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Influencing electroosmotic flow and selectivity in open tubular electrochromatography by tetrakis(pentafluorophenyl)porphyrin as capillary wall modifier

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Abstract

A physically adsorbed and covalently bonded porphyrin derivative, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, H_2TPFPP, has been used as a fused-silica capillary wall modifier in open tubular capillary electrochromatography (OT-CEC), and its influence on the electroosmotic flow (EOF) velocity and on the selectivity of OT-CEC separations of a set of model aromatic carboxylic acids has been tested. Whereas most of the coatings of this category bring about an increase in selectivity with a concomitant slow down of the EOF, H_2TPFPP coating, depending on pH of the background electrolyte used, resulted both in decreasing of EOF at pH 8.5 by 5% and in increasing of EOF by 10–43% at pH 6 and 5, respectively. The separation efficiency and the resolution of aromatic carboxylic acids separation in coated capillaries, namely in that one with covalent coating, were better than in the bare fused-silica capillary. The perspectives of H_2TPFPP as capillary wall modifier are visualized in introducing well defined electroosmotic properties of materials used for miniaturized separation channels preparation in chip-based electromigration devices.

Keywords: Electroosmotic flow; Selectivity; Electrochromatography; Coated capillaries; pH effects; Tetrakis(pentafluorophenyl)porphyrin; Carboxylic acids

1. Introduction

Synthetic porphyrin derivatives and expanded porphyrins with specific modifications of their structure exhibit distinct selectivity for a number of functionalities, e.g. typically phosphate, nitrate and amino groups [1–4]. This property of porphyrin derivatives has been broadly exploited in analytical chemistry, including their application for analysis of different types of compounds by chromatographic and electromigration separation techniques, for a review see Ref. [5]. The selectivity of porphyrin moieties has been exploited also in the constructions of specific sensors [6,7] as well as for both covalent and dynamic coatings of the inner fused-silica capillary walls in open tubular capillary electrochromatography, OT-CEC, [8–11].

Coating of the inner fused-silica capillary surface is known to slow down the electroosmotic flow (EOF) by shielding the silanol groups of the inner

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capillary surface. However, in a systematic investigation of oligopyrrolic macrocycles as inner capillary wall modifiers in OT-CEC we have observed that physically adsorbed or covalently bonded tetrakis(pentafluorophenyl)-porhyrin, H_2TPFPP, just on the contrary to a set of other porphyrin derivatives, is capable of increasing the EOF of the background electrolyte (BGE). To demonstrate this effect we have used a set of aromatic moiety possessing carboxylic acids as model compounds.

Carboxylic acids are relatively easy to separate by different capillary electromigration methods, zone electrophoresis, isotachophoresis and electrokinetic chromatography [12–15]. The only problem is detection, which is in an zone elelectrophoretic mode usually solved by indirect detection, however, other detection methods can be applied as well [16–19]. For derivatized carboxylic acids naturally other than purely electrophoretic mechanism have to be exploited [20]. This approach increases the detectability of the solutes, however, other than ionic interactions need to be exploited for their separation [21]. Consequently, electrochromatographic techniques appear the methods of choice. In capillary electrochromatography (CEC) fatty acids can be analyzed as free acids, phenacyl- or methyl esters [20]. For free fatty acids reversed-phase liquid chromatography separation with acetonitrile–50 m*M*–2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 6.0 (9:1, v/v) can be successfully applied [21]. Basically the same reversed-phase system can be used also for fatty acid-phenacyl esters (for other electrochromatographic systems see Refs. [22–29]). Aromatic moiety possessing carboxylic acids can be separated both in the purely electrophoretic or electrochromatographic mode as neither their detection nor the separation mechanisms exploited do not bring about any further complications.

During the present investigation we have tried to elucidate the influence of porphyrin derivative, tetrakis(pentafluorophenyl)porphyrin (H_2TPFPP), on the selectivity of OT-CEC separations of a model set of aromatic moiety possessing carboxylic acids. For preparation of chemically bonded phase we have used the known affinity of H_2TPFPP toward nucleophilic attack, our synthetic strategy for coating was based on generation of anion silanol groups on inner capillary surface which in turn were employed for nucleophilic substitution at para position of H_2TPFPP.

2. Experimental

2.1. Instrumentation

All experiments were performed using SpectraPhoresis 500 apparatus from Thermo Separation Products (TSP, Riviera Beach, FL, USA), controlled with PC 1000 Version 2.6 software (supported on OS2 2.1), equipped with the on-line, variable-wavelength UV-absorption detector set to 215 nm.

The separations were run in three types of capillaries. The first type was an untreated fused-silica capillary, 50 μm I.D.×375 μm O.D. fitted into the TSP cartridge. The total capillary length was 43 cm, effective length (to the detector) 35.5 cm. The other two capillaries were modified with 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin derivative (for the formula of the porphyrin derivative see Fig. 1). Other characteristics of the modified capillaries were the same as specified above for the bare fused-silica capillary. The separations were run at 15 kV (14–33 μA depending on the BGE composition), at 25 °C and the analysis time was up to 30 min; detection was done at 215 nm.

An uncoated fused-silica capillary was conditioned by sequential washings for 5 min with water, 10 min with 1 mol/l NaOH, 5 min with water and finally 5 min with the running buffer. For reconditioning the capillary was washed with the running buffer after each run for 5 min. The modified capillaries were conditioned by sequential washings with the running buffer for 5 min.

2.2. Reagents

Sodium tetraborate dihydrate, sodium dihydrogenphosphate, sodium hydroxide, hydrochloric acid and methanol, all of them with analytical grade purity, were obtained from Lachema (Brno, Czech Republic). Carboxylic aromatic acids: acetylsalicylic acid, 4-aminobenzoic acid, salicylic acid, benzoic acid, 4-hydroxybenzoic acid, 4-nitrophthalic acid, phthalic acid, 5-nitroisophthalic acid, terephthalic acid, isophthalic acid, 2-sulphobenzoic acid and thiourea

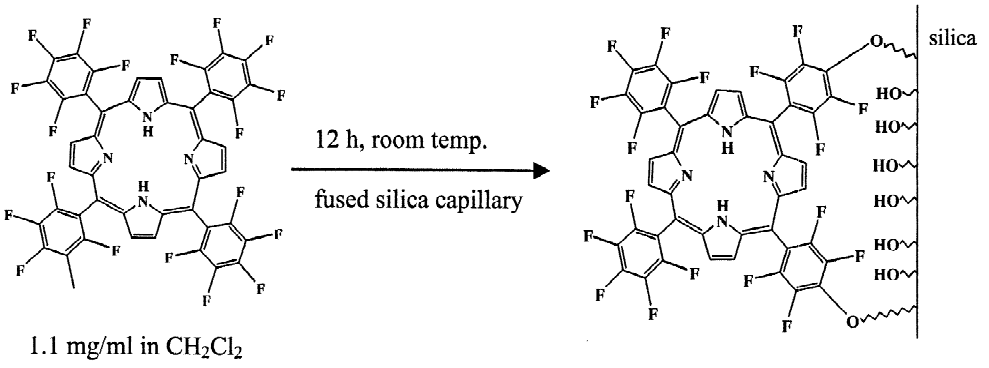

Fig. 1. The structure and the manner of covalent attachment of porphyrin derivative, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, H_2TPFPP, onto the fused-silica capillary surface.

were obtained from Sigma–Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland). The porphyrin derivative, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, was obtained from Fluka (Buchs, Switzerland).

Samples of carboxylic acids for analysis were prepared by dissolving of appropriate amount (1.2–4 mg) of each solute in 200 μl of Milli-Q water and stored below 4 °C. Isophthalic (1.3 mg) and 5-nitroisophthalic (1.5 mg) acids were dissolved in 200 μl of Milli-Q water and 50 μl of methanol. The volumes of injected stock solutions of amino acids were in the range of 1–3 μl. All samples were injected hydrodynamically for 2 s.

2.3. Coating of the capillary wall

A new fused-silica capillary was sequentially washed with water (10 min), 1 mol/l NaOH (3 h) and methanol (10 min). Next it was dried in the air stream for 10 min. Then the capillary was filled with the solution of the porphyrin derivative in dichloromethane (1.1 mg/ml), both ends of capillary were closed and the capillary was left overnight at the room temperature. We presume that the immobilization reaction proceeds according to scheme presented in Fig. 1 [30]. Actually we do not exactly know if the bond to silica is of single- or multitopic character. After this procedure the capillary was washed with methanol (5 min) and flushed with air for 10 min. Further the capillary was washed with water (10 min) and the running buffer (10 min). Running the separation buffer in the capillary at 15 kV for a period of 30 min was used for capillary coating stabilization and equilibration.

In addition, capillary modified simply by physical adsorption of our modifier onto the capillary wall was prepared as well. The fused-silica capillary was pretreated by subsequent washing with water, 1 mol/l NaOH, water and methanol. Each step took 10 min. Then the capillary was dried in air stream for 10 min. Further, the capillary was filled with the solution of porphyrin derivative in dichloromethane (1.1 mg/ml), and left in the vacuum oven for 2 h at 60 °C. Finally the capillary was flushed with methanol, water and the running buffer, each step for 5 min. Capillary was again stabilized using background electrolyte and applying 15 kV for 30 min.

2.4. Background electrolytes

The first BGE tested was 0.025 mol/l sodium borate buffer, pH 8.5. In addition, 0.025 mol/l sodium phosphate buffers at pH 5.0 and 6.0 were used as BGEs. Separations were performed both in the unmodified, bare fused-silica capillary and in the physically adsorbed and covalently porphyrin derivative modified capillaries, and the results were compared.

3. Results and discussion

During the experimental part of this study we have investigated 5,10,15,20-tetrakis(pentafluorophenyl)-porphyrin bonded fused-silica capillaries for the OT-

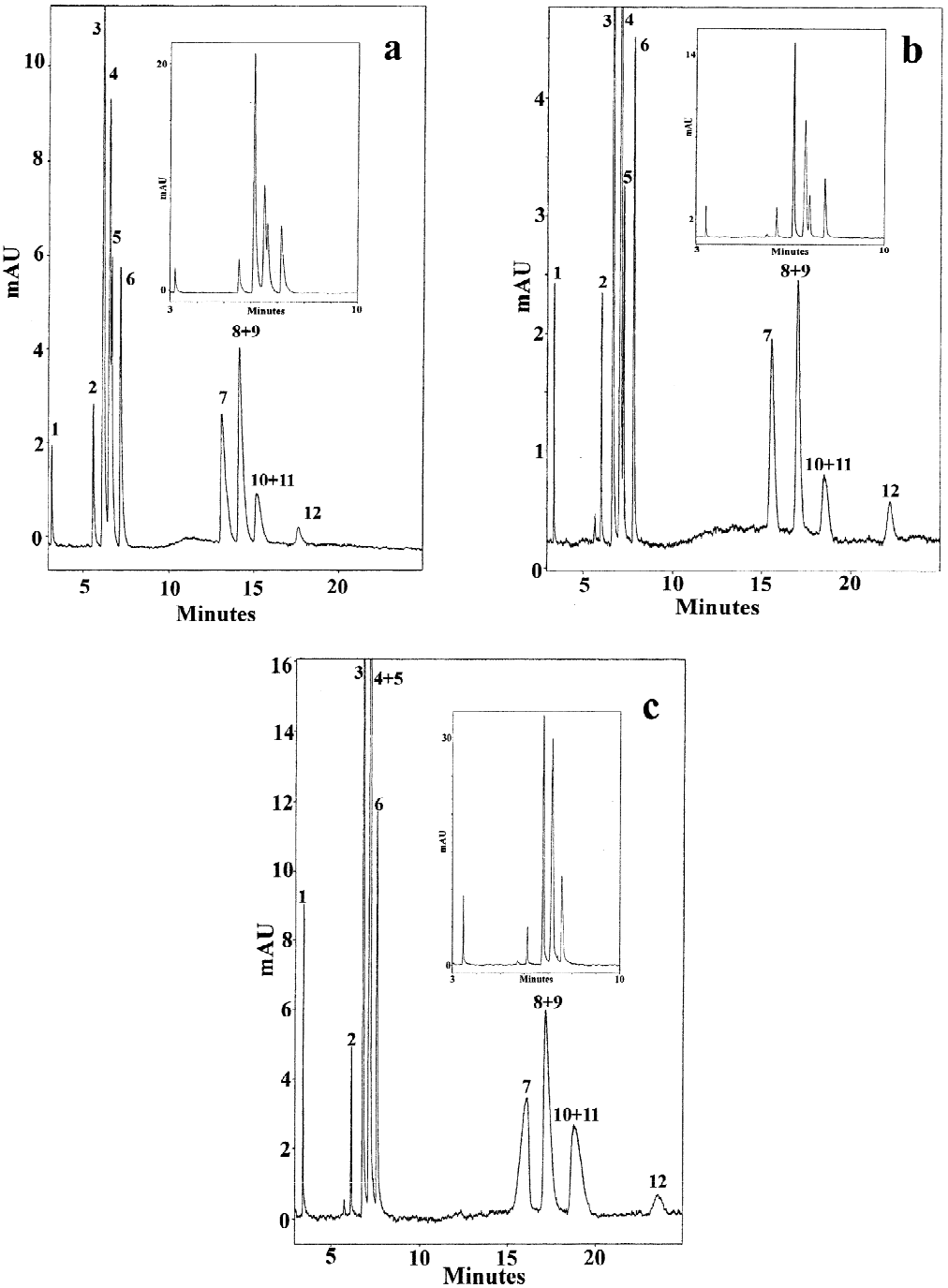

Fig. 2. Comparison of the CZE and OT-CEC separations of the model set of aromatic carboxylic acids in the bare fused-silica (a), covalently (b) and physically (by adsorption) (c) modified fused-silica capillaries at pH 8.5 (0.05 *M* borate BGE). Peak identification: (1) thiourea, (2) acetylsalicylic acid, (3) 4-aminobenzoic acid, (4) salicylic acid, (5) benzoic acid, (6) 4-hydroxybenzoic acid, (7) 4-nitrophthalic acid, (8) phthalic acid, (9) 5-nitroisophthalic acid, (10) terephthalic acid, (11) isophthalic acid, (12) 2-sulphobenzoic acid. For experimental conditions see the text.

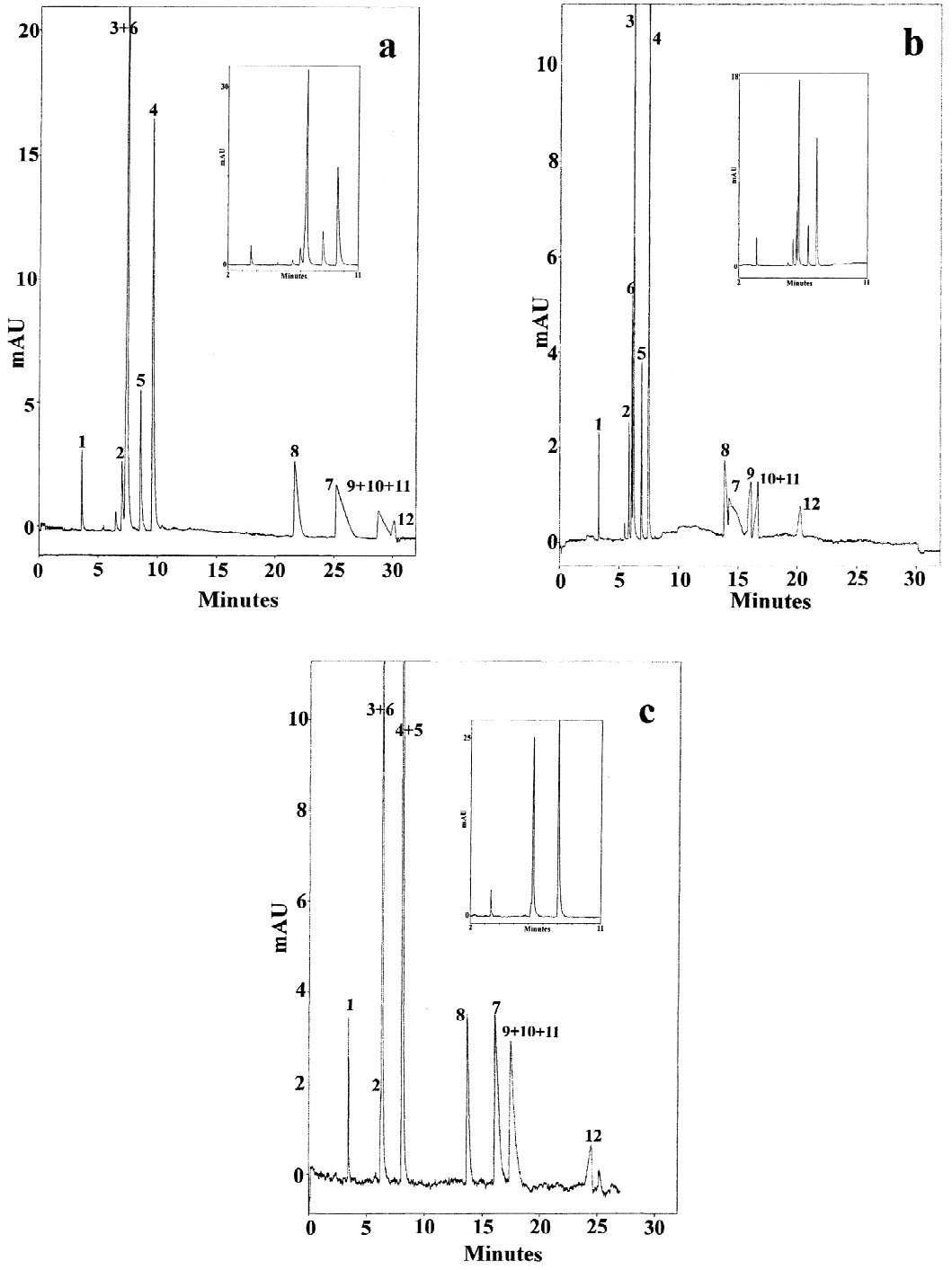

Fig. 3. Comparison of the CZE and OT-CEC separations of the model set of aromatic carboxylic acids in the bare fused-silica (a), covalently (b) and physically (by adsorption) (c) modified capillaries at pH 6.0 (phosphate BGE). Peak identification and other conditions as in Fig. 2.

CEC separations of a model set of aromatic carboxylic acids. The separations were done both in the capillary with chemically bonded modifier and in the capillary with physically adsorbed modifier at three different pH values (8.5, 6.0 and 5.0). Typical separations are presented in Figs. 2–4. At pH 8.5 and 6.0, respectively, the separations were done in the standard polarity mode (injection at the anodic side of the capillary). On the contrary, at pH 5 the separations had to be done in the reversed polarity mode, i.e. with sample injection at the cathodic end of the capillary.

While at the alkaline pH capillary coating resulted (as expected) in a decrease of the electroosmotic flow (EOF), al lower pH values (in particular pH 5.0) an increase of the EOF, roughly by 40% was observed (see Table 1). No matter whether the separation was materialized at alkaline (8.5) or acid (5.0, 6.0) pH values, the coated capillary exhibited nearly always a considerably higher number of theoretical plates (see Tables 2 and 3). On the other hand, there were critical pairs that were not resolved neither by a pH change nor by the chemical modification of the inner capillary surface. In alkaline pH (8.5) the unresolved pairs were phthalic/5-nitroisophthalic and terephthalic/isophthalic acids, for resolution in alkaline BGE, pH 8.5, see Table 4. The resolution of benzoic acid from salicylic acid was always less than one; though in covalently modified

Table 1
Comparison of electroosmotic flow mobilities m_{EOF} in uncoated fused-silica capillaries and in capillaries covalently coated with tetrakis(pentafluorophenyl)porphyrin at three different pH values of the BGE

pH of BGE	m_{EOF} ($m^2\ V^{-1}\ s^{-1}$)		EOF change in coated capillary[a] (%)
	Uncoated capillary	Covalently coated capillary	
8.5	53.6	50.7	5.4 (decrease)
6.0	47.3	52.2	10.4 (increase)
5.0	28.7	41.0	42.9 (increase)

[a] Electroosmotic flow mobility (m_{EOF}) in the covalently coated capillary related to that in the uncoated capillary.

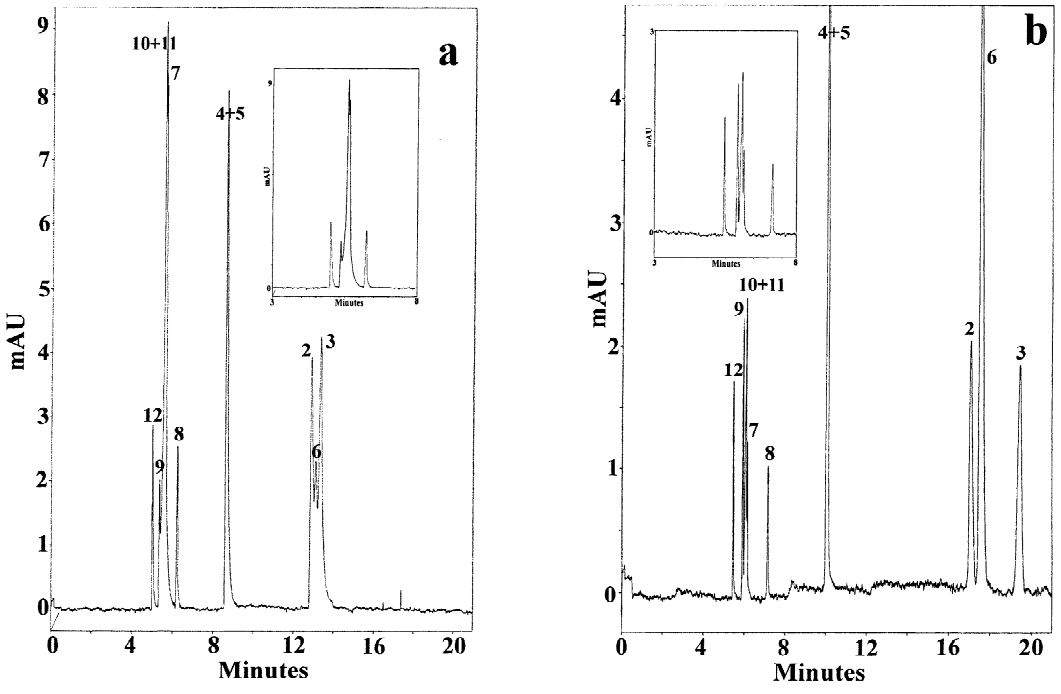

Fig. 4. Comparison of the CZE and OT-CEC separations of the model set of aromatic carboxylic acids in the bare fused-silica (a) and in the covalently modified (b) capillaries at pH 5.0 (phosphate BGE). Peak identification and other experimental conditions as in Fig. 2. Reversed polarity (injection at the cathodic capillary end). In OT-CEC separation (b) the sample was spiked with 4-hydroxybenzoic acid (peak no. 6).

Table 2
Number of theoretical plates obtained for separation of aromatic carboxylic acids in alkaline BGE, pH 8.5, in uncoated and in H_2TPFPP covalently coated fused-silica capillary

Compound	Peak. No.	Number of theoretical plates	
		Uncoated capillary	Covalently coated capillary
Thiourea	1	2805	3463
Acetylsalicylic acid	2	16 154	29 793
4-Aminobenzoic acid	3	5851	15 562
Salicylic acid	4	7180	10 175
Benzoic acid	5	15 646	60 691
4-Hydroxybenzoic acid	6	12 062	42 902
4-Nitrophthalic acid	7	5912	11 723
Phthalic acid	8	10 243	20 258
5-Nitroisophthalic acid	9	Unresolved	Unresolved
Terephthalic acid	10	6616	8000
Isophthalic acid	11	Unresolved	Unresolved
2-Sulphobenzoic acid	12	12 151	12 238

For separation conditions see the text.

capillary it was considerably better ($R_s = 0.98$) as compared to the separation in bare silica capillary column ($R_s = 0.60$ in untreated capillary). In the capillary with adsorbed stationary phase these two acids co-eluted.

At pH 5.0 (see Table 5) in the uncoated capillary the unresolved carboxylic acids were terephthalic/isophthalic/4-nitrophthalic acids (unresolved triplet) and benzoic/salicylic acid. In porphyrin coated capillary (covalent attachment) only terephthalic/isophthalic and benzoic/salicylic acids represented the critical pairs though, admittedly, the resolution of nitrophthalic acid from the terephthalic/isophthalic peak was incomplete ($R_s = 0.58$).

When comparing resolution obtained at all pHs investigated, both with coated and uncoated capillaries, the coated capillaries offered always better results, provided that the modifier was chemically bonded to the capillary wall. In adsorbed coating the results obtained at pH 8.5 were mostly worse than with chemically modified capillary but better than with the untreated capillary column (except for the separation of salicylic and benzoic acids which co-eluted). The survey of these results is presented in the quoted Tables 4 and 5. It is feasible to assume that changes in selectivity and EOF introduced by using expanded porphyrins as wall modifiers can be purposefully exploited in miniaturized, chip-based separation systems.

4. Conclusion

Coating of the inner capillary surface with 5,10,15,20-tetrakis(pentafluorophenyl)-porphyrin offered better resolution of the test mixture of aromatic carboxylic acids at all pHs tested (5.0, 6.0, 8.5). Both the covalent coating and the physical adsorp-

Table 3
Number of theoretical plates obtained for separation of aromatic carboxylic acids in acid BGE, pH 5, in uncoated and in H_2TPFPP covalently coated fused-silica capillary

Compound	Peak No.	Number of theoretical plates	
		Uncoated capillary	Covalently coated capillary
2-Sulphobenzoic acid	12	7790	21 988
5-Nitroisophthalic acid	9	11 469	18 158
Terephthalic acid	10	2328	8864
Isophthalic acid	11	Unresolved	Unresolved
4-Nitrophthalic acid	7	Not determined	84 497
Phthalic acid	8	23 406	153 581
Benzoic acid	5	15 100	29 120
Salicylic acid	4	Unresolved	Unresolved
Acetylsalicylic acid	2	20 039	46 032
4-Hydroxybenzoic acid	6	21 002	32 634
4-Aminobenzoic acid	3	13 241	26 760

For separation conditions see the text.

Table 4
Resolution (from the preceding peak) of the separation of carboxylic acids in alkaline BGE, pH 8.5, using uncoated, covalently coated and physically coated fused-silica capillaries

Compound	Peak No.	Resolution		
		Uncoated capillary	Covalently coated capillary	Physically coated capillary
Thiourea	1			
Acetylsalicylic acid	2	10	19.5	20.7
4-Aminobenzoic acid	3	1.8	3	4.1
Salicylic acid	4	1.27	2	1.25
Benzoic acid	5	0.6	0.98	Unresolved
4-Hydroxybenzoic acid	6	1.75	2.71	1.25
4-Nitrophthalic acid	7	10.87	19.6	15.9
Phthalic acid	8	1.58	2.7	1.16
Nitroisophthalic acid	9	Unresolved	Unresolved	Unresolved
Terepthalic acid	10	1.43	2.63	1.69
Isophthalic acid	11	Unresolved	Unresolved	Unresolved
2-Sulphobenzoic acid	12	4.18	6.35	4.96

tion of the modifier to the capillary wall resulted in similar results at alkaline (pH 8.5) and slightly acidic (pH 6.0) BGEs, however, the results were better with covalently coated capillaries. At pH 5.0 only the chemical, covalent modification can be used since physically adsorbed coating lead to irreproducible results. Regarding separation both the efficiency (number of theoretical plates) and resolution were better in the coated than in the uncoated capillary. Differences in the EOF were also observed. While EOF was slowed down at alkaline pH, at acid pH a distinct speed up of the electroosmotic flow was observed in covalently modified capillary.

Table 5
Resolution (from the preceding peak) of the separation of the aromatic carboxylic acids in acid BGE, pH 5, using uncoated and covalently coated fused-silica capillaries[a]

Compound	Peak No.	Resolution	
		Uncoated capillary	Covalently coated capillary
2-Sulphobenzoic acid	12		
5-Nitroisophthalic acid	9	1.78	2.83
Terephtahlic acid	10	0.56	0.92
Isophthalic acid	11	Unresolved	Unresolved
4-Nitrophthalic acid	7	Unresolved	0.58
Phthalic acid	8	1.61	8.78
Benzoic acid	5	5.35	10.58
Salicylic acid	4	Unresolved	Unresolved
Acetylsalicylic acid	2	7.36	18.76
4-Hydroxybenzoic acid	6	0.44	1.19
4-Aminobenzoic acid	3	0.48	3.88

[a] No reproducible results were obtained at this pH with physically adsorbed coating.

Acknowledgements

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