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Separation of structurally related peptides by open-tubular capillary electrochromatography using (metallo)porphyrins as the adsorbed stationary phase

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Abstract

Several (metallo)porphyrins, particularly the porphyrin derivative tetraphenylporphyrin, and complexes of porphyrin derivatives with metal ions (Zn^{2+} , Cu^{2+} , Ni^{2+} , Co^{2+} , Co^{3+}) have been employed as the stationary phase physically adsorbed onto the inner fused-silica capillary surface for open-tubular capillary electrochromatography, and applied for the separation of structurally related peptides. Four octapeptides, derivatives of the B23–B30 fragment of the B-chain of human insulin with minor changes in their sequences (presence of lysine or ornithine in position B-29, presence or absence of phenylacetyl protecting group on the amino group of lysine/ornithine or N-terminal amino group of glycine), were studied as model analytes. Separations were performed both in alkaline (pH 9.0) and in acidic (pH 2.25) background electrolytes, and the changes in the migration/retention behaviour of the model set of peptides were investigated with respect to the porphyrin periphery/central metal atom and the charge of the octapeptides modified. The key moment of successful separation of these peptides seems to be the accessibility of functional groups of the peptides to the interaction with the modifiers tested herein. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Recognition of the distinct moieties and/or differences in the peptide structures represents one of the utmost goals in contemporary analytical chemistry of synthetic and natural biologically active peptides. In addition to classical, but rather laborious, time-consuming and expensive immunochemical approaches,

new ligands capable to form biospecific complexes with peptides are looked for to achieve this goal. Porphyrins, the naturally occurring macrocyclic compounds taking part in many vitally important processes of living organisms, are considered potential candidates for molecules specifically recognizing different types of biomolecules and their particular moieties. The porphyrins and their derivatives possess different types of binding forces necessary for specific interactions. The tetrapyrrolic porphyrin ring is very stable and exhibits the aromatic character suitable for hydrophobic π – π stacking interactions,

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the porphyrin core forms stable 1:1 complexes with almost all transition metal ions through which they are able to form coordination bonds with other ligands, and via the ionic interactions of their ionogenic groups they are able to bind oppositely charged molecules. Owing to these properties, porphyrins and their derivatives and metallo-complexes have begun to be widely used as selective agents in analytical chemistry, including the area of separation techniques [1].

In one of their first studies, Kibbey and Meyerhoff showed that zinc(II)-tetraphenyl-porphyrin (Zn-TPP) retained imidazoles and histidine via a strong cation–solute interaction [2]. This has implied the application of porphyrin stationary phases for the chromatographic separation of amino acids and low-molecular-mass peptides [3,4]. Various metalloprotoporphyrins [4] and metallotetraphenylporphyrins [5] covalently linked to the silica supports were used as immobilized metal-ion affinity chromatography (IMAC) stationary phases for the separation of amino acids and oligopeptides using phosphate buffer as eluent. The modes of retention mechanism have been observed: coordinative interactions via metallic centre of the porphyrin and π – π interactions between π -electrons of the porphyrin macrocycle and π -electrons of the analyte.

Protoporphyrin IX, providing a less hydrophobic phase than TPP when used as metallated stationary phase, showed a cumulative binding affinity for small peptides containing amino acids that individually exhibited strong interactions with a given stationary phase [3]. In other studies on separation of peptides with metallated TPP silicas it was shown for several peptides containing histidine and phenylalanine residues, that retention decreases with the increasing distance between these two amino acids in the peptide molecule [5]. The separation of several tyrosine containing dipeptides and tripeptides and insulins was investigated with unmetallated and with Zn(II)- and Cu(II)-metallated TPP [6]. Greater retention factors obtained on metallated column as compared to the unmetallated version confirm the coordinative interaction between the metallic centre of the porphyrin and the separated peptide. It was found that the retention diversification of tripeptides containing tyrosine or phenylalanine residues was greater than for individual Tyr and Phe molecules,

obtained on the same Zn-TPP column with 1% acetonitrile addition to the phosphate eluent [4]. It was shown that the differences in the retention of tripeptides depend also on the structural conformation of their molecules.

In our recent papers we have shown that porphyrin derivatives with different central metal atoms and peripheral substituents used as modifiers of the fused-silica capillary surface in open-tubular capillary electrochromatography (OT-CEC) influenced the separation of some aliphatic and aromatic amino acids [7] and aromatic amino acids possessing peptides [8].

The aim of our present study was to employ porphyrin derivatives and their metallo-complexes, (metallo)porphyrins, as the recognition and separation-enhancing element for the distinct functional groups in the structurally related peptides, in particular the synthetic derivatives of the octapeptide–B23–B30 fragment of the C-terminal part of the B-chain of human insulin, differing in the presence or absence of protecting phenylacetyl group on the N-terminal amino group of glycine residue in position B-23, ϵ -amino group of lysine and δ -amino group of ornithine residues in position B-29 of the insulin B-chain. OT-CEC [9–13] with the stationary phase formed by the porphyrin derivatives and metalloporphyrins physically adsorbed onto the inner wall of the fused-silica (FS) capillary has been used to study the influence of these ligands on the migration/retention behaviour of the four octapeptides with minor changes in their sequences. Separations were done both in alkaline (pH 9.0) and acidic (pH 2.25) background electrolytes (BGEs) and the OT-CEC separations were compared with those obtained by capillary zone electrophoresis (CZE) in the bare FS capillaries.

2. Experimental

2.1. Instrumentation

All OT-CEC and CZE experiments were carried out in the a laboratory-constructed device for capillary electromigration methods equipped with a single-beam UV-photometric detector operating at 206 nm [14]. The bare FS capillaries [30–31 cm (effec-

tive length 19–20 cm)×50 μm I.D.×200 μm O.D.] were obtained from the Institute of Glass and Ceramic Materials of the Academy of Sciences of the Czech Republic (Prague, Czech Republic), the preparation of FS capillaries with (metallo)porphyrin-derivatives-based physically adsorbed stationary phases is described below. The applied separation voltage was 10 kV, the electric current was 12.6–13.1 μA for the alkaline BGE and 44.5–46.5 μA for the acidic BGE. Experiments were carried out at the ambient laboratory temperature 22–24 °C, without active capillary cooling. The samples were injected hydrodynamically at 700 Pa for 2–5 s.

2.2. Materials and methods

All chemicals used for preparation of the BGEs were of analytical purity grade. Tris(hydroxymethyl)aminomethane (Tris) was purchased from Serva (Heidelberg, Germany), phosphoric acid, boric acid and sodium hydroxide were obtained from Lachema (Brno, Czech Republic). Peptide samples, derivatives of the B23–B30–octapeptide fragment of B-chain of human insulin were provided by one of us, T.B. Porphyrin derivative, 5,10,15,20-tetraphenylporphyrin, H₂TPP, and metalloporphyrins (see Fig. 1) were synthesized in the laboratory by one of us (V. Král), and characterized by ¹H nuclear mag-

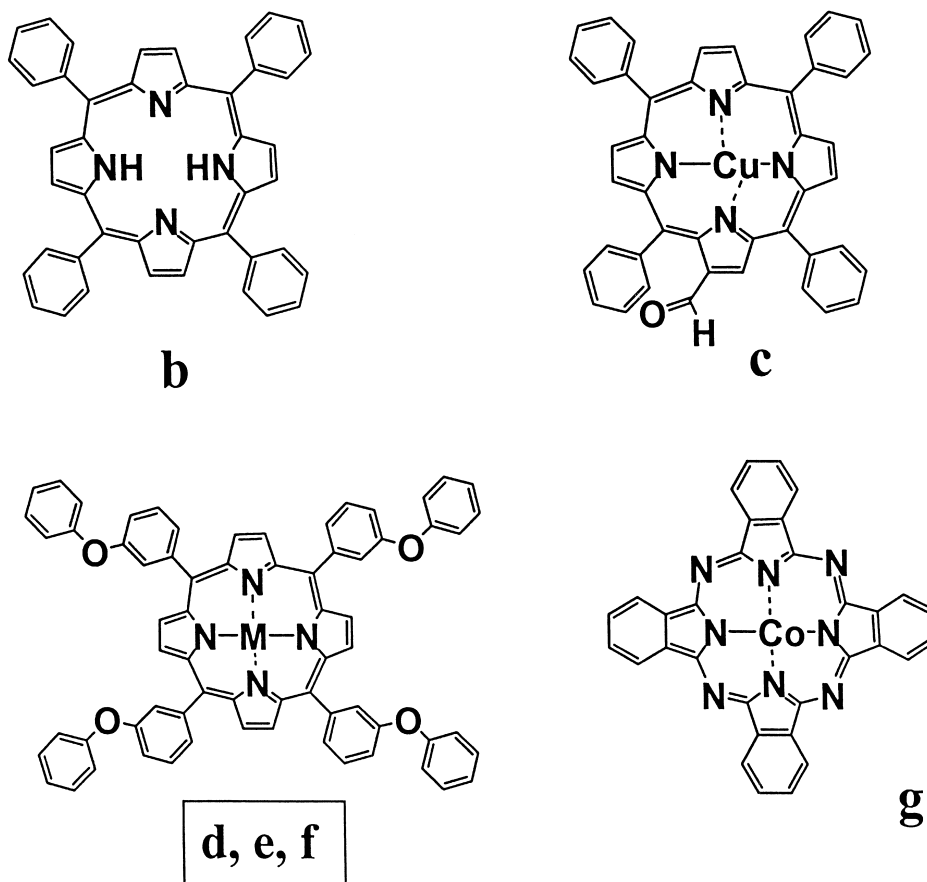


Fig. 1. Structures of (metallo)porphyrin derivatives: (b) 5,10,15,20-tetraphenylporphyrin, TPP; (c) 3-formyl-5,10,15,20-(tetraphenyl)porphyrinate Cu(II), Cu(II)TPP(HCO); (d, e, f) 5,10,15,20-tetrakis(phenoxyphenyl)porphyrinate metal (M), (d) M=Rh(III), Rh(III)TPP(*m*-OPh)₄, (e) M=Co(III), Co(III)TPP(*m*-OPh)₄; (f) M=Ni(II), Ni(II)TPP(*m*-OPh)₄; (g) Co(II) phthalocyanine.

netic resonance (NMR), mass spectrometry (MS) and IR spectrometry [15].

Coating of the inner FS capillary surface by (metallo)porphyrin derivatives is described in our previous paper [7]. Briefly, after a pretreatment with sodium hydroxide and water the FS capillary was washed with methanol and dried by the air stream. Next the capillary was flushed with a dichloromethane solution of the appropriate porphyrin derivative for 10 min and dried in the air stream. Further washing was performed with methanol and water. Before each set of experiments the capillaries were washed with water and BGE and stabilized at 10 kV for 20 min. Both bare FS capillary and the (metallo)porphyrins-modified capillaries were washed with the BGE for 2 min between runs.

2.3. Background electrolytes and sample solutions

Both CZE and OT-CEC experiments were performed in two BGEs, in alkaline and acidic pH regions, respectively:

BGE I (alkaline): 0.05 mol/l boric acid, 0.025 mol/l NaOH, pH 9.0.

BGE II (acidic): 0.1 mol/l phosphoric acid, 0.05 mol/l Tris, pH 2.25.

BGE solutions were prepared from the deionized

water and filtered through 0.45 μm membrane filter (Millipore, Bedford, MA, USA) prior to use.

Stock solutions of octapeptides were prepared by dissolving 0.14–0.25 mg of each octapeptide in 200 μl of deionized water and stored below 4 $^{\circ}\text{C}$. Sample mixture for injection was prepared by mixing 40 μl of stock solution of each octapeptide to give final volume 160 μl . The final concentration of peptides in the sample was in the range 0.175–0.313 mg/ml. Prior to the injection, all samples were centrifuged at 5000 rpm for 3 min (1845 relative centrifugal force). As a model mixture the following octapeptides were used (sequences given in three and single letter code, for structure see Fig. 2):

Peptide 1: H–Gly–Phe–Phe–Tyr–Thr–Pro–Orn(Pac)–Thr–OH, GFFYTPOrn(Pac)T.

Peptide 2: Pac–Gly–Phe–Phe–Tyr–Thr–Pro–Orn–Thr–OH, Pac-GFFYTPOrnT.

Peptide 3: H–Gly–Phe–Phe–Tyr–Thr–Pro–Lys(Pac)–Thr–OH, GFFYTTPK(Pac)T.

Peptide 4: Pac–Gly–Phe–Phe–Tyr–Thr–Pro–Lys–Thr–OH, Pac-GFFYTTPKT.

3. Results and discussion

3.1. Separations in alkaline BGE

Under the alkaline pH conditions of CZE and OT-CEC separations all four octapeptides possess a negative charge originating from fully dissociated C-terminal carboxyl group which is only partially compensated by the positive charge from the partially charged amino groups. Possible binding sites of our peptides 1–4 to the (metallo)porphyrins ligands include: (i) phenyl or hydroxyphenyl moieties for π – π stacking interaction, (ii) amino and hydroxyl groups for coordinating binding, (iii) amino, hydroxyl and carbonyl groups for hydrogen bonding, and (iv) methyl groups for hydrophobic interactions.

CZE and OT-CEC separations of the four peptides in the bare FS capillary and in the (metallo)porphyrin derivatives coated FS capillaries in the alkaline BGE, 50 mM boric acid, 25 mM NaOH, pH 9.0, are presented in Fig. 3. Migration times, effective mobilities, resolutions of the octapeptides and migration times and electroosmotic flow (EOF) mobilities are summarized in Table 1. As can be seen, in most

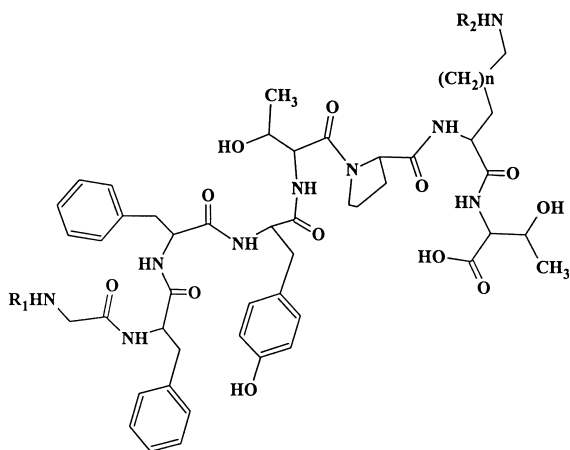


Fig. 2. Structures of analyzed octapeptides. Peptide 1: $R_1=H$, R_2 =phenylacetyl (Pac), $n=0$. Peptide 2: R_1 =phenylacetyl (Pac), $R_2=H$, $n=0$. Peptide 3: $R_1=H$, R_2 =phenylacetyl (Pac), $n=1$. Peptide 4: R_1 =phenylacetyl (Pac), $R_2=H$, $n=1$.

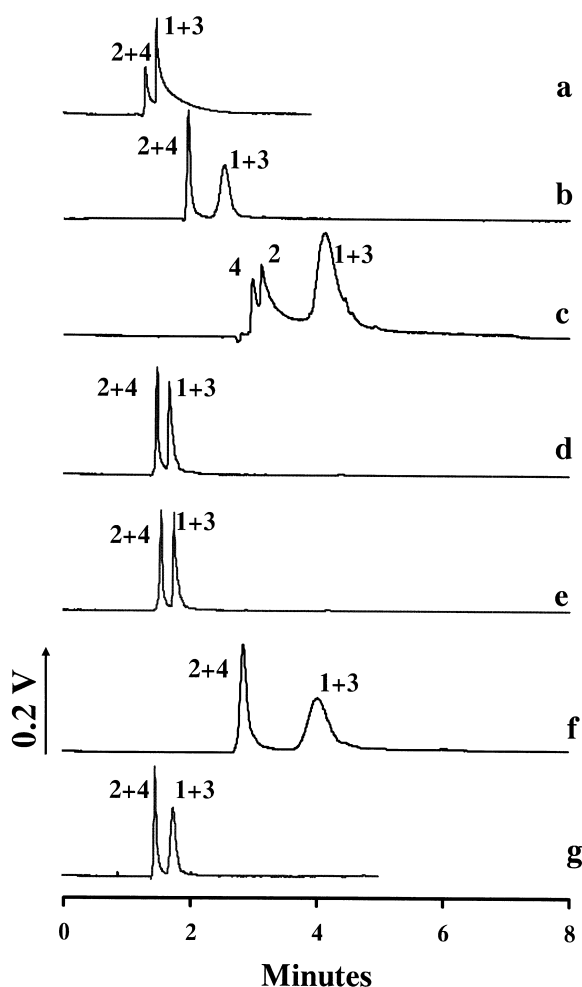


Fig. 3. CZE (a) and OT-CEC (b–g) separations of the model mixture of octapeptides 1–4 in alkaline BGE I, 50 mM H_3BO_3 , 25 mM NaOH, pH 9.0, 10 kV (14 μA); 22 °C; detection at 206 nm, injection for 2 s at 700 Pa. Peaks: 1 = GFFYTPOrn(Pac)T; 2 = (Pac)GFFYTPOrnT; 3 = GFFYTTPK(Pac)T; 4 = (Pac)GFFYTTPKT. Letter symbols at each line represent capillaries modified with the porphyrin derivatives: a = uncoated; b = H_2TPP ; c = Cu(II)TPP(HCO) ; d = $\text{Rh(III)TPP}(m\text{-OPh})_4$; e = $\text{Co(III)TPP}(m\text{-OPh})_4$; f = $\text{Ni(II)TPP}(m\text{-OPh})_4$; g = $\text{Co(II)phthalocyanine}$.

cases peptide 1 comigrated with peptide 3, and peptide 2 comigrated with peptide 4. The best resolution of these doublets was achieved in capillaries modified with $\text{Ni(II)TPP}(m\text{-OPh})_4$ ($R_s = 2.73$) and H_2TPP ($R_s = 1.86$). Moreover, at least partial separation of octapeptides 2 and 4 was achieved when the FS capillary was modified with the

Cu(II)TPP(HCO) derivative. In general, migration times of all components of the test mixture were always longer (EOF was slower) in the (metallo)porphyrin-modified FS capillaries than in the bare FS capillary, being the longest when Cu(II)TPP(HCO) was employed to cover the fused-silica surface.

Comparing the effective mobilities obtained under alkaline pH it was observed that in all (metallo)porphyrin-modified capillaries higher values of effective mobilities were obtained for peptides 1 and 3 (protecting group on the amino group of Lys or Orn) than in the bare FS capillary (see Table 1). Relative changes of effective mobilities of peptides 1–4 in the modified capillaries, related to the mobilities in the bare FS capillary, are presented in Fig. 4. Increase of the effective mobilities of peptides 1 and 3 is in the range of 5–20%. That implies that none of the above mentioned interactions occurs between the peptides 1 and 3 and the (metallo)porphyrin modifiers in alkaline pH. On the other hand effective mobilities for the solutes 2 and 4 (having the protecting group on amino group of glycine) were both increased [capillaries modified with $\text{Co(III)TPP}(m\text{-OPh})_4$ and $\text{Rh(III)TPP}(m\text{-OPh})_4$] about 40%, and decreased [capillaries modified with H_2TPP , $\text{Ni(II)TPP}(m\text{-OPh})_4$, $\text{Co(II)phthalocyanine}$] of 13–40%. A decrease in the EOF mobility was observed in all modified capillaries and the extent of this decrease was in the range 5–50% as compared to the unmodified capillary.

Considering the structure of modifiers we can conclude that the application of modifiers with the overall (1+) charge and identical peripheral substituents [$\text{Co(III)TPP}(m\text{-OPh})_4$ and $\text{Rh(III)TPP}(m\text{-OPh})_4$] result in an increase of effective migrations of octapeptides 2 and 4 as well as of octapeptides 1 and 3. Conversely, when the modifier possessed zero overall charge [H_2TPP , $\text{Ni(II)TPP}(m\text{-OPh})_4$, $\text{Co(II)phthalocyanine}$], the effective migration was considerably decreased. It is feasible to speculate that it is effected by the axial ligand binding between the spare electron pair of nitrogen in the Lys or Orn amino group and the metal atom in the porphyrin cavity [considering the two metalloporphyrins, $\text{Ni(II)TPP}(m\text{-OPh})_4$ and $\text{Co(II)phthalocyanine}$]. The major decrease of electrophoretic mobility of octapeptides 2 and 4 in H_2TPP modified capillary may be explained by hydrogen bonding between Lys

Table 1

Migration times, effective mobilities and resolutions of octapeptides **1–4**, and migration times and mobilities of EOF obtained from CZE and OT-CEC separations in alkaline BGE, 50 mM boric acid, 25 mM NaOH, pH 9.0

Capillary		Peptide 1		Peptide 2		Peptide 3		Peptide 4		EOF		Resolution
Type	Effective/ total length (cm)	t_m (min)	μ_{eff}	t_m (min)	μ_{eff}	t_m (min)	μ_{eff}	t_m (min)	μ_{eff}	t_m (min)	μ_{eff}	
Uncoated	19/30	1.513	11.0	1.345	3.2	1.513	11.0	1.345	3.2	1.280	74.2	$R_s=0.75$
H ₂ TPP	19/30	2.537	12.8	1.967	1.9	2.537	12.8	1.967	1.9	1.892	50.2	$R_s=1.86$
Cu(II)TPP(HCO)	20/31	4.130	12.5	3.127	4.5	4.130	12.5	2.990	3.0	2.753	37.5	$R_s=1.73$
Rh(III)TPP(<i>m</i> -OPh) ₄	19/30	1.680	11.6	1.490	4.4	1.680	11.6	1.490	4.4	1.394	68.2	$R_{s,4,2}=0.53$ $R_s=1.00$
Co(III)TPP(<i>m</i> -OPh) ₄	20/31	1.753	12.2	1.553	4.6	1.753	12.2	1.553	4.6	1.453	71.1	$R_s=1.2$
Ni(II)TPP(<i>m</i> -OPh) ₄	19/30	4.037	11.9	2.86	2.2	4.037	11.9	2.86	2.2	2.680	35.4	$R_s=2.73$
Co(II)phthalocyanine	19/30	1.760	13.3	1.473	2.8	1.760	13.3	1.473	2.8	1.413	67.3	$R_s=1.69$

R_s = Resolution of the doublet peaks (peptides **1** and **3** comigrating; peptides **2** and **4** comigrating), $R_{s,4,2}$ = resolution of peptides **4** and **2**.
 t_m = Migration time, μ_{eff} = effective mobility ($\cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$).

(Orn) amino group and nitrogens/(NH groups) in the porphyrin ring, which is under these conditions uncharged. In general, the non-protected Lys (Orn) amino group in the octapeptide structure plays the key role in the interactions with the (metallo)por-

phyrins tested. The preference of binding of the Orn and Lys amino group over the same group of the N-terminal amino acid (glycine) can be explained by the longer side chain of Lys and Orn and, consequently, a more favourable sterical accessibility to

Comparison of effective mobilities

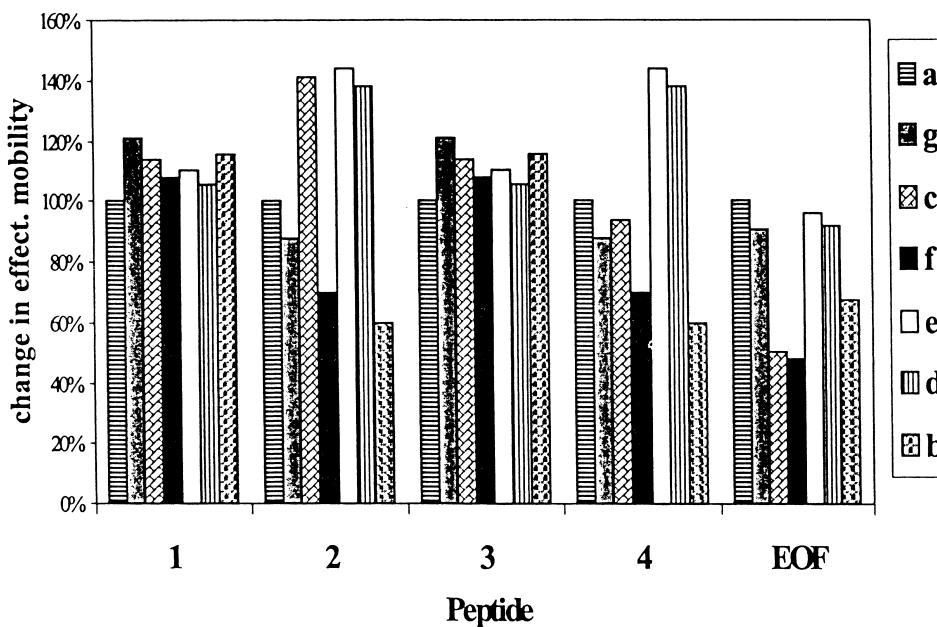


Fig. 4. Relative effective mobilities of octapeptides **1–4** obtained from CZE and OT-CEC separations (effective mobility obtained by CZE in the bare FS capillary is considered as 100%). Legend: FS capillary modified by: (a) unmodified; (b) H₂TPP; (c) Cu(II)TPP(HCO); (d) Rh(III)TPP(*m*-OPh)₄; (e) Co(III)TPP(*m*-OPh)₄; (f) Ni(II)TPP(*m*-OPh)₄; (g) Co(II)phthalocyanine.

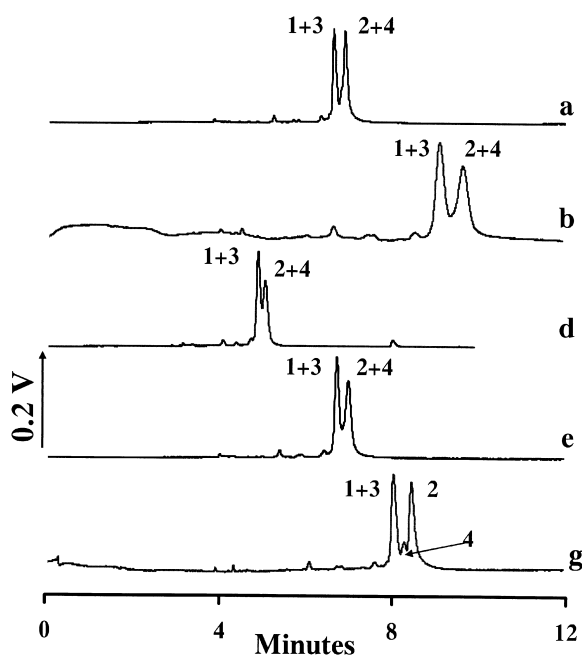


Fig. 5. CZE (a) and OT-CEC (b, d, e, g) separations of the model mixture of octapeptides in acidic BGE, 100 mM H_3PO_4 , 50 mM Tris, pH 2.25; 10 kV (43 μA). Other conditions and identifications as in Fig. 3.

the (metallo)porphyrin modifiers. An interesting point of our results is that the effective migration of octapeptide **2** is increased when using $\text{Cu(II)TPPCH}_2\text{OH}$ modified capillary, while the effective migration of octapeptide **4** in the same capillary is decreased.

Considering the type of possible interactions, it is unlikely that π - π interactions are involved owing to the sterical hindrance of the bulky analyte molecules.

3.2. Separation in acidic BGE

In the acid BGE, 100 mM phosphoric acid, 50 mM Tris, pH 2.25, all the tested oligopeptides exist in the cationic form, with ca. +0.75 elementary charge, resulting from the fully protonated amino groups of both Lys/Orn side chains and N-terminal glycine, and only partially dissociating carboxyl group of the C-terminal threonine. However, one of the above mentioned amino groups is always protected in the octapeptide molecules.

The records of CZE and OT-CEC separations of octapeptides **1–4** in the acid BGE are presented in Fig. 5. At these rather acidic conditions, pH 2.25, the EOF is very low, the migration times of the EOF marker were longer than several tens of minutes, so the migration times of the peptides, presented together with their resolutions in Table 2, can be considered as the reciprocal measure of their effective mobilities. Similarly as in the alkaline pH of the BGE, only partial separation of the four peptides mixture was obtained in most cases, peptides **1** and **3** comigrated in the faster peak and peptides **2** and **4** comigrated in the slower peak. The best resolutions were obtained in H_2TPP and $\text{Co(II)phthalocyanine}$ modified capillaries. Other resolutions were nearly the same as observed in the unmodified fused-silica

Table 2

Migration times and resolutions of octapeptides **1–4**, obtained from CZE and OT-CEC separations in acidic BGE, 100 mM phosphoric acid, 50 mM Tris, pH 2.25

Capillary	Effective/total length (cm)	Migration time (min)				Resolution
		Peptide 1	Peptide 2	Peptide 3	Peptide 4	
Uncoated	19/30	6.600	6.863	6.600	6.863	$R_s=0.66$
H_2TPP	19/30	9.123	9.663	9.123	9.663	$R_s=0.92$
Cu(II)TPP(HCO)	20/31	n.d.	n.d.	n.d.	n.d.	n.d.
$\text{Rh(III)TPP}(m\text{-OPh})_4$	19/30	4.917	5.083	4.917	5.083	$R_s=0.66$
$\text{Co(III)TPP}(m\text{-OPh})_4$	20/31	6.707	6.970	6.707	6.970	$R_s=0.87$
$\text{Ni(II)TPP}(m\text{-OPh})_4$	19/30	n.d.	n.d.	n.d.	n.d.	n.d.
$\text{Co(II)phthalocyanine}$	19/30	8.033	8.457	8.033	8.283	$R_s=0.40$ $R_{s,4,2}=0.92$

R_s = Resolution of the doublet peaks (peptides **1** and **3** comigrating; peptides **2** and **4** comigrating), $R_{s,4,2}$ = resolution of octapeptides **4** and **2**, n.d. = not determined (due to irreproducible results).

surface. Similarly to the alkaline BGE also in the acid buffer at least partial resolution of octapeptides **4** and **2** was achieved in one case, particularly when using Co(II)phthalocyanine as the capillary wall modifier. It seems that the key point under both alkaline and acidic conditions is the presence of unprotected amino group of Lys or Orn. It is also worth emphasizing that with Ni(II)TPP(*m*-OPh)₄ and Cu(II)TPP(HCO) used as wall modifiers, the interactions of the peptides with the capillary surface were much too strong and no reproducible results were obtained.

4. Conclusions

From the above results the following conclusions can be drawn:

(1) Modification of the bare fused-silica surface with all (metallo)porphyrin derivatives used resulted in the electroosmotic flow decrease (as compared to the bare fused-silica).

(2) In the alkaline BGE, pH 9, the best resolution was obtained when Ni(II)TPP(*m*-OPh)₄ was employed as the FS capillary inner surface modifier. When Cu(II)TPP(CHO) porphyrin was used partial resolution of octapeptides differing only in Lys/Orn amino acid side chain could be achieved.

(3) At least partial resolution of the same pair of octapeptides was obtained in the acid BGE as well, provided that the recognition macrocycle was Co(II)phthalocyanine.

(4) The unprotected amino group of Lys/Orn residues of the analysed octapeptides seems to play substantial role in the interaction of the solutes with the (metallo)porphyrin derivatives.

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References

- [1] M. Biesaga, K. Pyrzyńska, M. Trojanowicz, *Talanta* 51 (2000) 209.
- [2] C.E. Kibbey, M.E. Meyerhoff, *Anal. Chem.* 65 (1993) 2189.
- [3] J. Xiao, M.E. Meyerhoff, *Anal. Chem.* 68 (1996) 2818.
- [4] M. Biesaga, J. Orska, M. Trojanowicz, *Chem. Anal. (Warsaw)* 43 (1998) 647.
- [5] M. Trojanowicz, G.B. Martin, M.E. Meyerhoff, *Chem. Anal. (Warsaw)* 41 (1996) 521.
- [6] M. Biesaga, J. Orska, D. Fiertek, J. Izdebski, M. Trojanowicz, *Fresenius J. Anal. Chem.* 364 (1999) 160.
- [7] J. Charvátová, V. Kašička, V. Král, Z. Deyl, *J. Chromatogr. B* 770 (2002) 165.
- [8] J. Charvátová, V. Král, Z. Deyl, *J. Chromatogr. B* 770 (2002) 155.
- [9] J.J. Pesek, M.T. Matyska, *J. Cap. Electrophoresis* 4 (1997) 213.
- [10] Z. Liu, H.F. Zou, M.L. Ye, J.Y. Ni, Y.K. Zhang, *Electrophoresis* 20 (1999) 2891.
- [11] M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, *J. Chromatogr. A* 924 (2001) 211.
- [12] M.T. Matyska, J.J. Pesek, I. Boysen, M.T.W. Hearn, *Electrophoresis* 22 (2001) 2620.
- [13] J.J. Pesek, M.T. Matyska, S. Sentellas, M.T. Galceran, M. Chiari, G. Pirri, *Electrophoresis* 23 (2002) 2982.
- [14] V. Kašička, Z. Prusík, P. Sázelová, E. Brynda, J. Stejskal, *Electrophoresis* 20 (1999) 2484.
- [15] V. Král, J. Koutníková, K. Záruba, manuscript in preparation.