Porphyrin Based Affinity Interactions: Analytical Applications with Special Reference to Open Tubular Capillary Electrochromatography

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Summary: This review offers a brief summary of results obtained from separations of the sets of biologically relevant compounds (carboxylic acids, amino acids, peptides, nucleotides and organic phosphates) using open tubular capillary electrochromatography (OT-CEC), in which oligopyrrolic macrocycles serve as the modifiers of the inner fused silica capillary wall surface. Types of interactions leading to improved selectivity of the open tubular capillary electrochromatographic systems and/or to the change of the speed of the electroosmotic flow are also discussed. In addition, selected comparative data on the application of oligopyrrolic macrocycles in other analytical methods, particularly chromatographic and spectrometric techniques, are presented.

Keywords: Oligopyrrolic macrocycles / non-covalent interaction / open tubular capillary electrochromatography / OT-CEC / review.

1. INTRODUCTION

Owing to their multifunctional structure and complex formation abilities, porphyrins play not only a very important role in metabolic processes, but a number of the chemical features of this category of compounds can be exploited in analytical chemistry as well. Spectrophotometric, potentiometric and voltammetric methods for the analysis of a broad series of anions and cations can be found in the literature (for review see ref. [1]); scattered information refers to the analytical methods (voltammetric) for selected drugs, alcohols, amines (neurotransmitters) and DNA preparations. Peptides, saccharides, fullerenes and permanent gases represent other possible applications. Using porphyrins for construction of artificial receptors and sensors has been reviewed in depth in ref. [2].

Regarding chromatographic and electromigration techniques, the application of various porphyrin types as affiants is at hand. The current trends towards miniaturization favor the capillary electrochromatographic approach (see ref. [3-5]); this is why electrochromatographic approaches are emphasized in this review. On the other hand, purely chromatographic approaches offer similar possibilities and this is why adequate attention is paid to these techniques on the next pages. Some perspective complexation and binding interactions not yet exploited neither in the chromatographic nor electrochromatographic set-ups are paid marginal attention as well.

Several different ways of preparing columns for capillary electrochromatography (CEC) have been developed over the

monolithiclast years including packed capillaries, open tubular and columns. Several exhaustive reviews have been published about the fundamental aspects and column technologies [6-8] as well as the special reviews concerning CEC with physically and dynamically adsorbed phases [9,10], separation of enantiomers [11-14], application of etched columns [15] and application of CEC in analysis of different categories of compounds [16, 17], advances in sol-gel columns for CEC [18], polymeric monolithic stationary phases for CEC [19, 20] and microchannel wall coatings for protein separations by capillary and chip electrophoresis [21]. Summarizing monographs about CEC modes [22] and a special monograph dedicated to monolithic columns [23] have been published as well. The aim of this review is not to present another summary about used stationary phases; in this respect the potential reader is referred to the reviews of his particular field of interest and the literature cited herein [19-23].

The following pages are dedicated to the relatively new type, porphyrin derived modifiers used to change the inner capillary surface character for open tubular capillary electrochromatography (OT–CEC). Oligopyrrolic macrocycles seem to be promising candidates to broaden the family of commonly used modifiers since the application of oligopyrrole based receptors has been widely exploited in many branches of analytical chemistry, as mentioned above [1, 2].

The idea of using metalloporphyrins as capillary wall modifiers (stationary phases) in open tubular capillary electrochromatography opens wide possibilities for introducing specific solute-stationary phase interactions which can bring about considerable improvements in the selectivity of the separation system. In addition the non-covalent interaction of the solutes involved can improve the analytes' detectability as well. This has been clearly demonstrated in a recent paper of Charvatova *et al*. [66] for the case of amino acid separations. While for the amino acid separations a

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number of well established methods is available, the separation of peptides, in particular the separation of their complex mixtures still needs badly an improvement in selectivity. The general guidelines for selection of a particular (metallo) porphyrin derivative for the separation of a given set of analytes runs as follows: First, a bulky periphery substitution of the porphyrin core provides sufficient lipophilicity of the derivative which in turn offers a long term stability of the capillary coating and, consequently, offers an easy way of modifying the inner capillary surface. A modifier (for instance a metallocomplex) can provide simultaneously axial ligand binding; multiple ligand binding can be easily materialized which in turn enables efficient separation. Typically a combination of - stacking and hydrophobic interaction in combination with axial ligand binding offers a selectivity increase for aromatic amino acids and low molecular mass peptides as discussed in detail in sections 4.3 and 4.4 of this review.

2. PORPHYRIN BASED SYSTEMS

2.1. Basic Chemistry

The general description of porphyrins can be found in ref [22,24]. Porphin is the parent heterocycle which possesses eight peripheral (or "beta") pyrrolic hydrogens and four interpyrrolic (meso, methine) hydrogen atoms (Fig. **1**). Porphin has very low solubility in almost all common solvents and thus it has been only sparingly used in chemical or spectroscopic studies of the porphyrin systems. Porphyrins are the porphin derivatives substituted with various groups at the -pyrrol peripheral and/or methine positions. Such derivatives are not only more soluble, but also resemble more closely the naturally occurring compounds.
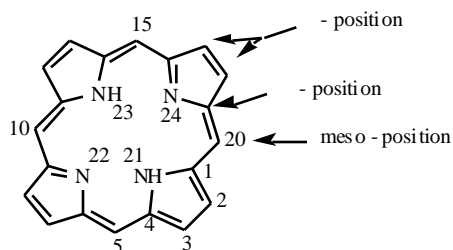
Fig. (1). Porphin structure and its numbering.

What kind of substitution can be introduced in order to influence separation behavior in terms of specific interaction and what kind of proton transfer equilibrium can play role? There are the following possibilities: the porphyrin ring can be substituted to various extents either in the "beta" or meso-positions. The porphyrin macrocycle is an ampholyte with two pyrrolenine (=N–) nitrogen atoms capable of accepting protons, and two NH groups capable of deprotonation. The most useful scheme is as follows:

$$PH^- \qquad P^{2-} \qquad + H^+ \qquad (pK_{1,0})$$
$$PH_2 \qquad PH^- \qquad + H^+ \qquad (pK_{2,1})$$
$$PH_3^+ \qquad PH_2 \qquad + H^+ \qquad (pK_{3,2})$$
$$PH_4^{2+} \qquad PH_3^+ \qquad + H^+ \qquad (pK_{4,3}),$$

where metal-free porphyrin is abbreviated PH_2, the dianion P^{2-}, and the dication PH_4^{2+}.

Porphyrins behave as very weak acids, strong bases such as alkoxides, can remove the two protons ($pK_{2,1}$, $pK_{1,0}$ 16) on the inner nitrogen atoms to form a dianion. On the other hand, the two pyrrolenine nitrogen atoms ($pK_{3,2}$ and $pK_{4,3}$ 5-5.5) can be protonated easily with acids such as trifluoroacetic acid. Generally, $pK_{3,2}$ and $pK_{4,3}$ are very similar.

The porphyrin core is a perfect ligand for a large set of cations; complexes with most metals in the periodic table have been prepared and characterized [24-26]. Example of their chromatographic separation is shown in Fig. **2**. The facility of metal complexes formation is underscored by the fact that most systems with any type of physiological function occur as metal complexes (*e.g.* Fe in hemoglobins, myoglobins, cytochromes; Mg in chlorophylls and bacteriochlorophylls, Co in vitamin B_{12}). Insertion of metals into the porphyrin core occurs with variable ease (and difficulty), and dramatically affects the reactivity and binding ability of the porphyrin macrocycle. For example, for efficient electrophilic substitution of the ring, metals can be chosen which effectively release electron density to the organic porphyrin ligand, while reduction is best carried out on metalloporphyrins in which the metal tends to deplete the porphyrin ligand of its electron density by way of back-bonding [24].
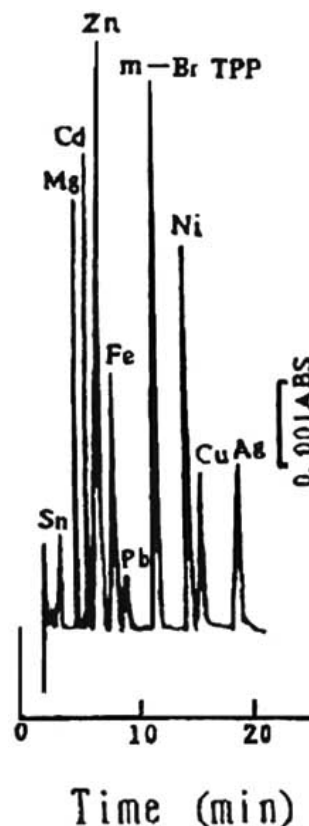
Fig. (2). Chromatogram of metal-*m*–BrTPP [metal-*meso*-tetrakis(3-bromophenyl)porphine] complexes. Column, YQG-C$_{18}$ (150 × 5 mm i.d., 5 μm); mobile phase, methanol-acetonitrile (65:35, v/v); flow rate 1.0 ml/min and 3.0 mol/min after Pb has eluted; detection at 420 nm; sensitivity 0.05 a.u.f.s.; injection volume 20 μl containing 10 ng Mg and Zn, 55 ng Cd and Fe, 65 ng Sn, 80 ng Ni, 85 ng Pb, 130 ng Cu and 145 ng Ag. (Reprinted with permission from [26], copyright (1997) Elsevier Sciences B.V.).

Table 1. Modification Procedures For Immobilization of Oligopyrrolic Macrocycles To The Fused Silica Capillary [35, 64-70]

Type of modification procedure		
Adsorption	**Covalent attachment**	**Covalent attachment via a spacer**
Methanol wash (20 min)	Pretreatment with 1M NaOH prolonged to 3 h	Acetone wash 20 min
Drying step (30 min)	Methanol wash 10 min	Drying
Flushing with the solution of oligopyrrole in organic solvent (~1mg/ml)	Drying 10 min	Capillary filled with 15% (v/v) APS in toluene, left to stand overnight at room temperature
Drying	Filling the capillary with the oligopyrrole solution in organic solvent, (~1mg/ml), sealing both ends, left in the capillary overnight at room temperature	Toluene and acetone wash, each step 10 min
Methanol wash (10 min)	Methanol wash 5 min	Rinsing with 2% solution (v/v) of TEA in acetone 15 min
	Drying	Acetone wash 30 min
		Drying
		Rinsing the capillary with the solution of oligopyrrole in DMF containing 2% TEA
		Sealing both ends, standing in 50°C bath for 3 h, left at room temperature overnight
		Acetone and water rinsing, each step 30 min
		Drying

Stabilization step - Final rinsing with water (10 min), running buffer (at least 15 min), stabilization of the capillary under high voltage drying was usually performed by flushing the air stream through the capillary or the modified column was dried in the oven.

In the most general sense, porphyrin analogues may be defined as consisting of all pyrrole-containing macrocycles that are not naturally occurring (for review see ref. [27]). This definition has generally been further restricted by the porphyrin community to include systems that either (1) contain four or more pyrroles and some degree of conjugation or (2) possess only four pyrroles but little or no conjugation. Traditionally, the first of these two groups has been represented by porphyrin analogues that are contracted, expanded or isomeric with respect to porphyrin itself. As examples of the former type of system, sapphyrins and phthalocyanines can be noticed. General structures of the latter two are shown on Fig. **3**.

Sapphyrin based macrocycles have been widely presented as anion binding agents [28-33], phthalocyanines have been found to function well as photosensitizers in photodynamic therapy (PDT) of cancer [34].

3. IMMOBILIZATION PROCEDURES

For the immobilization of oligopyrrolic macrocycles to the fused silica (FS) capillary wall three modes were applied the main steps of which are summarized in Table **1**. Prior to each type of modification the capillaries were pretreated as follows:

Drying was usually performed by flushing the air stream through the capillary or the modified column was dried in the oven

i.   Rinsing with water for 5 min

ii.   Rinsing with 1 M NaOH solution for 30 min

iii.   Flushing with water for 10 min

Three spectral methods have been employed to investigate whether and to what extent the porphyrin/porphyrin analogue is immobilized on the capillary wall, namely:

i) Laser induced fluorescence measurements (LIF)

ii) Raman spectroscopy measurements

iii) Diffuse reflectance infrared Fourier transformation (DRIFT)

Details of these measurements are beyond the scope of this review, they are described in [35].
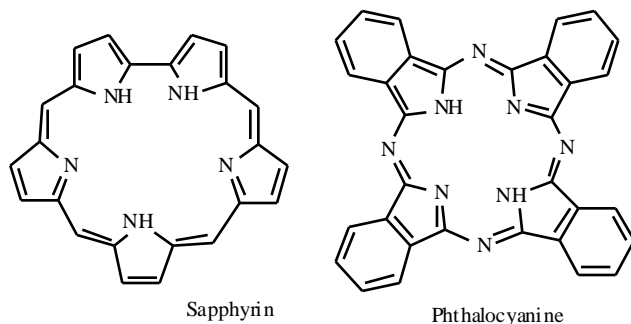

Sapphyrin   Phthalocyanine

Fig. (3). Structures of porphyrin analogues.

4. APPLICATIONS

4.1. General

Applications of porphyrins in analytical chemistry have been comprehensively reviewed [1, 2] including metal chelation experiments, spectrophotometric methods, potentiometry, voltametry, self-assembled monolayers and separation techniques. Porphyrin based stationary phases represent a specific category of column packings or inner capillary surface modifiers. Regarding separation techniques, they have been so far used to modify the properties of the sorbents/packings used in HPLC and for direct assays of a number of solutes as well. Deeper insight to all of these applications is outside the scope of this review. Table **2** offers a brief overview of the analytical application of porphyrins; two applications are illustrated by real chromatograms, particularly separation of eight aromatic sulfonates on the [tin(IV) 5-(*p*-carboxyphenyl)-10,15,20-triphenyl porphine] column on Fig. **4**, and analysis of imidazol on the reduced, Fe (II), and oxidized, Fe(III), [Fe-protoporphyrin]-silica sorbent on Fig. **5**.

4.2. Carboxylic Acids

The separation of eleven aromatic carboxylic acids using tetrakis(pentafluorophenyl)-porphyrin (PFFP) derivative as the inner capillary wall modifier is shown on Fig. **6** [64]. The modification was achieved either by simple physical adsorption or by the covalent attachment of the porphyrin derivative via the ether bond to the fused silica. Separations were done in 25 mmol/l borate and phosphate buffers at different pHs (8.5 for borate buffer, and 5.0 and 6.0 for phosphate buffers). In alkaline buffer, the effective mobilities of the solutes were slightly decreased in the modified capillaries (as compared to the unmodified one), apparently due to the shielding of the ionizable silanol groups. The peak tailing observed in bare capillary was reduced in both the modified capillaries. The resolution of the acids was better in covalently modified as compared to the unmodified

capillaries. Considering the adsorbed coating the resolution was a little worse than in uncoated capillary (benzoic and salicylic acids comigrated).

Decreasing pH to 6.0 brought about an interesting feature. While decreasing of EOF was expected in all three capillaries, in coated capillaries higher EOF mobility was observed. Moreover, more efficient separation was achieved in a capillary with adsorbed modifier in shorter time (20 min) as compared to 30 min in bare fused silica (FS) capillary used as control. Separation of nitroisophthalic acid from isophthalic and terephthalic acids (these three otherwise comigrated) was achieved as compared to the other two capillaries. Almost baseline resolution of aminobenzoic acid from hydroxybenzoic acid was observed in covalently modified capillary, while in uncoated and physically modified capillaries these acids coeluted. Benzoic and salicylic acids were not resolved at all when analyzed in the capillary with adsorbed porphyrin.
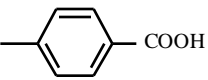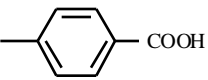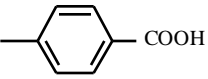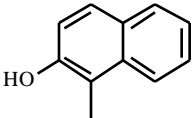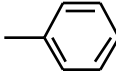
When the separations were run at pH 5.0, the polarity had to be changed to the reversed mode (cathode at the injection end) since at normal polarity (anode at the injection end) the separation of the test mixture in bare capillary was too long and only three peaks were observed within 40 min. On the contrary, nine peaks appeared in front of the detector window when the solutes were separated in the capillary with the covalent porphyrin attachment. In the reversed polarity mode the separation with the capillary containing adsorbed modifier gave irreproducible results. The main difference between the bare silica and the covalently modified FS capillaries was that in the latter complete separation of acetylsalicylic, hydroxybenzoic and amino-benzoic acids was observed, while in the bare capillary the separation of these three acids was much poorer. Moreover, nitroisophthalic acid was almost baseline resolved from the terephthalic-isophthalic acid doublet, while in the untreated capillary this separation was only indicated. Generally, covalently modified column containing the tested porphyrin derivative gave better resolution and higher number of theoretical plates as compared to the physically adsorbed phase and the unmodified silica capillaries.
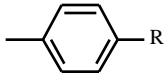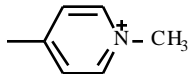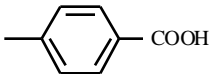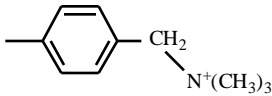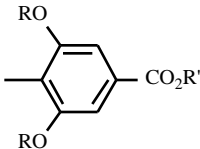
4.3. Amino Acids

Two slightly different approaches were applied to the separation of amino acids in porphyrin modified capillaries [65]. Firstly, only a six-membered set of the common amino acids was tested in capillaries with various adsorbed porphyrin derivatives. The model set consisted of three aliphatic (-Ala, Val, Pro) and three aromatic (Trp, Phe, Tyr) amino acids, and two oligopeptides, diglycine and triglycine. Capillaries were modified by adsorption with various porphyrins that differ in the peripheral substituents and the central metal in the porphyrin cavity. Based on the preliminary screening three porphyrins were selected for detailed investigation, H_2TPP, Co(II)Pc and Rh(III)TPP(*m*-OPh)$_4$.

The runs were done both in acidic (50 mmol/l Tris-100 mmol/l phosphate, pH 2.1) and alkaline (50 mmol/l disodium tetraborate, pH 9.7) background electrolytes. As expected, the presence of a macrocycle in which a large - electron system was available had a higher effect on the
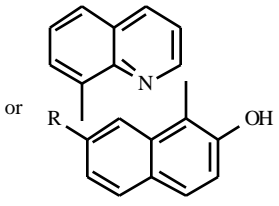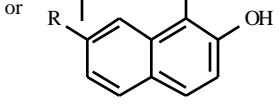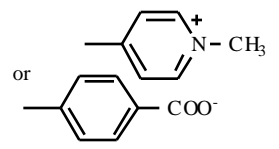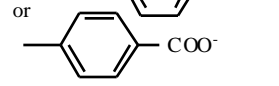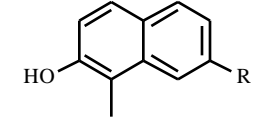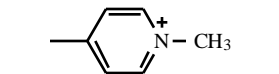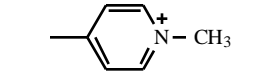
Table 2.   Applications of Porphyrin and Porphyrin-like Macrocycles in Analytical Chemisty (The Derivatives are Derived from the Basic Structure Described in Fig. 1., Only Peripheral Substituents and their Positions are Given in the First Column of the Table)

Porphyrin derivative	Analytical technique	Tested analytes	Conditions (solvents, mobile phases, background electrolytes)	Note/Type of interaction	Ref.
Zn(II)–5- [structure] —COOH -5,15,20-triphenyl	HPLC stationary phase	C_{60}, C_{70} and higher fullerenes	CS_2, toluene, p-xylene	- interaction	36
Zn(II)–5- [structure] —COOH -10,15,20-triphenyl	HPLC stationary phase	Metallofullerenes C_{60-84}	CS_2-toluene (25:75, v/v)	- interaction	37
Fe(III), Ni(II), Cu(II), Zn(II), Cd(II) metalloprotoporphyrins IX	HPLC stationary phase	Polyaromatic hydrocarbons (PAHs)	Acetonitrile-water (100-60:0-40, v/v)	Shape selectivity of phases depending on the central atom, - interaction	38
Sn(IV) and In(III) 5- [structure] —COOH -10,15,20 triphenyl	HPLC stationary phase	PAHs	Methanol-water (70-80:30-20, v/v) Acetonitrile-water (50-60:40-50, v/v)	Shape selective phases, planar PAH were more retained than non-planar ones, - interaction	39
Copper phthalocyanine trisulfonate	HPLC stationary phase	PAHs	Methanol-water, 80:20, v/v	Baseline separation of anthracene derivatives, shape selectivity between isomers *o*-terphenyl/triphenylene	40
5-*p*-carboxyphenyl-10,15,20-triphenylporphyrin, In(III) and Sn(IV) complexes	RP- and anion exchange HPLC	PAHs, aromatic sulfonates, carboxylates and inorganic ions	10 mM succinate, pH 5.5 10 mM acetate, pH 4.5, 15% methanol methanol-water (70:30) for PAHs	– interaction, shape selectivity for PAH, (Fig. 4)	41
Fe(II) or Fe(III) protoporphyrin, reduction and re-oxidation of stationary phase made *in situ*	HPLC stationary phase	Amino acids, aromatic anions, imidazole and its derivative	50 mM phosphate, pH 7.0, methanol up to 10%	- stacking, coordinative interaction with metal atom, hydrophobic interactions (Fig. 5)	42
Rh(III)-5,15- [structure] HO— 8,12-$CH_2CH_2CO_2CH_3$ 2,3,17,18-CH_2CH_3 7,13-CH_3	Separation of complexes (diastereomers) by HPLC	Amino acid methyl esters	Hexane-$CHCl_3$-CH_3OH (95:3:2, v/v)	Coordinative interaction, hydrogen bonding	43
Bis(hydroxypropyl) sapphyrin derivative	HPLC ^{1}H NMR complexation	Inorganic/organic anions, carboxylic acids	Sulfate or acetate mobile phase, pH 7.0	Hydrogen bonding	44
Zn (II), Cu (II), and metal free [structure] 5,10,15,20-	HPLC stationary phase	Trp, Phe, Tyr, Tyr-containing dipeptides and tripeptides, insulins	50 mM phosphate, pH 7.0, acetonitrile 0-60%	- stacking, coordinative interaction with metal atom	45

(Table 2). contd.....

Porphyrin derivative	Analytical technique	Tested analytes	Conditions (solvents, mobile phases, background electrolytes)	Note/Type of interaction	Ref.
Fe(III), Ni(II), Cu(II), Zn(II), Cd(II) metalloprotoporphyrins IX	RP-HPLC stationary phase	Amino acids, peptides	50 mM Phosphate, pH 2.5 or 7.0, acetonitrile 10-40%	- and coordinating interaction of Trp, Phe, His and related peptides	46
R=SO_3H, positions 10,15,20 R=NH_2, position 5 —⬡— R	Electrophoresis	Nitrite detection	20 mM phosphate, pH 7.0, 5 mM - CD	Diazotation reaction	47
Coproporphyrin, uroporphyrin, protoporphyrin	Affinity electrophoresis	Human serum albumin	Unspecified	Quick formation of 1:1 complex	48
Zn(II)–5,10,15,20– —⬡—$\overset{+}{N}$– CH_3	UV-Vis complexation	Amino acids	pH 8.9-11.8	Electrostatic interaction, hydrophobic interaction	49
Polyazacryptand-phenylporphyrin conjugates	UV-Vis binding studies	Nucleotides	HEPES, pH 7.4	Stacking interaction, preference for pyrimidine bases	50
5,10,15,20- —⬡— COOH	UV-Vis binding study	Synthetic peptides, derived from antibodies binding sites	20 mM Tris-HCl, pH 7.4, methanol up to 50 %	Hydrophobic interactions, affinity to the porphyrin was improved by the restriction of the conformation of peptide	51
Zn(II) porphyrins connected with binaphthyl or biphenyl spacers	UV-Vis, CD binding studies	Amino acid enantiomers Amino acid chiral derivatives	CH_2Cl_2	Coordinative interaction, association constant depend on the diameter of dimer cavity, *i.e.* the solute had to fit in it	52
Rh(III)-13,17-bis(trimethylammonium propyl)mesoporphyrin	UV-Vis, CD binding studies	Nucleotides	13 mM phosphate, pH 5-10	Coordinative interaction, Coulombic interaction	53
5,10,15,20- —⬡— CH_2 \ $N^+(CH_3)_3$	UV-Vis, CD	DNA, nucleotides, nucleosides	Not specified	Non-intercalative binding	54
Zn(II)–5,10,15,20– RO —⬡— CO_2R' RO R=$(CH_2)_n$ COOR' R'=Me or K, n=1,4 or 10	^{1}H NMR UV-Vis binding studies	Amines, -amino acid esters, oligopeptides	2.3 M Borax, pH 9.0, Methanol-borax, 10:1 (v/v)	Coordinative bond to Zn atom, electrostatic interaction, hydrophobic interaction (increasing with increasing alkyl chain of the receptor)	55

(Table 2). contd....

Porphyrin derivative	Analytical technique	Tested analytes	Conditions (solvents, mobile phases, background electrolytes)	Note/Type of interaction	Ref.
Zn(II)–5,15– R=OH or is not	^{1}H NMR UV-Vis ICD binding studies	Carbohydrates, *e.g.*, glucose, galactose, N-acetylglucosamine	CHCl$_3$, water, pyridine, phenol, p-nitrophenol etc. as polar additives	Lewis acid/base interaction, hydrogen bridges	56
Zn(II)- 5,10,15,20- or	^{1}H NMR binding studies	Amino acids	Deuterated 25 mM borax, pH 10.5	Electrostatic interaction, stacking interaction	57
Zn(II)-2,3,7,8,12,13,17,18-octaethylporphyrin or Zn(II)- 2,3,7,8,12,13,17,18-octaethyl-5,15- R=OH or is not	^{1}H NMR binding studies	Amino acid esters	CDCl$_3$	Coordinative interaction, hydrogen bond (one or two point), increased value of association constants with multiple binding	58
Tetraphenylporphyrin-saccharide conjugates (glucose, galactose)	spectrofluori-metric measurements	Liposome binding	Methanol, phosphate buffer, pH 7.4	Porphyrin bearing three glucose units was found to have the largest affinity for liposomes	59
Mg(II)meso-and Mg(II)-protoporphyrins	CD, ORD complexation	Binding studies with apomyoglobin, apohemoglobin and amino acids	Unspecified	Coordinative interaction, – interaction, hydrogen bonding	60
5,10,15,20–	SPR-chip affinity study	Synthetic peptide binding	Porphyrin deposited in buffer of pH 7.4	Stacking interaction	61
Protoporphyrin IX, and 5,10,15,20–	SPR-chip affinity study	Synthetic peptide binding	Porphyrin deposited in buffer of pH 7.4	No response for protoporphyrin IX,	62
Tetraphenylporphyrin-saccharide conjugates (xylose, glucose, galactose)	Differential scanning calorimetry	Liposome binding	Chloroform, lipid:porphyrin ratio 20:1, after solvent evaporation dissolution in phosphate, pH 7.4, 10-40°C	Association constants increased with increasing lipophilicity of the porphyrin derivatives	63

separation of aromatic amino acids as compared to the aliphatic species. The resolution of Trp – Phe – Tyr migration order increased in the following manner: uncoated < H_2TPP < Co(II)Pc < Rh(III)TPP(m-OPh)$_4$ (pH 2.1) (Fig. **7**). Aliphatic amino acids and oligopeptides were baseline resolved in bare FS capillary and their separation was only partially improved in the modified capillaries at the above specified pH.

Interesting results were obtained in the alkaline buffer. Regarding the resolution of the aromatic solutes, the order of increasing resolution was uncoated < Rh(III)TPP(m-OPh)$_4$ < Co(II)Pc < H_2TPP capillaries. Interestingly, the migration order Trp – Phe – Tyr seen in the unmodified capillary was changed to Tyr – Trp – Phe in capillary modified with H_2TPP.

Further investigation of the porphyrin derivative Rh(III)TPP(m-OPh)$_4$ as the most perspective candidate from the previous study was concerned with the separation of an enlarged set of common underivatized amino acids containing seventeen members [66]. Sodium phosphate and Tris phosphate buffers were prepared as background electrolytes (BGEs). Although no dramatic changes in the separation profile of the species was observed, several interesting results emerged.
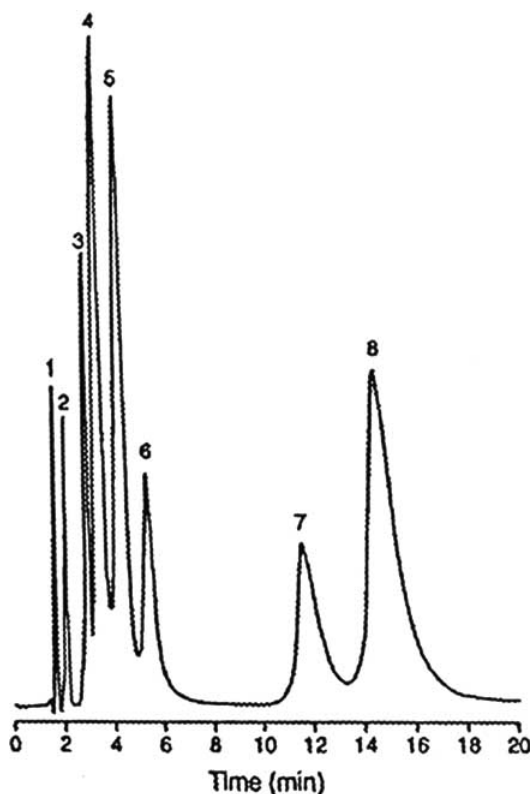

Fig. (4). Separation of eight aromatic sulfonates on the SnCTPP [tin(IV) 5-(p-carboxyphenyl)-10,15,20-triphenyl porphine] column: sulfanilic acid (1), benzenesulfonate (2), p-toluenesulfonate (3), 1,5-naphthalenedisulfonate (4), 2,6-naphthalenedisulfonate (5), 4-amino-1-naphthalenesulfonate (6), 1-naphthalenesulfonate (7), and 2-naphthalenesulfonate (8). Mobile phase, 15% methanol/ 85% 10 mmol/l succinate, pH 4.5; flow rate 1.0 ml/min; column temperature 25°C, detection 220 nm (0.001 AUFS). (Reprinted with permission from [41]. Copyright (1993) American Chemical Society).
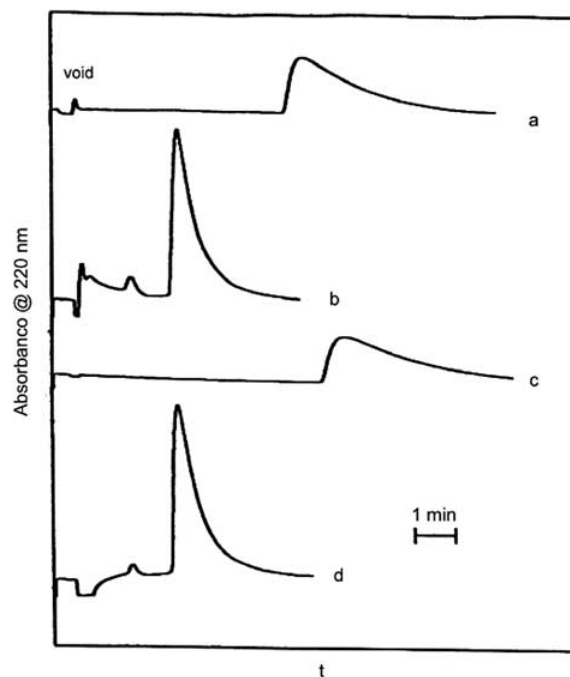

Fig. (5). Repeated tuning of ligation-based solute retention via FeProP [iron protoporphyrin]-silica redox state: chromatograms of imidazole on the ferric form on the stationary phase (a), after reduction of the central metal ion (b), following re-oxidation (c) and after additional reduction cycle (d); FeProP-silica stationary phases based on sterically protected aminopropyl-silica. (Reprinted with permission from [42], copyright (1999) Elsevier Sciences B.V.).

The best separations were obtained in [Rh(III)TPP(m-OPh)$_4$] coated capillary in 50 mmol/l Tris–100 mmol/l phosphate buffer at pH 2.25. Separation of the critical triplet Val – Ile – Leu was always at least indicated being better at higher BGE concentrations. Regarding the sensitivity of the method, lower concentration limit of detection (LOD) in the coated capillary was obtained for Thr, Gly, Tyr, and Val (114.5; 116.3; 24.6; 58.4 µg/ml, respectively); the other amino acids exhibited lower LOD in the uncoated capillary. The separation of acidic amino acids, aspartic and glutamic acids was not achieved.

4.4. Peptides

Coming to the gently more difficult model set, five aromatic amino acids possessing tripeptides as the model set in OT–CEC, experiments with capillaries modified again by Rh(III)TPP(m-OPh)$_4$ and H_2TPP(m-OPh)$_4$ derivatives were reported [67]. Though these two modifiers have identical peripheral substituents, their difference is in the state of the macrocycle core; *i.e.*, free-base or metal form. Both macrocycles were simply physically adsorbed on the capillary wall after the obvious column pretreatment.

Upon pH 2.5 and H_2TPP(m-OPh)$_4$ coated capillary using 100 mmol/l phosphate buffer, the running time of analysis was not changed, but almost baseline separation and peak narrowing was observed. If the metalled Rh(III) complex was used, complete baseline separation with good resolution was
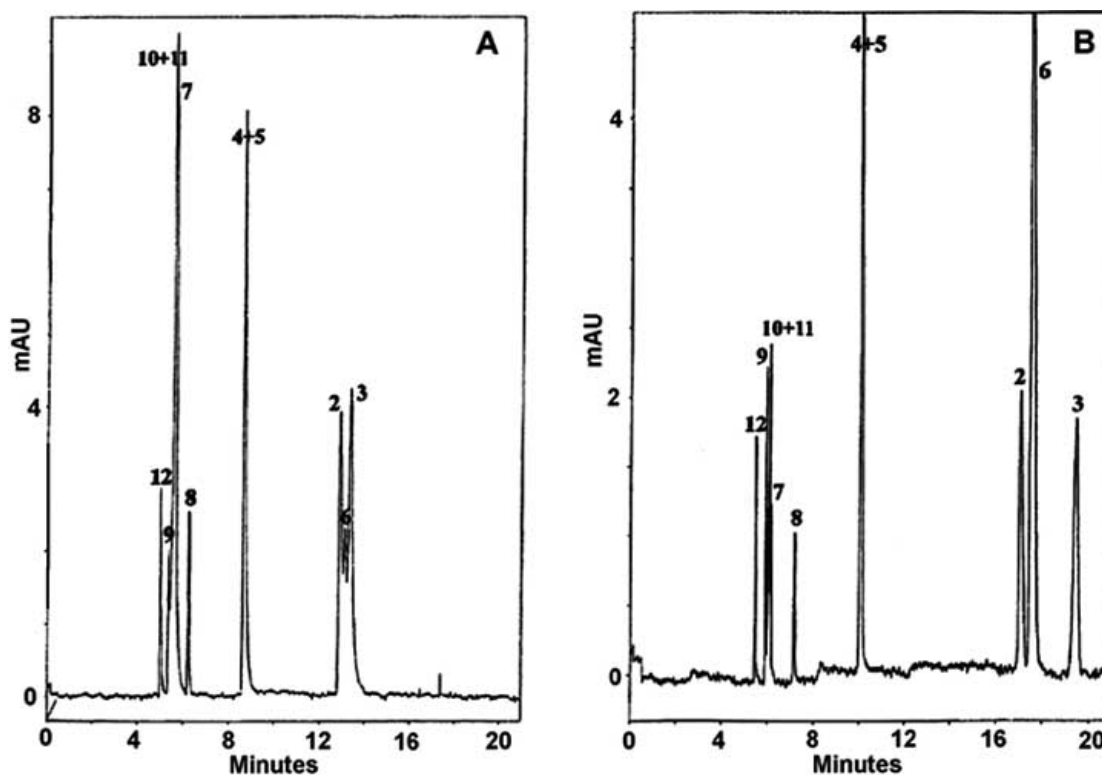
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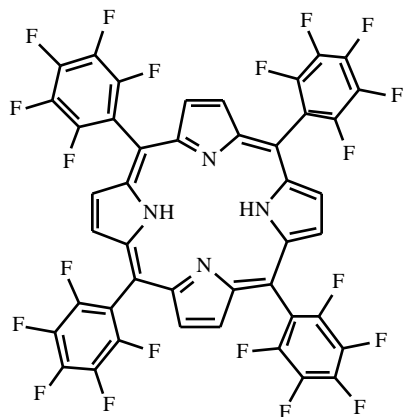
Tetrakis (pentafluorophenyl) porphyrin [PFFP]

Fig. (6). Separation of a set of aromatic carboxylic acids in the bare fused-silica (FS), (A) and in   tetrakis(pentafluorophenyl)porphyrin (PFFP) covalently coated FS capillary (B). Conditions: 25 mmol/l phosphate, pH 5.0, -15 kV (inversed polarity), capillaries 50 µm I.D., 43 cm (35.5 cm effective length), detection at 215 nm. Peak identification: **12**-2-sulfobenzoic acid, **9**-5-nitroisophthalic acid, **10**-terephthalic acid, **11**-isophthalic acid, **8**-phthalic acid, **4**-salicylic acid, **5**-benzoic acid, **2**-acetylsalicylic acid, **6**-4-hydroxybenzoic acid, **3**- 4-aminobenzoic acid. (Reprinted with permission from [64], copyright (2003) Elsevier Sciences B.V.).

achieved, particularly at the increased voltage (15 kV instead of 10 kV) and, consequently, shorter analysis time (Fig. **8**).

Changing the composition of the background electrolyte from pure phosphate to Tris-phosphate lead to even faster separation, however, with slightly reduced resolution. The speeding up of the overall time of analysis might have the origin in the overall positive charge of the porphyrin metallocomplex (+1) that enables repulsion of the positively charged peptides.

Regarding separations in alkaline pH, better resolution of a critical pair of peptides was observed in capillary modified

with metal-free porphyrin than that in the bare silica, but the analysis time was about 30% longer (Fig. **9**).

Capillaries that had their inner surface covered with various porphyrin derivatives [H_2TPP, Cu(II)TPP(CHO), Rh(III)-, Ni(II)–, Co(III)TPP(*m*-OPh)$_4$; Co(II)Pc] were applied also for the separation of model sets of octapeptides (Fig. **10**) [68]. The structural diversity of individual members of the set was very low, as the octapeptides (C-terminal fragments of B-chain of human insulin) differed only in the position of the protecting phenylacetyl (Pac) group (on amino group of N-terminus or Lys/Orn residue),
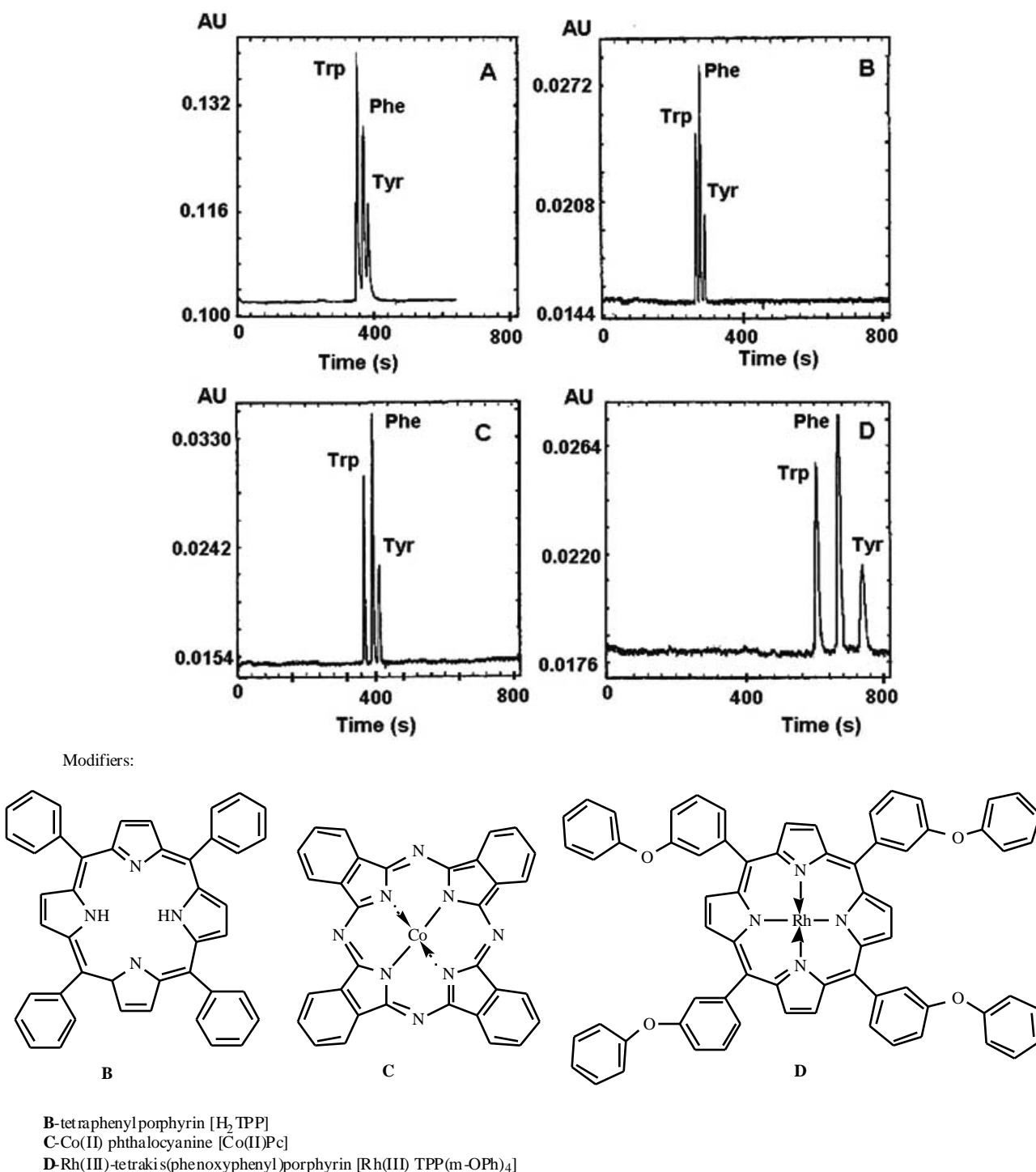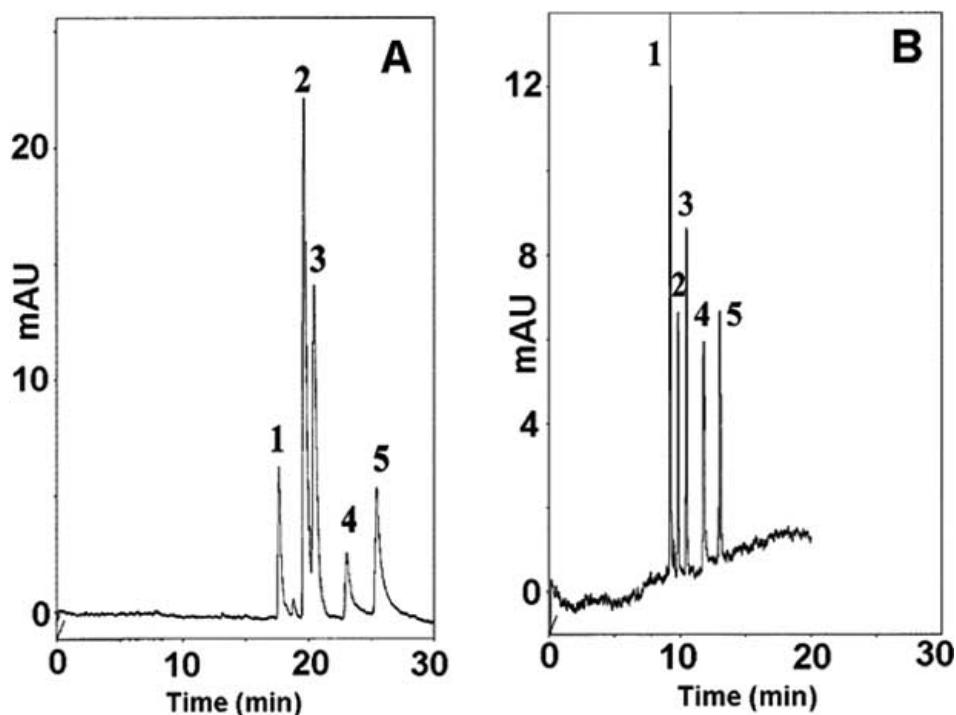
Modifiers:

B-tetraphenylporphyrin [H₂TPP]
C-Co(II) phthalocyanine [Co(II)Pc]
D-Rh(III)-tetrakis(phenoxyphenyl)porphyrin [Rh(III) TPP(m-OPh)₄]

Fig. (7). Separation of a model set of aromatic amino acids using uncoated (A) and modified capillaries (B-D). Separation conditions: 50 mmol/l Tris-100 mmol/l H_3PO_4, pH 2.1, 10 kV. Capillaries 50 μm I.D, 30-31 cm (19-20 cm effective length), detection at 206 nm. (Reprinted with permission from [65], copyright (2002) Elsevier Sciences B.V.).

and one amino acid residue change in the sequence (Lys or Orn). Separations were performed either in highly acidic pH (Tris-phosphate, 2.14) or in alkaline pH (sodium borate, 9.0).

Irrespectively of the pH of the buffers, similar molecules, *i.e.* those with the protecting group on the same position in the sequence, comigrated. At least partial separation of the octapeptides having the Pac group on N-terminus was observed using the capillary modified with Cu(II)TPP(*m*-OPh)₄. Similarly, the same doublet was partially separated in alkaline pH in Co(II)Pc column. As the conclusion of this investigation it was feasible to assume that the key role for successive separation of such intimately related compounds is played by the free Lys or Orn side chain possessing a free amino group allowing coordinative interaction with the porphyrin metallocomplexes.
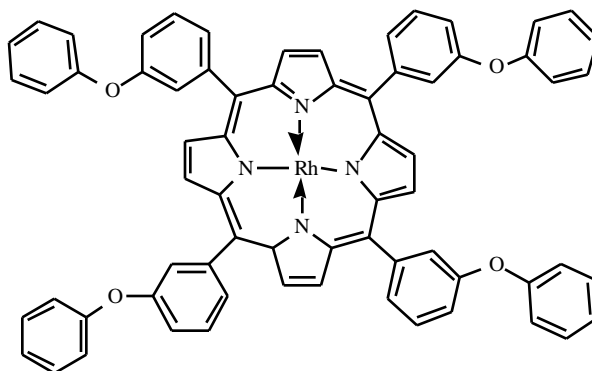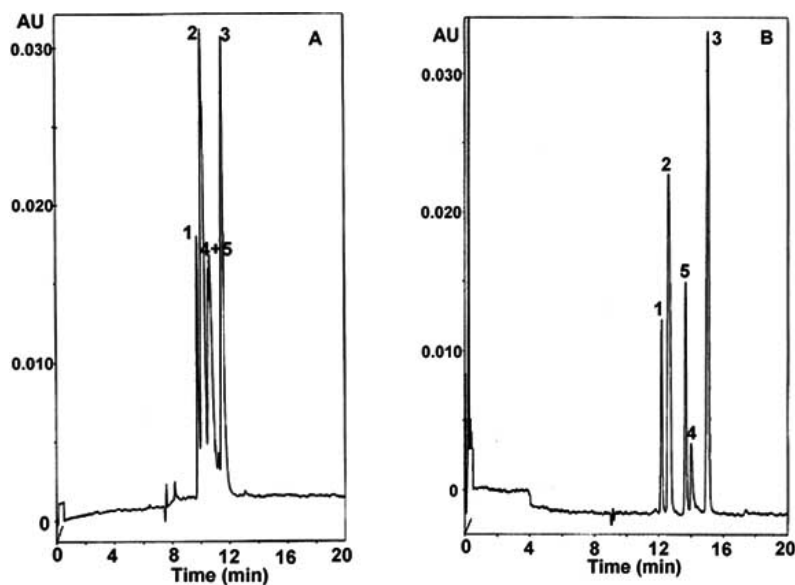
Modifier

Rh(III)-tetrakis(phenoxyphenyl)porphyrin [Rh(III)TPP(*m*-OPh

Fig. (8). Separation of a model set of peptides using uncoated (A) and modified capillary (B). Conditions for Fig. 8A:100 mmol/l phosphate, pH 2.5, 10 kV, 50 µm I.D. capillary, 42.5 cm (34.5 cm effective length). Fig. 8B 100 mmol/l phosphate buffer, pH 2.5, 15 kV, 50 µm I.D. capillary, 43 cm (35.5 cm effective length). Detection at 200 nm. Peak identification: **1**-Ala-Ala-Ala, **2**- Gly-Gly-Phe, **3**-Tyr-Gly-Gly, **4**-Phe-Phe-Phe, **5**- Tyr-Tyr-Tyr. (Reprinted with permission from [67], copyright (2002) Elsevier Sciences B.V.).

4.5. Nucleotides

The affinity of sapphyrin macrocycles towards phosphate moiety has been previously proved in several papers [26-30]. Based on these findings, the sapphyrin molecule was also tested as the possible nucleotide recognition element adsorbed onto the inner FS column surface [69]. Preliminary studies performed in borate-acetate buffer (pH 7.0) showed several features: (i) the electroosmotic mobility of the unretained solute (EOF marker) was decreased by about 25% as compared to the unmodified capillary, (ii) under identical voltage conditions (17 kV) the di- and triphosphates could not be eluted at all on the sapphyrin capillary, (iii) after increasing the voltage up to 20 kV, however, at least a

partial separation of nucleotide diphosphates (adenosine diphosphate ADP, cytidine diphosphate CDP, uridine diphosphate UDP) and adenosine triphosphate ATP was observed; (iv) the decrease in effective mobilities of the analytes was   20 % for monophosphates and   40% for di- and triphosphates (at 20 kV). The reduction of the effective mobilities could be ascribed to the interaction with the capillary surface. From the above mentioned results it is feasible to assume that under appropriate conditions selective separation of monophosphates from di- and triphosphates could be achieved (*i.e.* borate-acetate, voltage lower than 20 kV). Changing the buffer composition to Tris-HCl brought no improvement of the separation but increased EOF was observed.
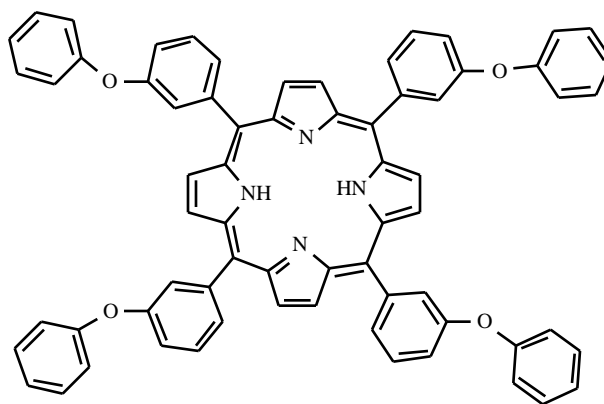
Modifier

Tetrakis (phenoxyphenyl) porphyrin [H_2TPP(m-OPh)4]

Fig. (9). Separation of model set of peptides in uncoated (A) and modified capillary (B). Conditions:100 mmol/l phosphate, pH 8.0, 10 kV. Peak identification: **1**-Ala-Ala-Ala, **2**- Gly-Gly-Phe, **3**-Tyr-Gly-Gly, **4**-Phe-Phe-Phe, **5**- Tyr-Tyr-Tyr. Other conditions as in Fig. 8. (Reprinted with permission from [67], copyright (2002) Elsevier Sciences B.V.).

In order to reduce the solute-wall interaction, borate-phosphate buffer (pH 7.0) was used for further investigations. As expected, the presence of phosphate in the background electrolyte decreased the retention of solutes in the sapphyrin-modified capillary, elution of all solutes was obtained, and the effective mobilities were decreased by only about 3% apparently owing to the competition of phosphates present in the buffer and phosphate residues bound to the solutes (Fig. **11**). Irrespective of the similar effective mobilities of the test solutes, much better resolution was observed in the modified capillary, *i.e.*, otherwise poorly resolved UDP and CDP were completely separated. On the other hand, separation of monophosphates was only marginally affected by the presence of the sapphyrin molecule on the capillary wall.

4.6. Organic Phosphates

The sapphyrin macrocycle as the inner column wall modifier was used for the separation of this category of compounds. Two test mixtures were used [70]. The first one (No. 1) comprised of serine, threonine, phosphoserine and phosphothreonine (all as PTH derivatives) and *p*-aminobenzylphosphonic acid. The second one (No. 2) contained polyphosphates of adenosine having 4-6 phosphate moieties per molecule. 20 mmol/l borate-phosphate and 25 mmol/l borate-acetate buffers (both pH 7.0) were used as the running electrolytes.

Regarding the separation of the mixture No. 1 in borate-acetate buffer, similar results as with the separation of nucleotides were obtained; *i.e.*, phosphorylated amino acids did not appear in front of the detection window within 30 min using the modified capillary, while complete separation of all the solutes was observed within 21 min using the uncoated column (Fig. **12**). Comparing the mobilities of the EOF marker in both capillaries, a decrease of 35% was observed in the modified column as compared to the unmodified capillary. On the other hand, effective mobilities of non-phosphorylated amino acids were not changed, but that of aminobenzylphosphonic acid decreased about 12%.

This could lead to the assumption that certain interaction of the phosphate group with the sapphyrin could occur. In borate-phosphate buffer, the interaction of solutes with the modifier is nearly abolished owing to the excess of phosphate in the electrolyte. Nevertheless, the effective mobility decreases observed for phosphorylated amino acids were still 5.96% and 8.75% for P-Ser and P-Thr, respectively, and 23% for aminobenzylphosphonic acid. It is feasible to conclude that some other non-covalent interaction is involved besides phosphoric acid residue complexing in the coated capillary. Since the aminobenzylphosphonic acid bears an aromatic cycle in its molecule, stacking interaction with the sapphyrin -electron rich macrocycle could be considered.

To evaluate the influence of the number of phosphate groups on the separation in the modified capillary, several polyphosphates bearing one or two adenosine residues were employed as the test solutes. It was revealed that for analytes possessing a single adenosine moiety (p_4A, p_5A) the increasing number of phosphates leads to the decrease of the
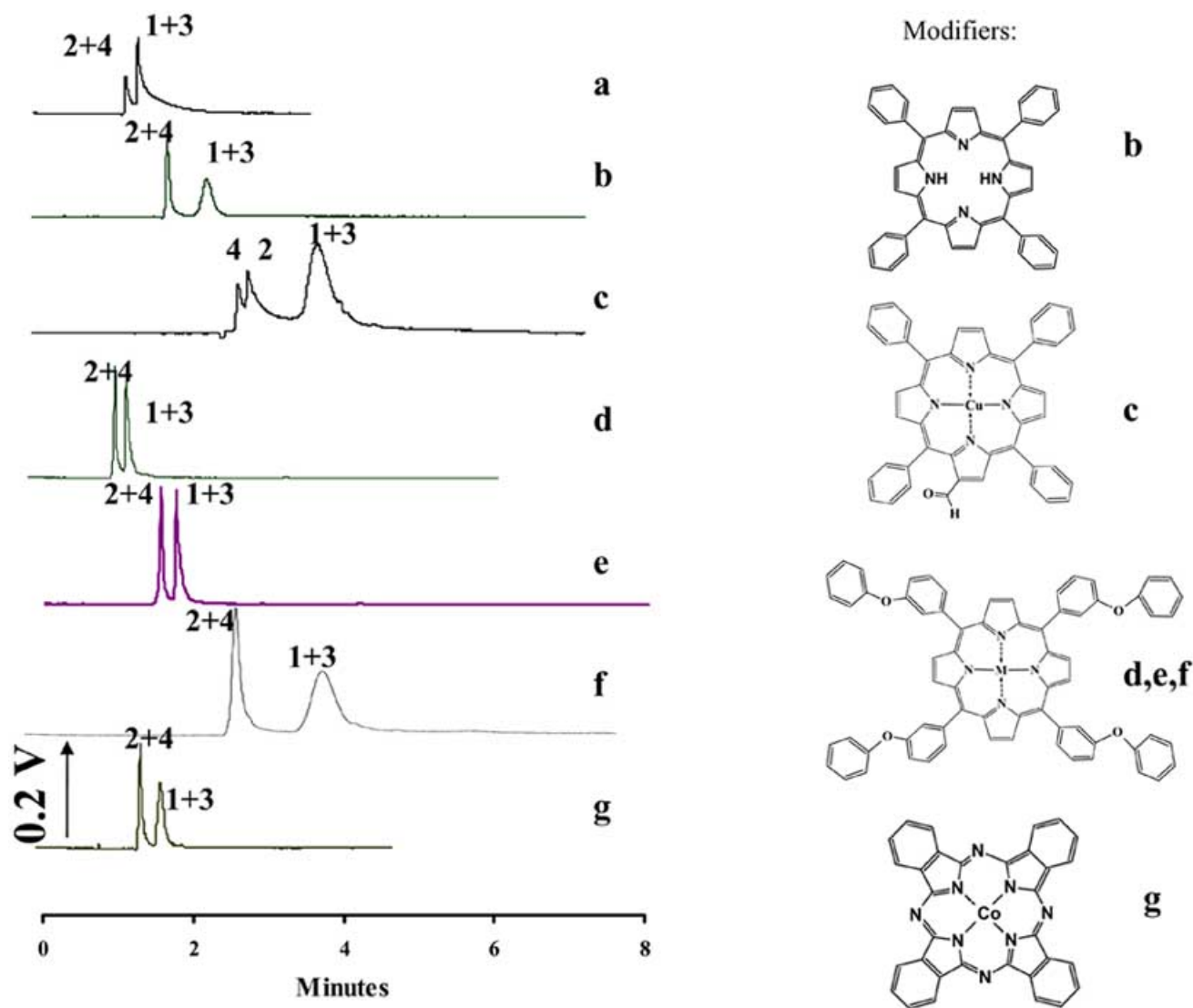

Fig. (10). CZE (a) and OT-CEC (b–g) separations of a model mixture of octapeptides **1**–**4** in alkaline BGE I, 50 mmol/l H_3BO_3, 25 mmol/l NaOH, pH 9.0, 10 kV, detection at 206 nm. Peaks: **1**–GFFYTPOrn(Pac)T; **2**–(Pac)GFFYTPOrnT; **3**–GFFYTPK(Pac)T; **4**–(Pac)GFFYTPKT. Letter symbols at each line represent capillaries modified with the porphyrin derivatives: **a**–uncoated; **b**–H_2TPP; **c**–Cu(II)TPP(HCO); **d**–Rh(III)TPP(*m*-OPh)$_4$; **e**–Co(III)TPP(*m*-OPh)$_4$; **f**–Ni(II)TPP(*m*-OPh)$_4$; **g**–Co(II)Pc. (Reprinted with permission from [68], copyright (2003) Elsevier Sciences B.V.).
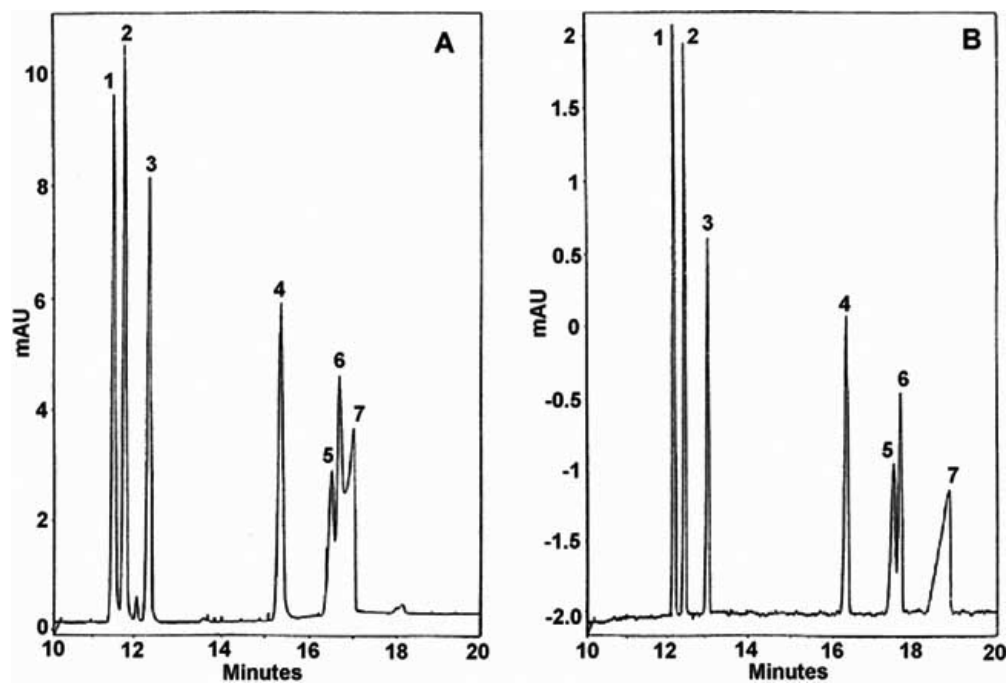
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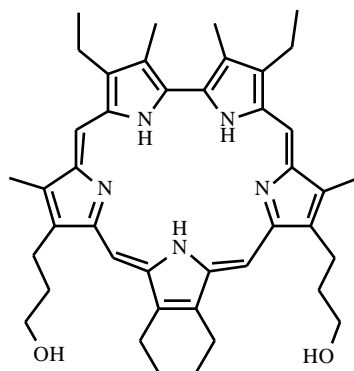
3,12,13,22-tetraethyl-2,7,18,23-tetramethyl-8,17-bis(hydroxypropyl)sapphyrin

Fig. (11). Separation of a test mixture of nucleotides in 25 mmol/l borate-phosphate buffer, pH 7.0, 20 kV. Uncoated capillary (A), modified capillary (B). Peak identification:**1**-GMP, **2**-AMP, **3**-UMP, **4**-ATP, **5**-ADP, **6**-CDP, **7**-UDP. Capillary 50 μm I.D., 70 cm (62.5 cm effective length). Detection at 254 nm. (Reprinted with permission from [69], copyright (2001) Elsevier Sciences B.V.).

interaction as compared to ATP (*vide supra*). If the solute contained two adenosine residues (Ap$_4$A, Ap$_5$A, Ap$_6$A) the interaction with the coated capillary was of similar magnitude as with the appropriate counterpart containing a single adenine only. Sterical conditions of interaction should also be taken into account, since the planar sapphyrin molecule is unable to accommodate the bulky polyphosphate compounds.

By comparing the set of compounds tested we can consider three types of interaction between the sapphyrin coating and the solute: (i) complexing of the phosphate residue (provided that it is sterically available), (ii) hydrophobic and (iii) - interactions.

5. CONCLUDING REMARKS

In this brief review we attempted to summarize different possibilities of immobilization of oligopyrroles onto the inner FS capillary surface for OT–CEC separation, and thus trying to enlarge the field of application of oligopyrroles in analytical chemistry. Basically, there are two ways how to modify a capillary column inner surface, namely by adsorption and covalent binding. Adsorption is generally easier to carry out; however, it is necessary to pay attention to the stabilization of the modified column (to prevent leakage of the adsorbent). Chemical bonding is considerably more time consuming in the laboratory practice, however it abolishes the problems with modified column stabilization to a considerable extent. Oligopyrrolic macrocycles (porphyrins and expanded porphyrins) represent another choice of perspective category of modifiers since they mimic the naturally occurring derivatives. These can be used both for the adsorption and chemical binding. The range of analytes separated by OT–CEC is broad indeed. Actually, the only limitation is to find a suitable pair of the modifier and the analyte (or category of analytes) to obtain the desired results.
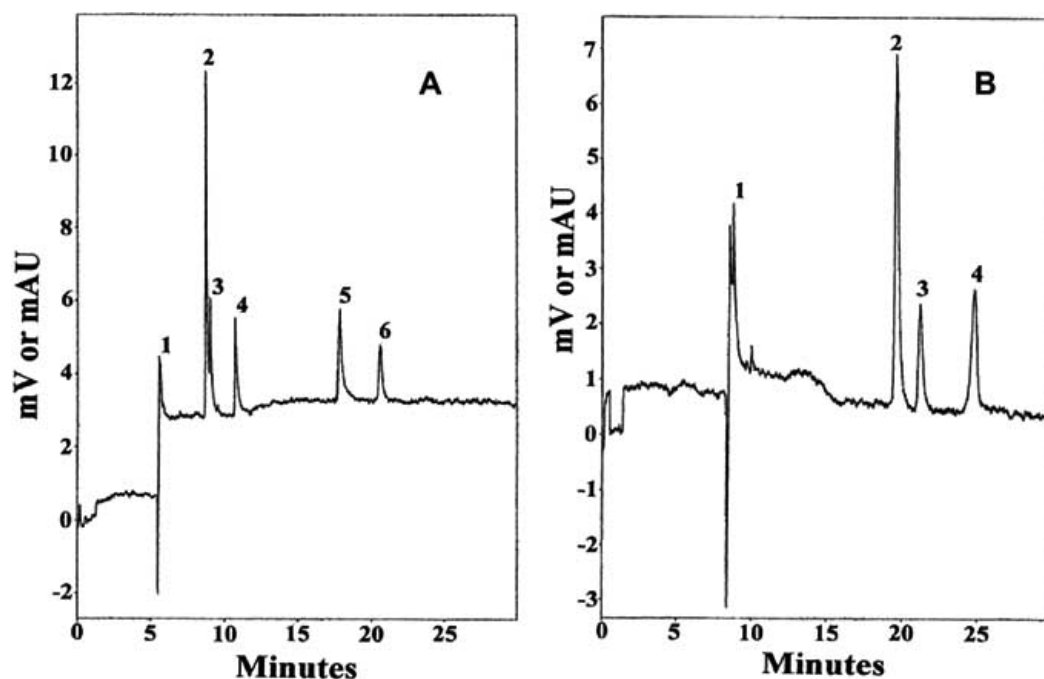
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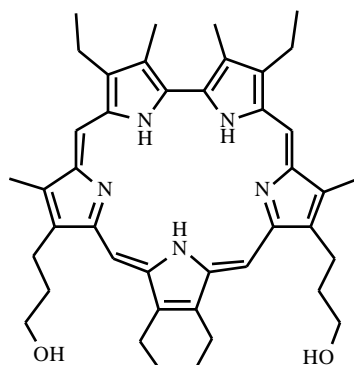
3,12,13,22-tetraethyl-2,7,18,23-tetramethyl-8,17-bis(hydroxypropyl)sapphyrin

Fig. (12). Separation of model set mixture No. 1 in 25 mmol/l borate-acetate buffer, pH 7.0, 10 kV. Uncoated capillary (A), coated capillary (B). Capillaries 50 μm I.D., 43 cm (35.5 cm effective length), detection at 205 nm. Peak identification: **1**–thiourea, **2**–PTH-threonine, **3**–PTH-serine, **4**–*p*-aminobenzylphosphonic acid (*p*-ABPA), **5**–PTH-phosphothreonine, **6**–PTH-phosphoserine. (Reprinted with permission from [70], copyright (2002) Wiley-VCH Verlag GmbH).

The following conclusions could be drawn from the published results:

1) It was shown that the preparation of adsorbed layers of the modifier on the inner surface of the FS capillary is easy by simple flushing the column (after its pretreatment) with the porphyrin/porphyrin analogue in an appropriate solvent. Modification of the inner capillary surface by covalent attachment of the studied modifier is more time consuming but it ensures more stable and long lasting coatings.

2) Regarding the analytes separated by OT–CEC in porphyrin/porphyrin analogue modified FS capillaries, the best candidates of biological interest were found to be those which (i) possess aromatic moiety in its molecule and/or (ii) include functionalities having a spare electron pair capable of

a coordinative interaction (relevant for metalloporphyrins).

3) The interactions of the separated species with the oligopyrrole–modified capillary are of four types: (i) previously mentioned coordinative interactions, (ii) – (stacking) interactions, (iii) hydrophobic forces, (iv) hydrogen bonding.

ABBREVIATIONS

Ap_4A	=	Adenosine (5')tetraphospho(5')adenosine
Ap_5A	=	Adenosine (5')pentaphospho(5')adenosine
Ap_6A	=	Adenosine (5')hexaphospho(5')adenosine
APS	=	Aminopropyltriethoxysilane
CD	=	Circular dichroism
CEC	=	Capillary electrochromatography
DMF	=	Dimethylformamide
DRIFT	=	Diffuse reflectance infrared Fourier transformation
FS	=	Fused silica
ICD	=	Induced circular dichroism
LIF	=	Laser induced fluorescence
ORD	=	Optical rotary dispersion
OT–CEC	=	Open tubular capillary electrochromatography
p_4A	=	Adenosine (5')tetraphosphate
p_5A	=	Adenosine (5')pentaphosphate
PAH	=	Polyaromatic hydrocarbons
PDT	=	Photodynamic therapy
SPR	=	Surface plasmon resonance
TEA	=	Triethylamine

REFERENCES

[1] Biesaga, M.; Pyrzń yska, K.; Trojanowicz, M. *Talant,* **2000**, *51*, 209-224.
[2] Záruba, K.; Setnicka, V.; Charvátová, J.; Rusin, O.; Tománková, Z.; Hrdeicka, J.; Sý kora, D.; Král, V. *Collect. Czech. Chem. Commun.,* **2001**, *66*, 693-769.
[3] Tsuda, T.; Nomura, K.; Nakagawa, G. *J. Chromatogr.,* **1982**, *248*, 241-247.
[4] Stachowiak, T.B.; Svec, F.; Frechet, J.M.J. *J. Chromatogr. A,* **2004**, *1044*, 97-111.
[5] Guihen, E.; Glennon, J.D. *J. Chromatogr. A,* **2004**, 1044, 67-81.
[6] Smith, N.W.; Carter-Finch, A.S. *J. Chromatogr. A,* **2000**, *892*, 219-255.
[7] Colón, L.A.; Burgos, G.; Maloney, T.D.; Cintrón, J.M.; Rogríguez, R.L. *Electrophoresis*, **2000**, *21*, 3965-3993.
[8] Tang, Q.; Lee, M.L. *Trends in Anal. Chem.*, **2000**, *19*, 648-663.
[9] Zou, H.; Ye, M. *Electrophoresis,* **2000**, *21*, 4073-4095.
[10] Liu, Z.; Wu, R.; Zou, H. *Electrophoresis,* **2002**, *23*, 3954-3972.
[11] Kang, J.; Wistuba, D.; Schurig, V. *Electrophoresis,* **2002**, *23*, 4005-4021.
[12] Wistuba, D.; Schurig, V. *Electrophoresis,* **2000**, *21*, 4136-4158.
[13] Wistuba, D.; Schurig, V. *J. Chromatogr. A,* **2000**, *875*, 255-276.
[14] Fanali, S.; Catarcini, P.; Blaschke, G.; Chankvetadze, B. *Electrophoresis,* **2001**, *22*, 3131-3151.
[15] Pesek, J. J.; Matyska, M.T. *J. Chromatogr. A,* **2000**, *887*, 31-41.
[16] Altria, K.D.; Smith, N.W.; Turnbull, C.H. *J. Chromatogr. B,* **1998**, *717*, 341-353.
[17] Vanhoenacker, G.; Van den Bosch, T.; Rozing, G.; Sandra, P. *Electrophoresis,* **2001**, *22*, 4064-4103.
[18] Malik, A. *Electrophoresis,* **2002**, *23*, 3973-3992.
[19] Hilder, E.F.; Svec, F.; Fréchet, J. M. J. *Electrophoresis,* **2002**, *23*, 3934-3953.
[20] Legido-Quigley, C.; Marlin, N.D.; Melin, V.; Manz, A.; Smith, N.W. *Electrophoresis,* **2003**, *24*, 917-944.
[21] Doherty, E. A. S.; Meagher, R. J.; Albarghouthi, M. N.; Barron, A. E. *Electrophoresis,* **2003**, *24*, 34-54.
[22] Deyl, Z.; Svec, F. *Capillary Electrochromatography*, Elsevier : Amsterdam, **2000**.
[23] Svec, F.; Tennikova, T.B.; Deyl, Z. *Monolithic materials*, Elsevier:Amsterdam, **2003**.
[24] Smith, K.M. in *Comprehensive Heterocyclic Chemistry*, Bird C.W.; Cheeseman G.W.H. (Editors), Vol. *4*, Pergamon Press: Oxford, **1984**, pp. 377-442.
[25] Falk, J.E. *Porphyrins and Metalloporphyrins*, Elsevier: Amsterdam, **1964**.
[26] Shi, Z.; Fu, Ch. *Talanta,* **1997**, *44*, 593-604.
[27] Sessler, J.L. *J. Porphyrins and Phthalocyanines*, **2000**, *4*, 331-336.
[28] Iverson, B.L.; Schreder, K.; Král, V.; Sansom, P.; Lynch, V.; Sessler, J.L. *J. Am. Chem. Soc.*, **1996**, *118*, 1608-1616.
[29] Král, V.; Andrievsky, A.; Sessler, J.L. *J. Chem. Soc. Chem. Commun.*, **1995**, 2349-2351.
[30] Král, V.; Furuta, H.; Schreder, K.; Lynch, V.; Sessler, J. L. *J. Am. Chem. Soc*., **1996**, *118*, 1595-1607.
[31] Sessler, J.L.; Furuta, H.; Král, V. *Supramol. Chem.*, **1993**, 209-220.
[32] Král, V.; Schmidtchen, F.P.; Lang, K.; Berger, M. *Org. Lett.*, **2002**, *4*, 51-54.
[33] Sessler, J.L.; Davis, J.M. *Acc.Chem. Res*., **2001**, *34*, 989-997.
[34] Allen, C.M.; Sharman, W.M.; Van Lier, J.E. *J. Porphyrins and Phthalocyanines,* **2001**, *5*, 161-169.
[35] Charvátová J. PhD. Thesis, Institute of Chemical Technology, Prague, **2003**.
[36] Xiao, J.; Meyerhoff, M.E. *J. Chromatogr. A,* **1995**, *715*, 19-29.
[37] Xiao, J.; Savina, M.R.; Martin, G.B.; Francis, A.H.; Meyerhoff, M.E. *J. Am. Chem. Soc.*, **1994**, *119*, 9341-9342.
[38] Chen, S.; Meyerhoff, M.E. *Anal. Chem*., **1998**, *70*, 2523-2529.
[39] Kibbey, CH.E.; Meyerhoff, M.E. *J. Chromatogr.*, **1993**, *641*, 49-55.
[40] Hayatsu, H. *J. Chromatogr.*, **1992**, *597*, 37-56.
[41] Kibbey, C.E.; Meyerhoff, M.E. *Anal. Chem*., **1993**, *65*, 2189-2196.
[42] Rüdel, U.; Meyerhoff, M.E. *Anal. Chim. Acta,* **1999**, *392*, 191-200.
[43] Aoyama, Y.; Uzawa, T.; Saita, K.; Tanaka, Y.; Toi, H.; Ogoshi, H. *Tetrahedron Lett.,* **1988**, *29*, 5271-5274.
[44] Sessler, J.L.; Král, V.; Genge, J.W.; Thomas, R.E.; Iverson, B.L. *Anal. Chem*., **1998**, *70*, 2516-2522.
[45] Biesaga, M.; Orska, J.; Fiertek, D.; Izdebski, J.; Trojanowicz, M. *Fresenius J. Anal. Chem.*, **1999**, *364*, 160-164.
[46] Xiao, J.; Meyerhoff, M. E. *Anal. Chem*., **1996**, *68*, 2818-2825.
[47] Andrighetto, P.; Carofiglio, T.; Fornasier, R.; Tonellato, U. *Electrophoresis,* **2000**, *21*, 2384-2389.
[48] Zhang, W.; Zhang, L.; Ping, G.; Zhang, Y.; Kettrup, A. *J. Chromatogr. B*, **2002**, *768*, 211-214.
[49] Mikros, E.; Gaudemer, A.; Pasternac, R. *Inorg. Chim. Acta*,**1988**, *153*, 199-200.
[50] Král, V.; Cattani, A.; Sinica, A.; Smidtchen, F.P. *Tetrahedron*,**1999**, *55*, 7829-7834.
[51] Takahashi, M.; Ueno, A.; Uda, T.; Mihara, H. *Bioorg. Med. Chem. Letters,* **1998**, *8*, 2023-2026.
[52] Hayashi, T.; Aya, T.; Nonoguchi, M.; Mizutani, T.; Hisaeda, Y.; Kitagawa, S.; Ogoshi, H. *Tetrahedron*, **2002**, *58*, 2803-2811.
[53] Kuroda, Y.; Hatakeyama, H.; Inakoshi, N.; Ogoshi, H. *Tetrahedron Lett.,* **1993**, *34*, 8285-8288.
[54] Robic, N.; Bied-Charreton, C.; Perrée-Fauvet, M.; Verchère-Béaur, C.; Salmon, L.; Gaudemer, A.; Pasternack, R.F. *Tetrahedron Lett.,* **1990**, *31*, 4739-4742.
[55] Mizutani, T.;Wada, K.; Kitagawa, S. *J. Am. Chem. Soc*., **1999**, *121*, 11425-11431.
[56] Mizutani, T.; Kurahashi, T.; Murakami, T.; Matsumi, N.; Ogoshi, H. *J. Am. Chem. Soc*., **1997**, *119*, 8991-9001.
[57] Verchére-Bèaur, C.; Mikros, E.; Perrèe-Fauvet, M.; Gaudemer, A. *J. Inorg. Biochem*., **1990**, *40*, 127-139.
[58] Mizutani, T.; Murakami, T.;Ogoshi, H. *Tetrahedron Lett.,* **1996**, *37*, 5369-5372.
[59] Csík, G.; Balog, E.; Voszka, I.; Tölgyesi, F.; Oulmi, D.; Maillard, P.; Momenteau, M. *J. Photochem. Photobiol. B,* **1998**, *44*, 216-224.
[60] Choon, O.Ch.; Rodley, G.A. *Inorg. Chim. Acta*,**1983**, *79*, 166-167.
[61] Nakamura, Ch.; Inuyama, Y.;Shirai, K.; Nakano, S.; Sugimoto, N.; Miyake, J. *Synthetic Metals,* **2001**, *117*, 127-129.

[62]   Nakamura, Ch.; Inuyama, Y.; Shirai, K.; Sugimoto, N.; Miyake, J.; *Biosensors and Bioelectronics*, **2001**, *16*, 1095-1100.

[63]   Voszka, I.; Galántai, R.; Maillard, P.; Csík, G. *J. Photochem. Photobiol. B*, **1999**, *52*, 92-98.

[64]   Charvátová, J.; Kasicka, V.; Deyl, Z.; Král, V. *J. Chromatogr. A*, **2003**, *990*, 111-119.

[65]   Charvátová, J.; Kasicka, V.; Král, V.; Deyl, Z.; *J. Chromatogr. B*, **2002**, *770*, 165-175.

[66]   Charvátová, J.; Deyl, Z.; Kasicka, V.; Král, V. *J. Chromatogr. A*, **2003**, *990*, 159-167.

[67]   Charvátová, J.; Král, V.; Deyl, Z. *J. Chromatogr. B*, **2002,** *770*, 155-163.

[68]   Charvátová, J.; Kasicka, V.; Barth, T.; Deyl, Z.; Miksik, I.; Král, V. *J. Chromatogr. A,* **2003**, *1009*, 73-80.

[69]   Charvátová, J.; Mat ě jka, P.; Král, V.; Deyl, Z. *J. Chromatogr. A,* **2001**, *921*, 99-107.

[70]   Charvátová, J.; Král, V.; Deyl, Z. *Electrophoresis,* **2002***, 23*, 237-244.