m*M* KCl–20 m*M* phosphate buffer at pH 2.50–3.20, 30 m*M* KCl–20 m*M* acetate buffer at pH 4.00–5.30, 30 m*M* KCl–20 m*M* phosphate buffer at pH 6.50–7.50, 30 m*M* KCl–20 m*M* borate buffer at pH 8.30–9.50. Symbols: ■=uncoated capillary, ○=C_2 capillary, ●=C_6 capillary, △=C_8 capillary, ▲=C_{10} capillary, □=C_{12}capillary. Capillaries 30 cm (25.5 cm effective length)×50 μm I.D..

Table 1
Influence of the field strength on the number of theoretical plates per meter for four aromatic amines; C_{10} capillary 30 cm (25.5 cm effective length)×50 μm I.D., electrolyte: 40 m*M* phosphate buffer at pH 2.50

Substances	*N* m^{-1}			
	200 V cm^{-1}	266.7 V cm^{-1}	333.3 V cm^{-1}	400 V cm^{-1}
o-Tolidine	280 000	300 000	266 000	193 000
Benzidine	286 000	310 000	261 000	185 000
α-Naphthylamine	212 000	258 000	257 000	224 000
4-Biphenylamine	232 000	234 000	217 000	198 000

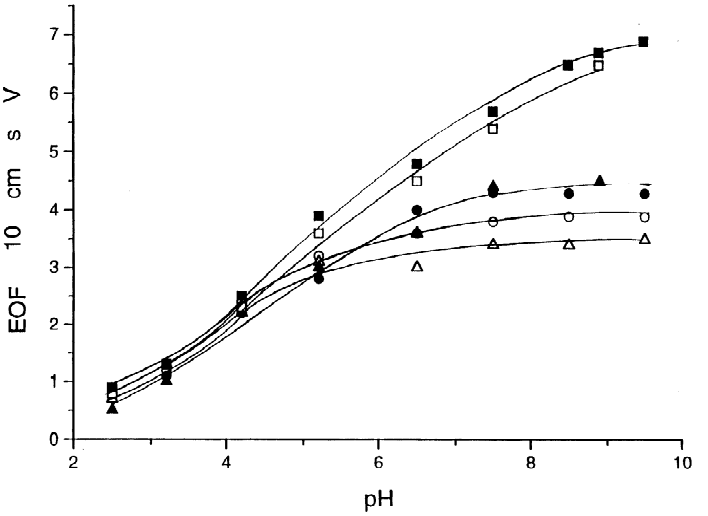

Fig. 2. Influence of the pH on the EOF of uncoated and hydrocarbon–EB coated capillary columns. Conditions: as in Fig. 1. Capillaries: 30 cm (25.5 cm effective length)×50 μm I.D. Symbols: ■=uncoated capillary, ○=C_2–EB capillary, ●=C_6–EB capillary, △=C_8–EB capillary, ▲=C_{10}–EB capillary, □=C_{12}–EB capillary.

capillary column coated with C_8 shows an intermediate decrease of the EOF at high pH (≈30% reduction compared to an untreated one).

The EOF measured for the capillary columns coated with *n*-hydrocarbons and EB is compared in Fig. 2 to the EOF obtained with the uncoated capillary column of the same batch and identical surface pretreatment. As can be seen in Fig. 2, the capillary columns coated with *n*-hydrocarbons and EB show a decrease of the EOF at high pH compared to that of the untreated capillary column. The column coated with C_8–EB shows a high decrease of the EOF at high pH (≈50% reduction compared to that of untreated one and ≈10% reduction compared to that treated with C_8 alone, Fig. 1). The column coated with C_{10}–EB shows a decrease of the EOF at high pH (≈30% reduction compared to that of untreated one and ≈25% reduction compared to that treated with only C_{10}, Fig. 1). Only the capillary column coated with C_{12}–EB shows an EOF similar to that of initial fused-silica capillary column and to that coated with only C_{12}, Fig. 1. In this case the few hydrocarbon chains that reacted with the silanol groups obstruct (steric hindrance) other chains from reacting and so many silanol groups are free. For this reason the EOF of the capillary columns coated with C_{12}, C_{10} or C_{12}–EB is similar to that of the

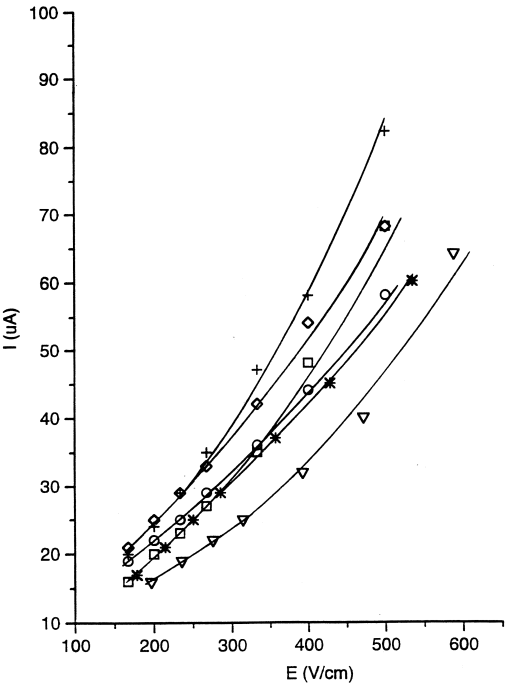

Fig. 3. Plot of field strength versus resulting current for the 40 m*M* phosphate buffer at pH 2.50. Symbols: □=untreated capillary, ○=C_2 capillary, *=C_6 capillary, ▽=C_8 capillary, ◊=C_{10} capillary, +=C_{12} capillary. Capillaries: 30 cm (25.5 cm effective length)×50 μm I.D., temperature, 25°C and UV detection at 200 nm.

untreated column (≈3% reduction compared to that of the untreated capillary column). The columns coated with C_6–EB and C_2–EB show a decrease of the EOF at high pH (≈30–40% reduction compared to that of the untreated one).

The EOF of the capillary columns coated with *n*-hydrocarbons show only a slight decrease with respect to that of untreated column at low pH (pH< 4). This proves that when the surface is treated with the hydrocarbon-phase the elution time for the neutral marker increases, indicating that EOF is decreased, since the bonds between the silanol groups and the marker decrease.

The reproducibilities of hydrocarbon capillary columns were investigated. The reproducibilities were evaluated by comparing the relative standard deviation (RSD) of the retention time of the o-T obtained on C_2 and C_2–EB columns for five replicate analyses, using 40 m*M* phosphate buffer at pH 2.50 and applied voltage, 266.7 V cm^{-1}. The RSD shown in the run-to-run, day-to-day and capillary-to-capillary analyses was ≤2.5% and good reproducibilities were obtained.

The capillaries coated with *n*-hydrocarbons and *n*-hydrocarbon–EB were continuously swept with the pH 10.0 buffer by the EOF. After every 2 h the buffer was changed and the EOF remeasured. Over a 50-h period of continuous treatment with buffer of pH 10.0, no EOF increase could be detected. This indicates that the layer is stable. One reason for this stability might be that the hydrophobic surface layer prevents the hydroxyl ions from attacking the Si–0–C bonds of the hydrocarbon groups to the surface. The achievable surface coverage with *n*-hydrocarbons and *n*-hydrocarbon–EB is good.

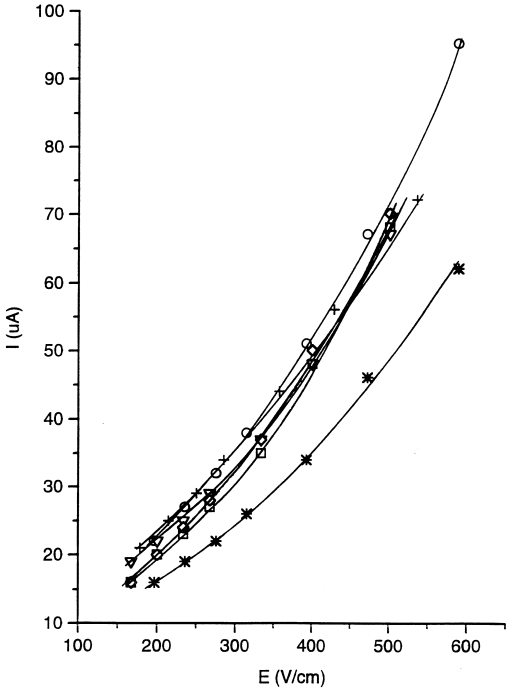

Fig. 4. Plot of field strength versus resulting current for the 40 m*M* phosphate buffer at pH 2.50. Symbols: □=untreated capillary, ○=C_2–EB capillary, *=C_6–EB capillary, ▽=C_8–EB capillary, ◊=C_{10}–EB capillary, +=C_{12}–EB capillary. Capillaries 28 cm (23.5 effective length)×50 μm I.D.; temperature, 25°C and UV detection at 200 nm.

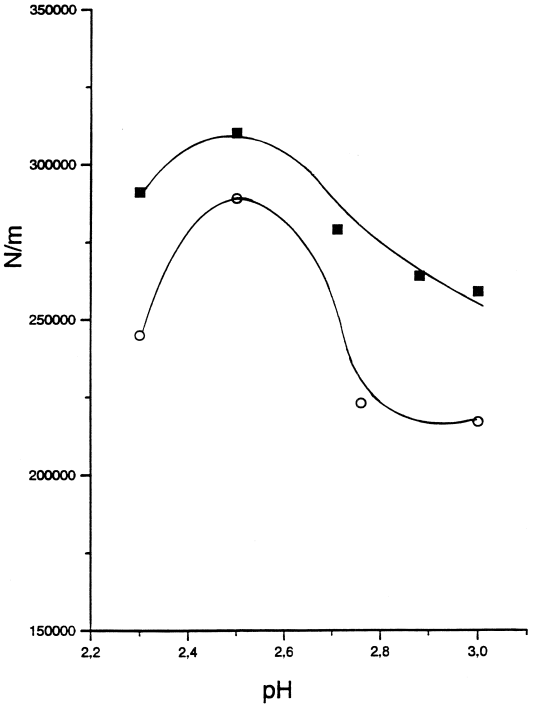

Fig. 5. Influence of the pH on the number of theoretical plates per meter for two amines. ○=Dopamine, ■=benzidine. C_{10} coated capillary column: 30 cm (25.5 cm effective length)×50 μm I.D.; 40 m*M* phosphate buffer; temperature, 25°C and UV detection at 200 nm.

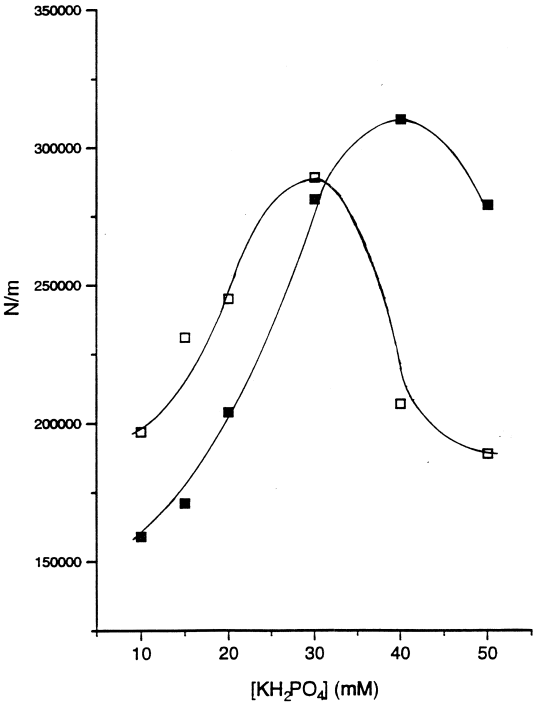

Fig. 6. Influence of the phosphate buffer concentration (pH 2.50, 25°C and 200 nm) on the theoretical plate numbers per meter for two amines. □=Dopamine, ■=benzidine; the same capillary column as Fig. 5.

3.2. Optimization of CZE analysis

The electrophoretic migration velocity and EOF velocity are directly proportional to the electric field. The highest field strengths will bring about the shortest analysis times. According to the electrophoretic theory one could imagine that the highest efficiency would be obtained by working at the highest possible field strengths. But the number of theoretical plates is proportional to the voltage only for low values of E, because heat production limits the application of high field strengths. The influence of voltage on the efficiency is demonstrated in Table 1.

Four amino compounds were separated at 200, 266.7, 333.3 and 400 V cm^{-1}. The number of theoretical plates per meter increased by increasing the field from 200 to 266.7 V cm^{-1}, while the efficiency decreased if field strength was further increased up to 400 V cm^{-1}. The influence of

Table 3
Number of theoretical plates per meter for pharmacological amines obtained on capillary columns coated with *n*-hydrocarbons and *n*-hydrocarbon–EB, 30 m*M* KH_2PO_4 buffer at pH 2.50

Capillary column	*N* m^{-1}			*E* (V cm^{-1})
	Dopamine	Cyclizine	Chetocaine	
C_2	263 000	285 000	235 000	267
C_6	265 000	283 000	115 000	286
C_8	61 000	22 000	54 000	308
C_{10}	289 000	258 000	123 000	267
C_{12}	296 000	267 000	169 000	267
C_2−EB	222 000	238 000	219 000	308
C_6−EB	192 000	141 000	146 000	308
C_8−EB	190 000	225 000	170 000	267
C_{10}−EB	197 000	194 000	79 000	267
C_{12}−EB	202 000	241 000	266 000	286

Table 2
Number of theoretical plates per meter obtained on capillary columns coated with *n*-hydrocarbons and *n*-hydrocarbon–EB, 40 m*M* KH_2PO_4 buffer at pH 2.50.

Capillary column	*N* m^{-1}				*E* (V cm^{-1})
	o-T	B	α-N	4-B	
C_2	320 000	293 000	210 000	219 000	267
C_6	311 000	327 000	241 000	228 000	286
C_8	266 000	297 000	228 000	204 000	308
C_{10}	300 000	310 000	257 000	233 000	267
C_{12}	324 000	277 000	265 000	225 000	267
C_2−EB	270 000	288 000	186 000	218 000	308
C_6−EB	230 000	226 000	156 000	170 000	308
C_8−EB	276 000	292 000	172 000	169 000	267
C_{10}−EB	295 000	275 000	235 000	225 000	267
C_{12}−EB	312 000	314 000	249 000	257 000	286

Table 4
Number of theoretical plates per meter for aromatic acids obtained on capillary columns coated with *n*-hydrocarbons and *n*-hydrocarbon–EB, 10 m*M* Na_2CO_3 buffer at pH 9.30

Capillary column	*N* m^{-1}		*E* (V cm^{-1})
	Pyromellitic acid	Isophthalic acid	
C_2	149 000	86 000	333
C_6	131 000	89 000	357
C_8	165 000	103 000	335
C_{10}	161 000	69 000	333
C_{12}	38 000	36 000	333
C_2–EB	180 000	95 000	385
C_6–EB	137 000	112 000	385
C_8–EB	107 000	63 000	333
C_{10}–EB	48 000	37 000	333
C_{12}–EB	173 000	114 000	357

excessive heat production can be visualized by plotting the applied field strength versus the resultant current, Figs. 3 and 4. According to Ohm's law these plots should be linear. In practice, however, the plot is linear only in the lower voltage range as shown in Fig. 3 and Fig. 4. Deviations from linearity are caused by increased heat generation at higher potential differences. The optimal field strength which should be applied corresponds to the point where the deviation from linearity begins. The same results were obtained by Jorgenson and Lukacs [18,19].

In Fig. 5, the number of theoretical plates (N m^{-1})

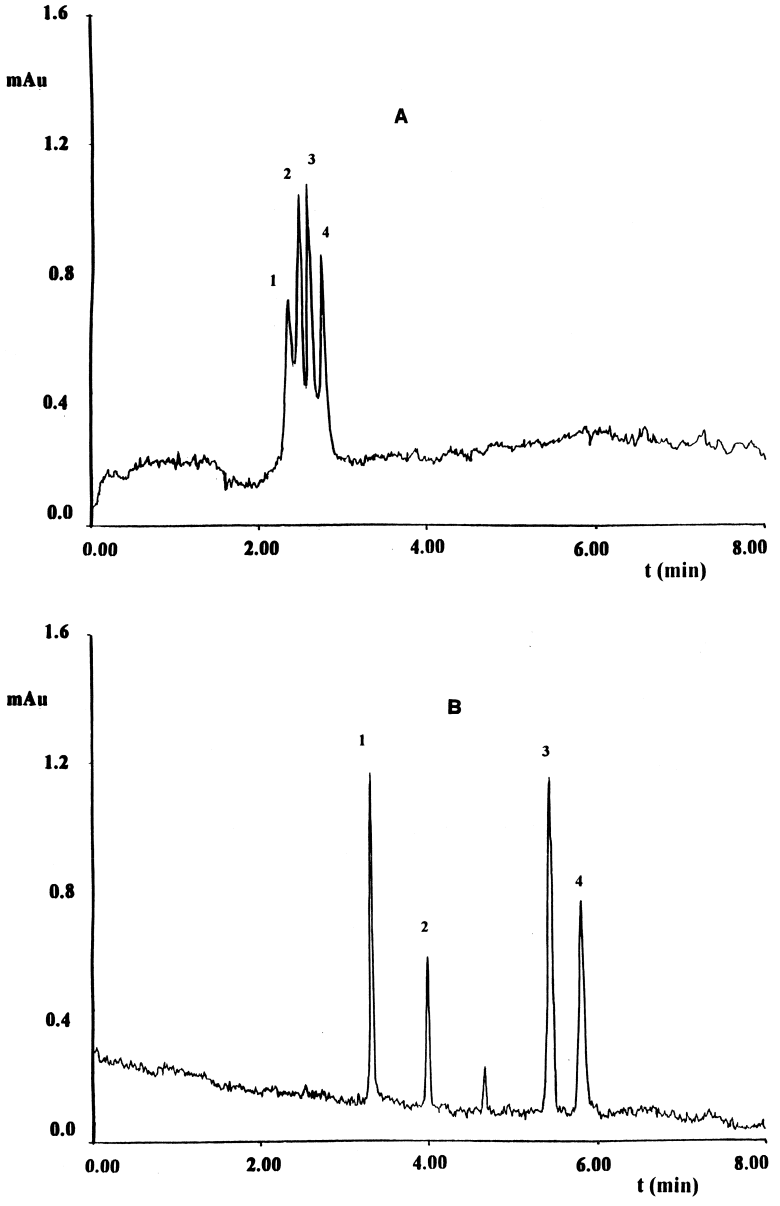

Fig. 7. Comparison of the electropherogram of the aromatic amines obtained from uncoated column (A) and C_6 coated column (B). Conditions: 40 mM phosphate buffer, pH 2.50, temperature 25°C; UV detection 200 nm, applied voltage, 286 V cm^{-1}. Peaks: 1=*o*-tolidine, 2=benzidine, 3=α-naphthylamine, 4=4-biphenylamine.

versus pH is reported. The plate number decreases when the pH increases and the optimum value, for benzidine and dopamine, was obtained at pH 2.50. In Fig. 6, the N m^{-1} versus buffer concentration is reported. For dopamine the optimum buffer concentration was 30 mM, while for benzidine the optimum buffer concentration was 40 mM.

In Table 2 is reported the efficiencies (N m^{-1}) of some aromatic amines obtained on different capillary columns, while in Table 3 are reported the efficien-

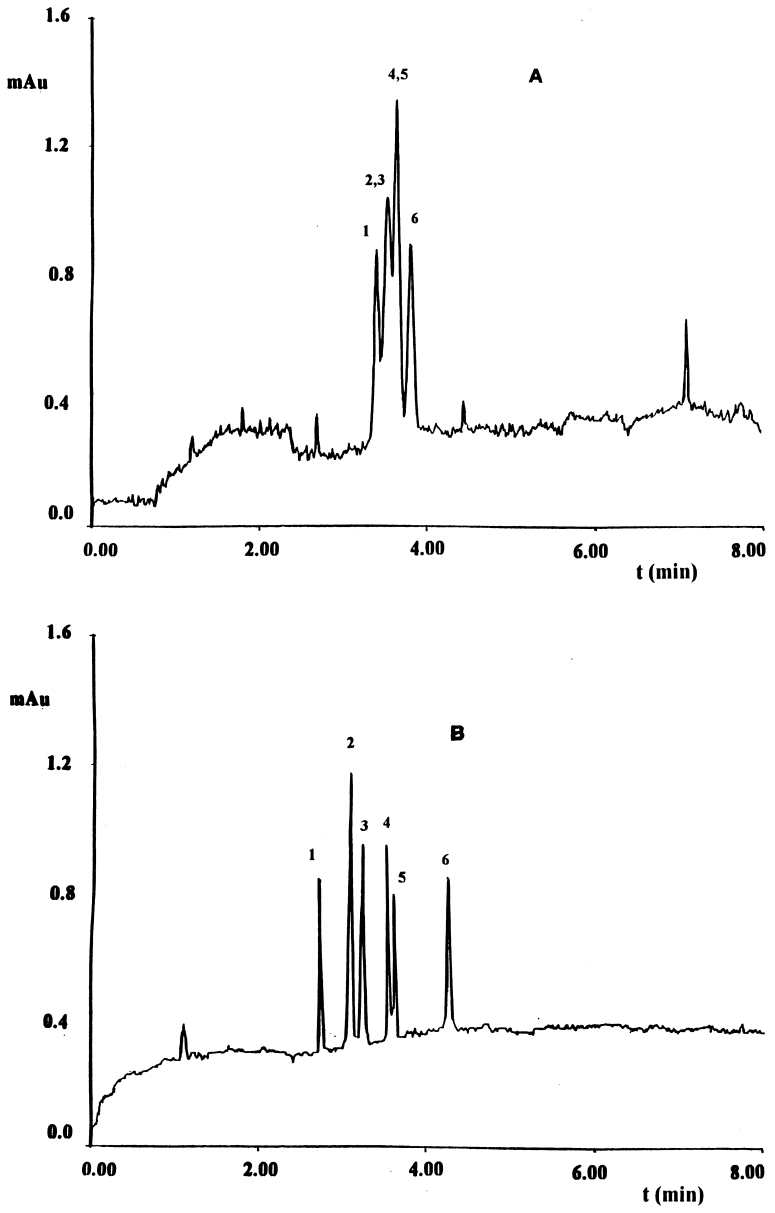

Fig. 8. Comparison of the electropherogram of the pharmacological amines obtained from uncoated column (A) and C_2 coated column (B). Conditions: 30 mM phosphate buffer, pH 2.50; temperature, 25°C; UV detection 200 nm, applied voltage, 267 V cm^{-1}. Peaks: 1=dopamine, 2=cyclizine, 3=4-methoxyphenyl-2-ethylpropylamine, 4=N-methyl-3,3-biphenylpropylamine, 5=diphenhydramine, 6=chetocaine.

cies (N m^{-1}) of some pharmacological amines obtained on the same capillary columns. In Table 4 are reported the efficiencies (N m^{-1}) of two aromatic acids obtained on the same hydrocarbon coated capillary columns. The results show that the highest efficiency using these modified columns was obtained in analysing the aromatic amines. The efficiency of the C_{12} and C_{12}–EB columns may be attributed to the hydrocarbon chains that prevent to the solutes bond with the silanol groups. The ability to perform the amines analyses is evaluated by comparing the separation of the amine test samples on the uncoated and C_6 coated columns. Figs. 7 and 8 show the electropherograms of the amines at pH 2.50.

In the case of the uncoated column the peaks of the amines were not separated, while using the same experimental conditions the solutes were completely separated on the C_6 coated column.

4. Conclusions

A coating procedure was developed to obtain stable hydrocarbon-modified capillaries. The capillary columns coated with the hydrocarbons and EB showed a substantial decrease of the EOF at high pH compared to untreated capillary column, while the EOF of the coated columns show only a slight decrease with respect to that of untreated column a pH$<$4. These capillary columns exhibited a high efficiency, good reproducibility and chemical stability. They can be used for the analysis and the separation of basic compounds such as the amines.

References

[1] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[2] H.H. Laurer, D. Mc Manigill, Anal. Chem. 58 (1986) 166.
[3] R.M. McCormirck, Anal. Chem. 60 (1988) 2322.
[4] M.M. Bushey, J.W. Jorgenson, J. Chromatogr. 480 (1989) 301.
[5] J.A. Lux, H. Yin, G. Schomburg, J. High Resolut. Chromatogr. 31 (1990) 145.
[6] J.K. Towns, F.E. Regnier, J. Chromatogr. 516 (1990) 69.
[7] H. Engelhardt, M.A. Cunot-Walter, J. Chromatogr. 716 (1995) 27.
[8] M. Huang, E. Dubrovcakova-Schneiderman, M. Novotny, H.O. Fatunmbi, M.J. Wirth, J. Microcol. Sep. 6 (1994) 571.
[9] K.A. Cobb, V. Dolnik, M. Novotny, Anal. Chem. 62 (1990) 2478.
[10] M. Huang, W.P. Vorkink, M.L. Lee, J. Microcol. Sep. 4 (1992) 233.
[11] D. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho, B.L. Karger, J. Chromatogr. 652 (1993) 149.
[12] M. Chiari, C. Micheletti, M. Nesi, M. Fazio, P.G. Righetti, Electrophoresis 14 (1994) 177.
[13] T. Izumi, T. Nagahori, T. Okuyama, J. High Resolut. Chromatogr. 14 (1991) 351.
[14] M.W.F. Nielen, J. High Resolut. Chromatogr. 16 (1993) 62.
[15] P.Z. Liu, A. Malik, M.C.J. Kuchar, W.P. Vorkink, M.L. Lee, J. Microcol. Sep. 5 (1993) 245.
[16] P.Z. Liu, A. Malik, M.C.J. Kuchar, M.L. Lee, J. Microcol. Sep. 6 (1994) 581.
[17] Z. Zhao, A. Malik, M.L. Lee, Anal. Chem. 65 (1993) 2747.
[18] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[19] J.W. Jorgenson and Lukacs, Anal. Chem. 53 (1981) 1298