Journal of Chromatography A, 1517 (2017) 185–194

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Full length article

Separation of oligopeptides, nucleobases, nucleosides and nucleotides using capillary electrophoresis/electrochromatography with sol–gel modified inner capillary wall

Jana Svobodová[a,*], Olga Kofroňová[c], Oldřich Benada[c], Vladimír Král[b], Ivan Mikšík[a]

[a] Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
[b] Department of Analytical Chemistry, Institute of Chemical Technology, Prague, Czech Republic
[c] Laboratory of Molecular Structure Characterization, Institute of Microbiology, v.v.i., Czech Academy of Sciences, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form 2 August 2017
Accepted 5 August 2017
Available online 7 August 2017

Keywords:
Capillary electrochromatography (CEC)
Open-tubular capillary electrochromatography (OT-CEC)
Nucleo-compounds
Oligopeptides
Sol–gel methods
Porphyrin
Scanning electron microscopy (SEM)

ABSTRACT

The aim of this article is to study the modification of an inner capillary wall with sol–gel coating (pure silica sol–gel or silica sol–gel containing porphyrin-brucine conjugate) and determine its influence on the separation process using capillary electrophoresis/electrochromatography method. After modification of the inner capillary surface the separation of analytes was performed using two different phosphate buffers (pH 2.5 and 9.0) and finally the changes in electrophoretic mobilities of various samples were calculated. To confirm that the modification of the inner capillary surface was successful, the parts of the inner surfaces of capillaries were observed using scanning electron microscopy. The analytes used as testing samples were oligopeptides, nucleosides, nucleobases and finally nucleotides.

1. Introduction

Capillary electrochromatography based on the sol–gel formation of stationary phases represent rapidly growing area in separation science [1,2]. The advantages of the sol–gel method in preparing of stationary phases for CEC are summarized in Ref. [2], and the focusing on OT-CEC technique is very broadly described in Ref. [1]. The entrapment of biomolecules in sol–gel matrices and their applications as biosensors and other wide applications was also published and is far beyond the scope of this paper [3,4]. Sol–gel glass offers an easy way to immobilize biomolecules within its porous optically transparent matrix and demonstrate functional activity of encapsulated biomolecules [5,6].

Porphyrins with their tetrapyrrolic macrocycle represent a group of various naturally occuring macrocyclic compounds, e.g. our lifelong never-ending contact with chlorophyll, hemoglobin, cytochrome c, catalase or vitamin B_{12} and many other poprhyrin derivatives in daily life [7]. Owing to their multifunctional structure and facility to form metallo complexes, porphyrins play an important role in metabolic processes of living organisms as well as they represent a group of compounds exploited in analytical chemistry [8]. Porphyrins, their wide group of expanded and substituted derivatives and their use in analytical chemistry is such a large and widespread field that it is found in almost every single branch of analytical research and for deeper insight the reader is referred to ref. [7].

The covalent bonding of porphyrin free bases or their respective complexes with different metals or substituent group on the walls of porous network producing translucent and monolithic silica xerogels was also described [9]. Furthermore, the size and possibly the shape of the silica cavities that were formed around the solvated macrocyclic species depended on the nature and position of the substituent groups in the porphyrin compounds [9]. Luminiscent porphyrinosilica obtained by the sol–gel method were studied using UV–vis, infrared spectroscopy, atomic force microscopy (AFM), nuclear magnetic resonance (NMR) and scanning electron microscopy (SEM) [10–12,13–15]. Real-time

Abbreviations: PB-conjugate, porphyrin-brucine conjugate; TEOS, tetraethyl orthosilicate; APTES, aminopropyl triethoxysilane; PDT, photodynamic theraphy; OT-CEC, open-tubular capillary electrochromatography.

* Corresponding author.
E-mail address: Jana.Svobodova@fgu.cas.cz (J. Svobodová).

http://dx.doi.org/10.1016/j.chroma.2017.08.014
0021-9673/

investigation of molecular recognition between a protein and the photosensitizer of photodynamic therapy (PDT) was carried out by a quartz crystal microbalance (QCM) sensor integrated into a flow injection analysis system. The photosensitizer meso-tetrakis(4-hydroxyphenyl)porphyrin was immobilized on the gold electrode of the QCM chip and exhibited specific interaction with hemoglobin [16]. Open-tubular capillary electrochromatography with the inner surface modified with covalently bonded metallo-porphyrins was also presented for separation of oligopeptides [17,18] and a thorough and widely informative review about porphyrins used in OT-CEC was also published with special attention paid on physically adsorbed porphyrins as stationary phases [19]. The broad reviews about porphyrins in analytical chemistry can be found in [8,20].

Brucine, the indole alkaloid closely related to strychnine, originates from India and South-East Asia. Both of these alkaloids are commonly used as agents for chiral resolution. The alkaloid brucine from *Strychnos-Nux Vomica* tree obtained from its seeds, bark, wood and roots, has numerous applications in traditional medicine in Asia and Europe. In India it is used in ayurvedic medicine as an appetizer, tonic, astringen, antipyretic and it is claimed to cure blood diseases, itching, piles, ulcers, anemia, urinary discharges and weaknes of limbs. In Europe its medicinal use dates back to 1540 CE. and was specifically used to treat nervous disorders and problems with digestive organs and the respiratory system. Nowadays it is mainly used in phytotherapy to treat upset stomach, abdominal pain, constipation, heart burn, circulatory problems, nervous conditions and respiratory diseases. Brucine alkaloid is 50–100 times less toxic than strychnine and the extract from Nux Vomica seeds exhibits significant analgesic, antiinflammatory, anti ulcer, cytotoxic, antioxidant and uterine stimulant activity [21].

Brucine alone was reported as a novel chiral selector bonded to the stationary phase in HPLC separation of binaphtyl-carboxylic acid derivatives [22]. The porphyrin-brucine conjugates were studied as receptors for oxoanions [23], a transporter of inorganic anions through artifical membrane [24], and when fixed to gold nanoparticles were also studied not only for anion binding [25] but also applied in PDT [26].

Therefore, we tried to combine these three fields: – sol–gel technology, porphyrin-brucine conjugate and electrophoresis/electrochromatography – for the analysis of biologically simple compounds.

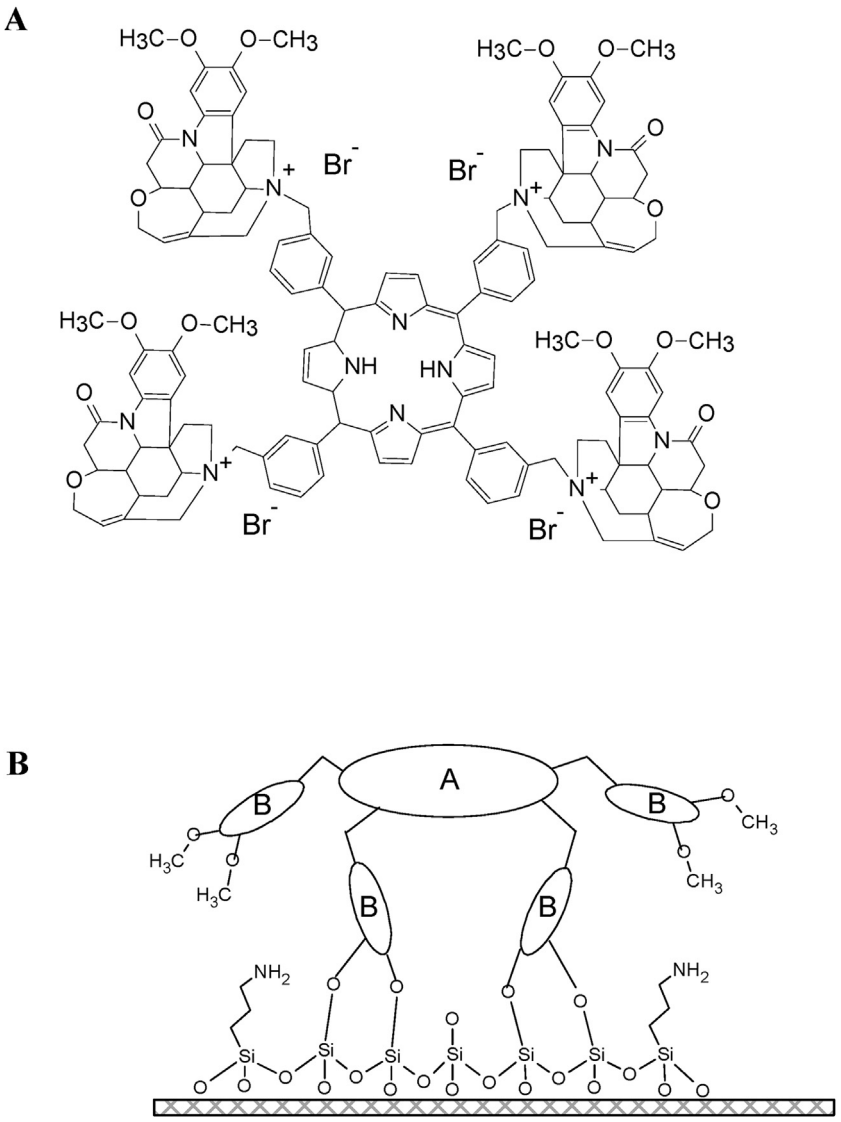

Fig. 1. (A) Structure of porphyrin derivative with brucine substituent –PB-conjugate. (B) Possible mode of the inner capillary wall modification, A – tetraphenyl porphyrin inner core, B – brucine substituent.

2. Methods

2.1. Electrophoretic analyses

The Beckmann Coulter P/ACE 5500 apparatus (Fullerton, CA, USA) was used during all the electrophoretic analyses. Detection by PDA (254 nm for nucleotides, nucleobases and nucleosides, 214 nm for oligopeptides; bandwidth 10 nm) was applied. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of total length 27 cm and 20.5 cm of the effective length, 75 μm I.D. and 375 μm O.D. were used for all separations. Injection was performed with applied pressure 0.5 psi (3.4474 kPa). The apparatus was cooled with liquid coolant (3 M Fluorinert™ FC-770, Zwijndrecht, Belgium).

2.2. Chemicals and accessories

Nucleobases, nucleosides and nucleotides were obtained from Lachema (Brno, Czech Republic), Sigma Aldrich (Munich, Germany) excluding AMP from Merck (Darmstadt, Germany). Tetraethyl orthosilicate (TEOS), aminopropyl triethoxysilane (APTES) and thiourea were obtained from Sigma Aldrich (Munich, Germany) as well as all oligopeptides except for L-glutathione (Alexis Corp., Lausen, Switzerland). Liquid chemicals i.e. ethanol (EtOH p.a); dimethyl sulfoxide (DMSO) were obtained from Lachema (Brno, Czech Republic). Chemicals for the preparation of phosphate buffers – dihydrogen sodium phosphate and hydrogen sodium phosphate – were from Lachema (Brno, Czech Republic).

Parafilm (Bemis, WI, USA) and plastic syringes (Chirana, Stará Turá, Slovakia) were also often needed and used. Porphyrin-brucine conjugate (PB-conjugate) was prepared at the Institute of Chemical Technology (Prague, Czech Republic). Plastic materials such as the pipette tips (Vertex, California CA, USA), eppendorf tubes (Hamburg, Germany) and nylon syringe filters (13 mm, 0.22 μm, Labicom, Olomouc, Czech Republic) were used in all experiments.

2.3. Preparation of modified capillaries for OT-CEC

2.3.1. Modification of the inner capillary surface with pure sol–gel

a) The fused silica capillary was prewashed with water, 1 mol/L NaOH, distilled water, 1 mol/L HCl, water, each step for 10 min.
b) The standard solution of sol–gel preparation contained as follows:

50 μl0.02 mol/LHCl, 50 μlTEOS, 200 μlEtOH, 200 μlH_2O,

all mixed together, vortexed and left to hydrolyze for 20 min. After this part of sol–gel preparation (a transparent clear solution was obtained) the capillary was washed with this solution using suction (with an injection syringe connected to the homemade tapering plastic tubing) for 10 min, the ends of capillary were sealed with parafilm and left at room temperature overnight.

c) The rest of the inner wall modifier was sucked out of the capillary with vacuum, then the capillary was blown with air and nitrogen (5 min each) and finally washed with a solution of APTES in ethanol (1:19 v/v, respectively) for 5 min. Then again blown with air and nitrogen for 5 min each, washed with EtOH for 2 min, blown again with air for 5 min and left open at room temperature.

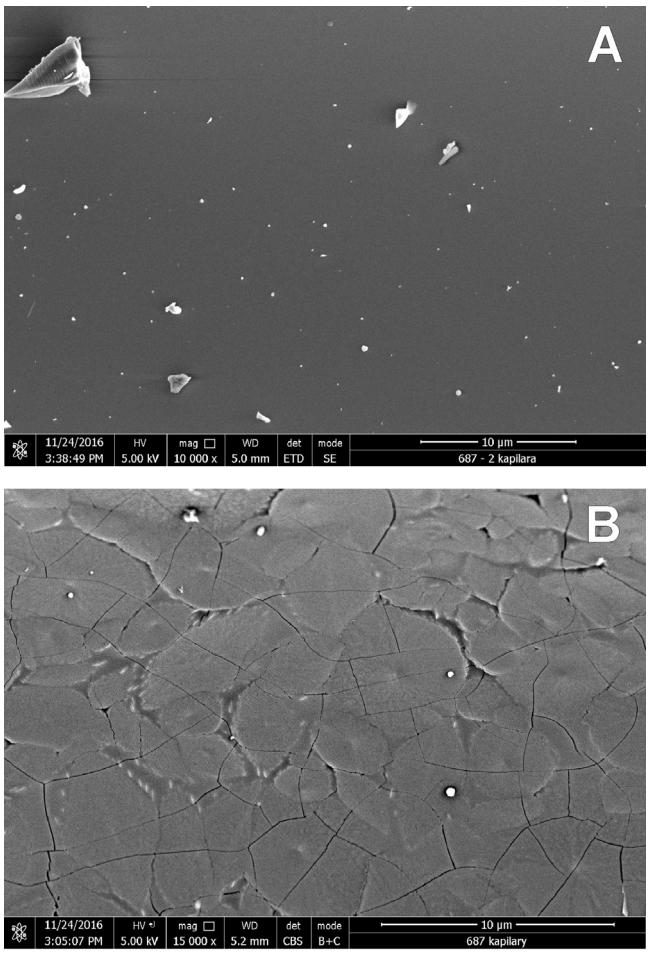

Fig. 2. SEM images of uncoated and modified capillary with sol–gel + PB- conjugate, A-uncoated, B-modified capillary.

2.3.2. Modification of the inner capillary surface with sol–gel which contains porphyrin-brucine conjugate

The initial cleaning of the capillary was done as well as described in the previous section.

The stock solution of PB-conjugate (Fig. 1A) was prepared by dissolving of the solid PB-conjugate (0.5 mg) in 100 μl of DMSO. Then 50 μl of this solution was diluted with 100 μl of EtOH, and thus diluted solution was pulled through a previously sol–gel modified capillary (see step a,b) plus first 5 min flushing with air, finally sealed with parafilm and left at room temperature overnight. The rest of the PB-conjugate solution was sucked out of the capillary with vacuum, then the capillary was washed with the solution of APTES (step c mentioned above) for 5 min and blown with air and nitrogen for 5 min each. Thus prepared capillary was left at room temperature until the experiments.

The solution of APTES in EtOH was finally used to accelerate the process of condensation of the sol–gel solution in both modified capillaries. The probable connection of the PB-conjugate to the sol–gel surface is drawn at Fig. 1B.

2.4. Conditioning (stabilization) of capillaries

After modification and resting at room temperature for several days, the capillaries were prepared for separation experimens. Before running with the buffer, each capillary was washed with water for 10 min and then finally with running buffer for 20 min.

All experiments were performed in two phosphate buffers – one at pH 2.50, the second at pH 9.0. In both cases the concentration was

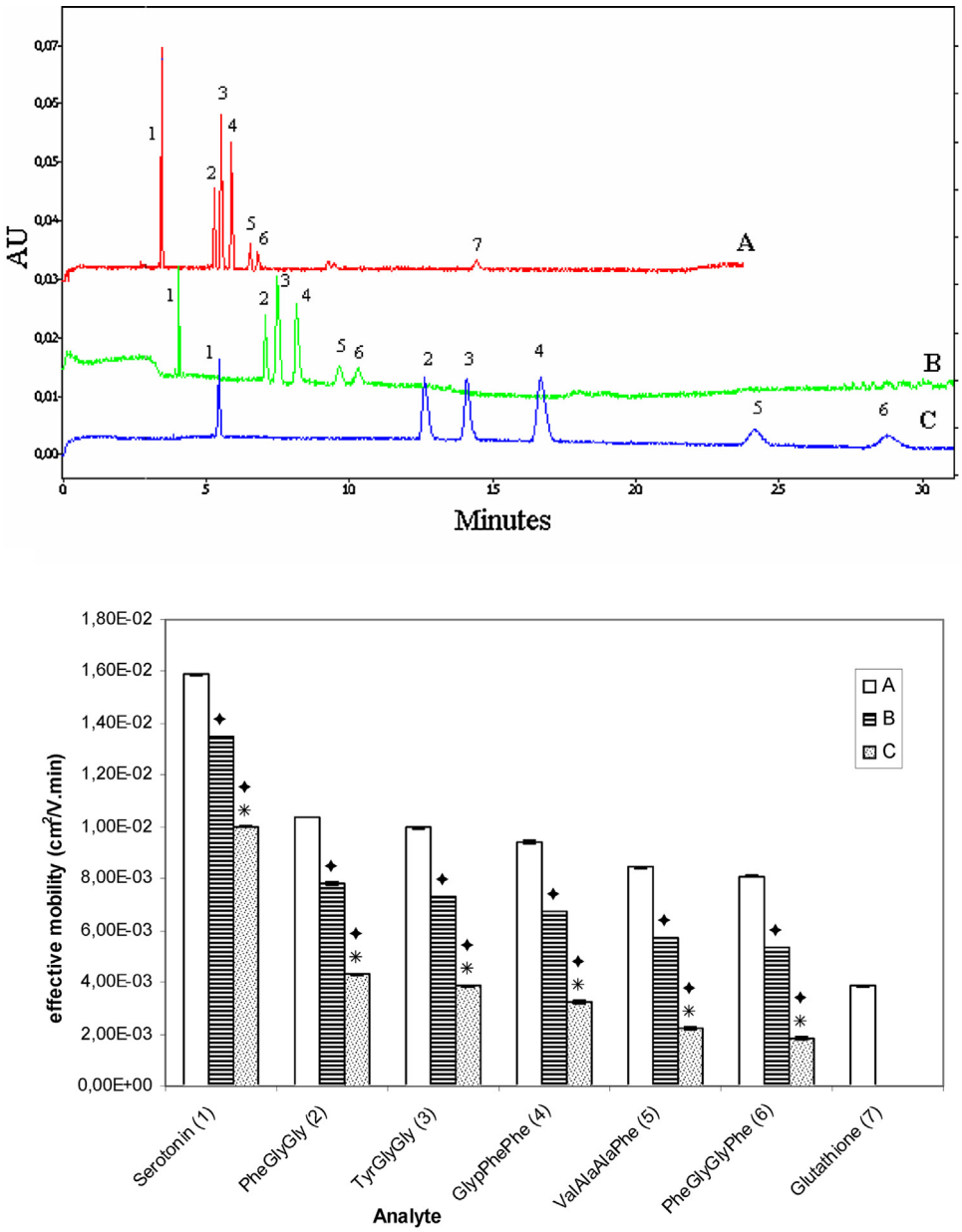

Fig. 3. Separation of oligopeptides under acidic conditions in 0.05 mol/L phosphate buffer, pH 2.50, detection 214 nm, 10 kV, normal polarity, 20 °C, injection for 3 s with pressure 0.5 psi.
Electropherogram: 1-serotonin, 2-PheGlyGly, 3-TyrGlyGly, 4-GlyPhePhe, 5-ValAlaAlaPhe, 6-PheGlyGlyPhe, 7-Glutathion; A- uncoated, B- pure sol–gel, C- sol–gel + PB-conjugate capillaries
Graph of effective mobilities: A-uncoated capillary (n = 3), **B**-sol–gel capillary (n = 4), **C**-sol–gel + PB-conjugate capillary (n = 3); Glutathion not detected using B and C capillary ⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary), ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary.

0.05 mol/L and the buffer was adjusted with 1 mol/L phosphoric acid.

2.5. Scanning electron microscopy (SEM)

Cut pieces of capillaries were mounted onto standard aluminum SEM mounts using SPI Die-Cut Carbon Conductive Double Sided Adhesive Discs (West Chester, PA, USA). The mounts were blown off with an air duster (Armor, České Budějovice, Czech Republic) to remove any non-mounted material. The samples were sputter coated with 3 nm of platinum in the high resolution turbo-pumped sputter coater Q150T (Quorum Technologies Ltd., Laughton, East Sussex, UK). Final samples were examined in the FEI Nova NanoSEM 450 (FEI, Brno, Czech Republic) scanning electron microscope at 5 kV using secondary (SED, TLD) and back-scattered (CBS) electron detectors. The high magnification images were recorded in immersion and beam deceleration mode of the microscope.

2.6. Analytes

The concentration of the tested analytes in stock solutions was 5 mg/ml (dissolved in distilled water), then diluted 10 times with water again to the final concentration 0.5 mg/ml. Usually, 2.5 μl of each analyte was added to the testing mixture (step by step, one after another), consisting of 20 μl of distilled water at the beginning as a starting solution.

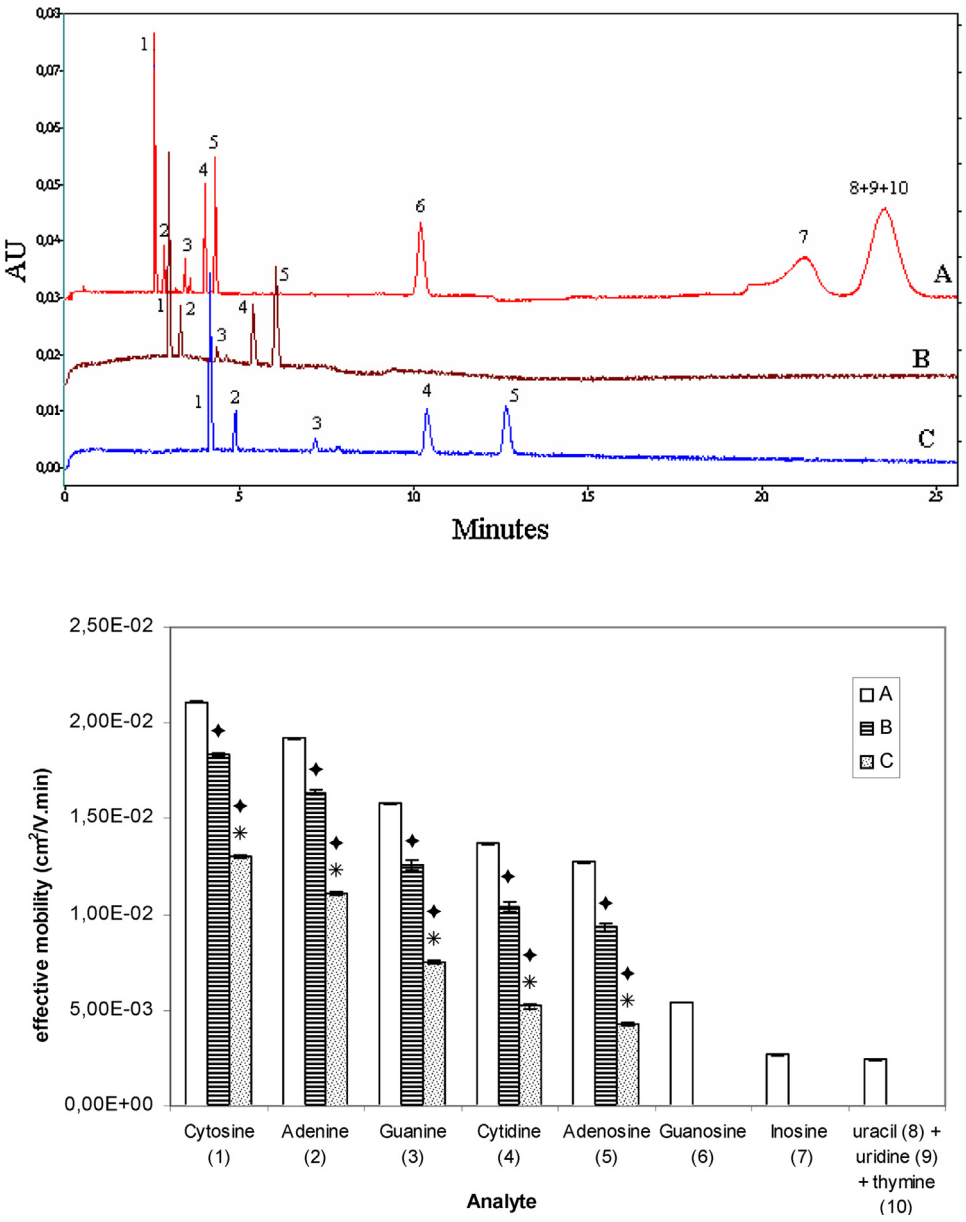

Fig. 4. Separation of nucleobases and nucleosides in acidic pH 2.50, 0.05 mol/L.
Phosphate buffer, 10 kV, normal polarity, detection 254 nm, 20 °C, injection for 3 s with pressure 0.5 psi
Electropherogram: 1- cytosine, 2- adenine, 3- guanine, 4- cytidine, 5- adenosine; 6- guanosine, 7- inosine, 8- uracil, 9- uridine, 10- thymine, A- fused silica, B –pure sol–gel, C- sol–gel + PB-conjugate capillaries
Graph of effective mobilities: **A**-uncoated capillary (n = 3), **B**-sol–gel capillary (n = 7), **C** –sol–gel + PB-conjugate capillary (n = 5); in B and C capillaries guanosine, inosine and the triplet uracil-uridine-thymine were not detected
⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary), ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary.

Oligopeptides – serotonin, glutathione, Tyr-Gly-Gly, Phe-Gly-Gly, Gly-Phe-Phe, Phe-Gly-Gly-Phe, Val-Ala-Ala-Phe

Nucleobases – adenine, guanine, thymine, uracil, cytosine

Nucleosides – adenosine, guanosine, uridine, cytidine, inosine

Nucleotides – uridine 5′-monophosphate (UMP), adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP), adenosine 5′-diphosphate (ADP), guanosine 5′-diphosphate (GDP), cytidine 5′-diphosphate (CDP), uridine 5′-triphosphate (UTP), adenosine 5′-triphosphate (ATP), guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP),

The number of analyses (calculated together for both electrolytes at pH 2.5 and 9.0) was more than 150 runs in each capillary.

It also needs to be pointed out that in contrast with the results presented in study [18], there was no metal ion in the porphyrin core – thus no axial-ligand coordinative interaction–, but on the other hand, there is a quarternized ammonium cation in the PB-conjugate that is available for appropriate non-covalent (expected) interaction.

All the changes in the electrophoretic mobilities of the samples obtained during the experiments were calculated according to *t*-test, (see appropriate graph).

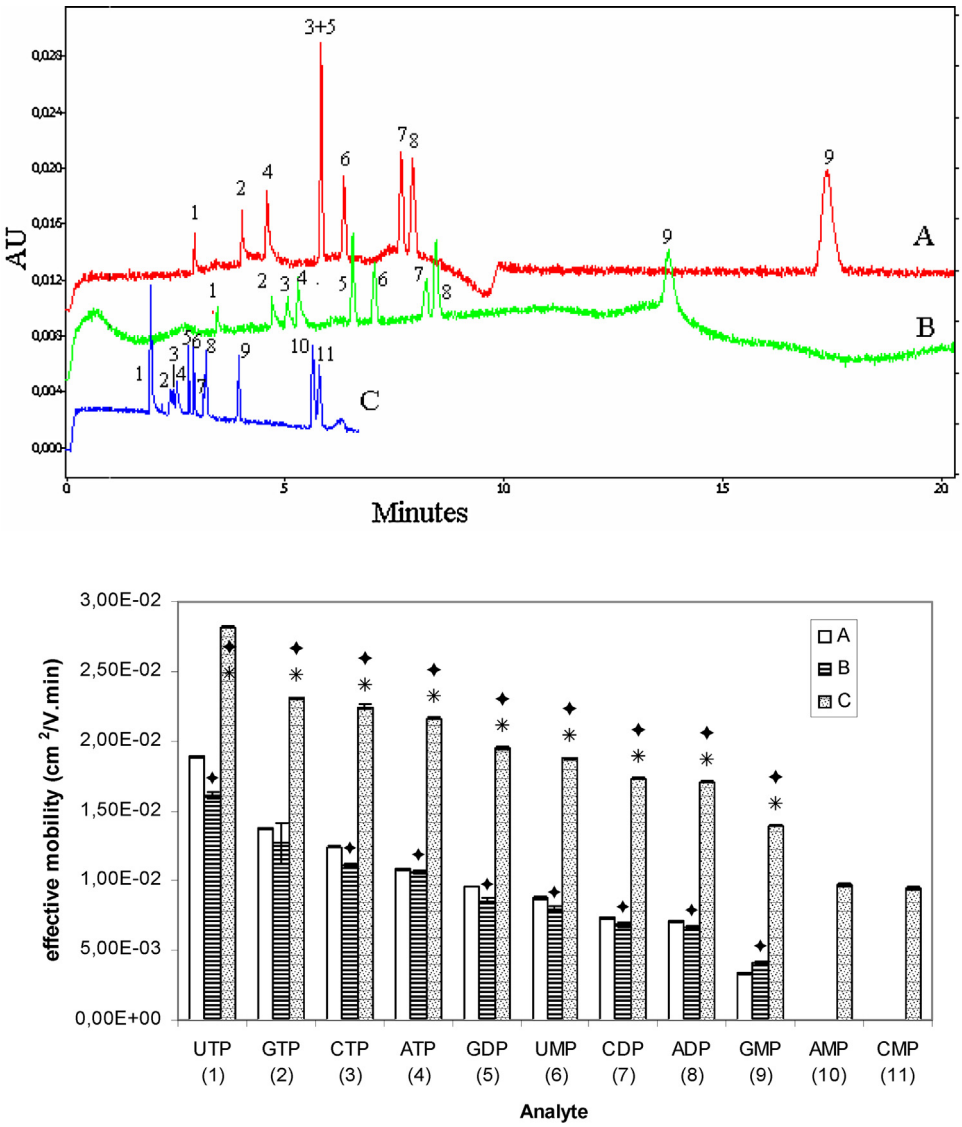

Fig. 5. Separation of nucleotides in acidic buffer solution pH 2.50, 0.05 mol/L phosphate, 10 kV, reversed polarity, detection 254 nm, 20 °C, injection for 3 s with pressure 0.5 psi.
Electropherogram: 1-UTP; 2-GTP; 3-CTP; 4-ATP, 5-GDP, 6-UMP; 7-CDP; 8-ADP; 9-GMP; 10-AMP; 11-CMP; A- Fused silica, B- sol–gel, C- sol–gel + PB-conjugate coated capillaries
Graph of effective mobilities: A-uncoated capillary (n = 6), **B**-sol–gel capillary (n = 4), **C**-sol–gel + PB-conjugate capillary (n = 5). AMP and CMP did not eluted in front of the detector at all in A and B capillaries.
⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary), ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary.

3. Results and discussion

3.1. SEM images

The successfulness of the modification of the inner capillary wall with the sol–gel was assessed using scanning electron microscopy (Fig. 2). From the image obtained with a bare fused silica capillary we can see that the surface of the capillary is smooth and showing no differences (Fig. 2A). On the other hand, the image of the inner capillary wall modified with the sol–gel containing PB-conjugate brought evident changes in the surface (Fig. 2 B).The inner capillary wall became rougher (creased) with numerous cracks over the whole surface, proving that modification occurred. (Images at lower and higher magnifications were also taken, but are not shown). Similar cracks were observed with the dryed sol–gel drops in the Petri bowl, which were made to confirm the formation of the sol–gel structure (images not shown). It should also be mentioned that all of the SEM images were taken after the end of separation process of all analytes, i.e. after 150 runs (as mentioned above).

3.2. Electrophoretic separations

3.2.1. Electrophoretic separations in acidic background electrolyte, pH 2.50

Since the electrophoretic mobility of the EOF is generally very low in acidic background electrolyte, the apparent mobilities of the individual samples were considered as the effective mobilities.

Experimental separation of the oligopeptides revealed some interesting phenomena. The first was the finding, that when using the sol–gel modified capillary (with or without the PB-conjugate), glutathion was not detected at all. The second was the much better resolution of peptides especially with the porphyrine-brucine modified sol–-gel (Fig. 3, C). The latter phenomenon could be explained by the interaction of aromatic parts of the oligopeptides with

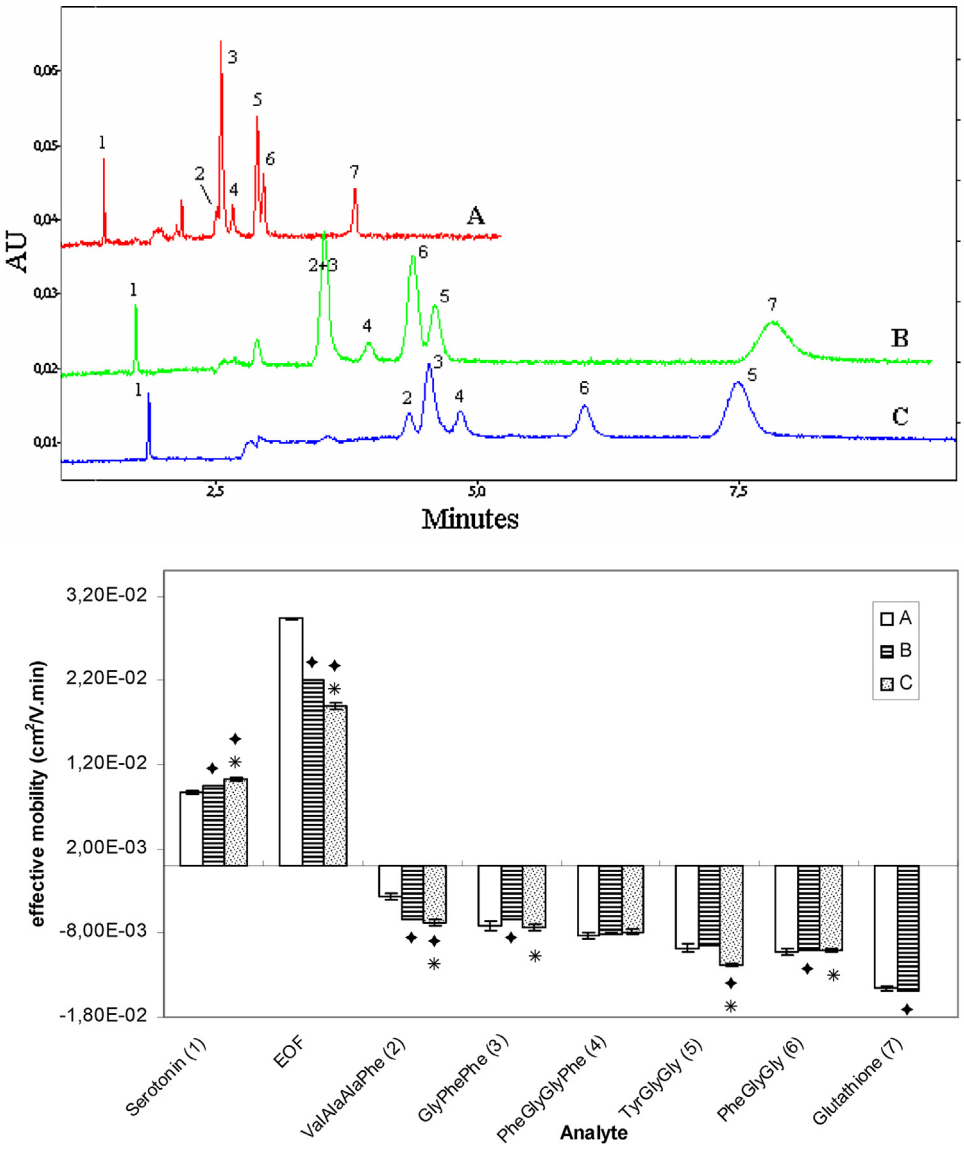

Fig. 6. Separation of oligopeptides in alkaline 0.05 mol/L phosphate buffer, pH 9.0, 10 kV, normal polarity, detection 214 nm, injection for 3 s with pressure 0.5 psi, 20 °C.
Electropherogram: 1-serotonin, 2-ValAlaAlaPhe, 3-GlyPhePhe, 4-PheGlyGlyPhe, 5-TyrGlyGly, 6-PheGlyGly, 7-glutathion; A – uncoated, B – sol–gel coated, C –sol–gel + PB-conjugate coated capillaries
Graph of effective mobilities: A-uncoated capillary (n = 8), **B**-sol–gel capillary (n = 3), **C**-sol–gel + PB-conjugate capillary (n = 4)
⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary), ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary

the planar aromatic structure of the porphyrin modifier and thus switching on the π-π interaction, hydrogen interaction and other non-covalent bonds. The two peptides with the chain containing four amino acid units could interact more in the planar layout over the structure of the porphyrin-brucine conjugate. In synergy with the other mentioned non-covalent interactions the separation of ValAlaAlaPhe and PheGlyGlyPhe gave much better resolution. The calculated changes of the effective mobilities of oligopeptides can be seen in graph at Fig. 3.

The separation of nucleobases and nucleosides (i.e. no phospho-group) took place with just the similar effect. Using a bare fused-silica capillary, all of the analysed samples were detected (see Fig. 4), no matter how well they were separated. When modified capillaries were used, only five of ten analytes could be detected and therefore non-covalent interactions can be considered. To resolve the reason of this mode of separation is quite difficult because of several included aspects, i.e. basic structure of the analyte, number of nitrogen atoms in the nucleobase molecule and the connection of the sugar unit. A graphical representation of the changes in the effective mobilities of samples is shown at Fig. 4.

Separation of nucleotides in pH 2.5 buffer exhibited several interesting aspects. First of all it has to be mentioned, that reversed polarity had to be applied because in this low pH value nucleotides still remain in the form of anions, and electroosmotic flow (EOF) is generally very slow and incapable to drag the anions to the detector. The electropherograms representing the separation of nucleotides are shown on Fig. 5. The interesting fact is that AMP and CMP were detected only in PB-conjugate modified capillary (Fig. 5, line C), while in fused-silica and pure sol–gel modified capillaries did not elute at all. Possible explanation could be that the quite wide structure of porphyrin-brucine conjugate, although having quaternized nitrogen in its structure, covers the original quartz and pure sol–gel surface of the capillary wall, therefore making impossible for the smaller anionic nucleotides AMP an CMP to interact with the pos-

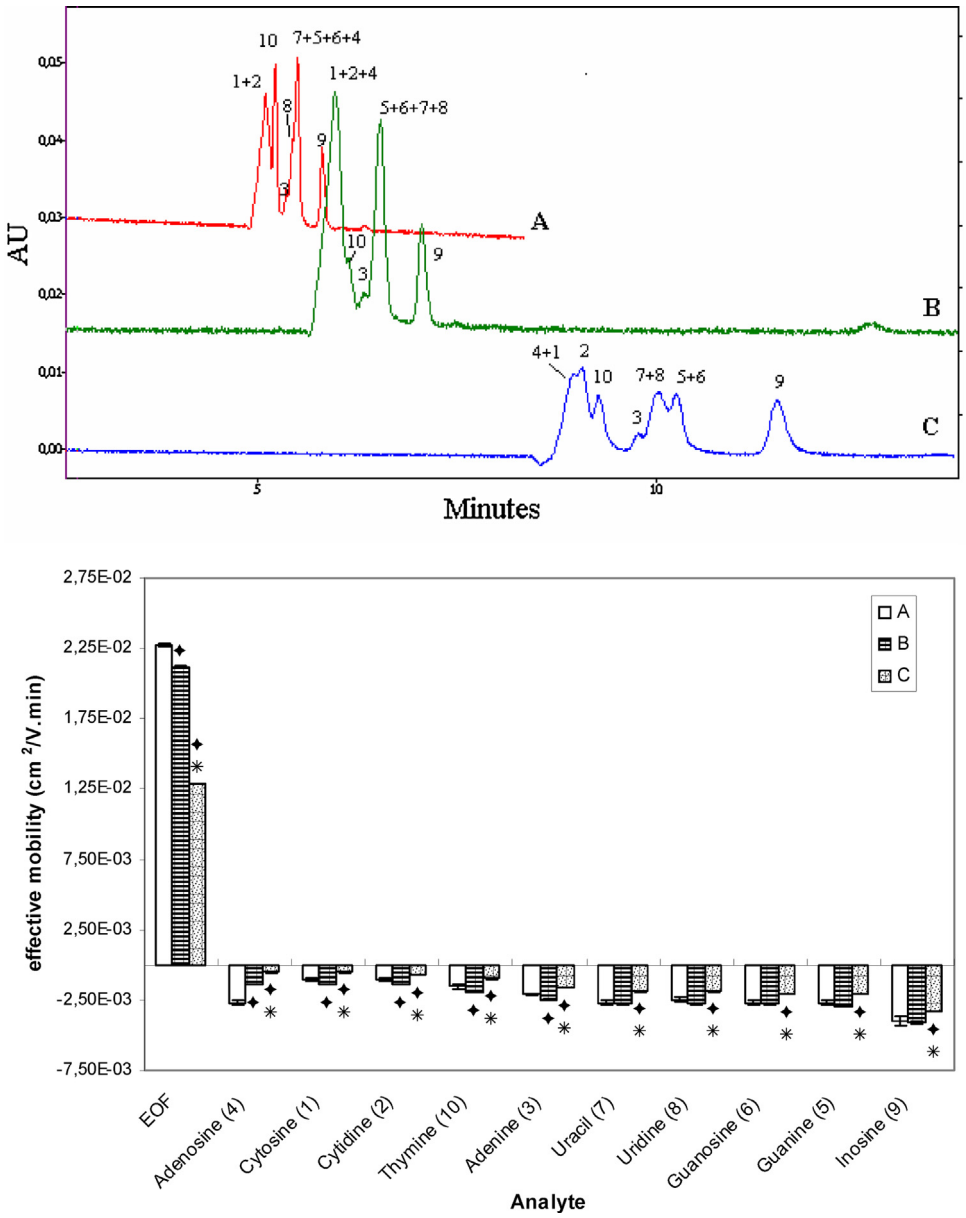

Fig. 7. Separation of nucleobases and nucleosides in alkaline buffer 0.05 mol/L phosphate, pH 9.0, normal polarity 5 kV, detection 254 nm, injection for 3 s with 0.5 psi, 20 °C.
Electropherogram: 1-cytosine, 2-cytidine, 3-adenine, 4-adenosine, 5-guanine, 6-guanosine, 7-uracil, 8-uridine, 9- inosine, 10-thymine; A- fused silica capillary, B –sol–gel coated, C –sol–gel + PB-conjugate coated capillaries
Graph of effective mobilities: A-uncoated capillary (n = 4), **B**-sol–gel capillary (n = 3), **C**-sol–gel + PB-conjugate capillary (n = 2)
⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary); ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary.

itively charged cationic surface to a larger extent. Calculation of analyte effective mobilities can be seen in the graphic description (Fig. 5).

3.2.2. Electrophoretic separation in alkaline background electrolyte, pH 9.0

If we take a look at the electropherograms of the oligopeptides, there were again some interesting findings (Fig. 6). First – the separation of oligopeptides takes the longer time the more complex is the inner wall modification. Second – the migration orders of Tyr-Gly-Gly and Phe-Gly-Gly are changed in both modified capillaries. The only difference in structure of these two tripeptides is the hydroxyl group bounded to the phenyl substituent of tyrosine so it is feasible to presume that this fact plays the key role in the interaction with the modified inner surface. Third – glutathion did not eluted to the detector at all using the PB-conjugate modified capillary. It could be explained by the interaction of anionic thiol group of glutathion with the qaternized nitrogen in PB conjugate. And finally the separation of oligopeptides is much better, in terms of the resolution of all peaks, in the PB-conjugate modified capillary. The differences in the effective mobilities of the oligopeptides are calculated in graph at Fig. 6.

As for the separation of nucleobases and nucleosides the first thing that can be seen is a slowing down of the EOF again. The second finding with these results was that the migration position of adenosine moves more to the beginning of the whole cluster of analytes with increasing modification of the inner capillary wall (see Fig. 7). Even thought the resolution of this group of analytes is not ideal in none of the three capillaries, it can be clearly seen at least better partial separation of the tested group of analytes,

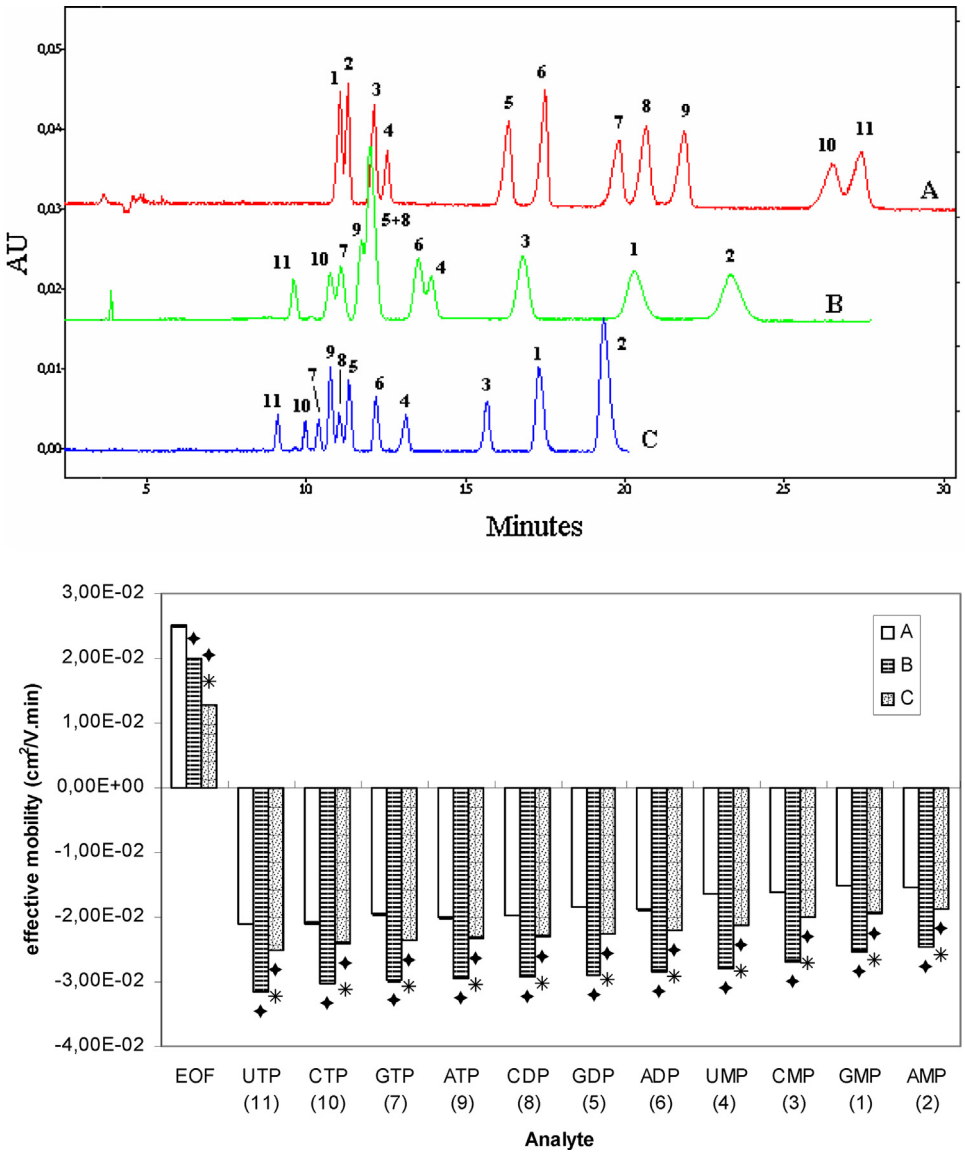

Fig. 8. Separation of nucleotides in alkaline 0.05 mol/L phosphate buffer, pH 9.0, 5 kV, normal polarity (A), reversed polarity (B, C), 20 °C, detection at 254 nm, injection for 3 s with 0.5 psi.
Electropherogram: 1-GMP, 2-AMP, 3-CMP 4-UMP, 5-GDP, 6-ADP, 7-GTP, 8-CDP, 9-ATP, 10-CTP, 11-UTP; A- uncoated capillary, B- sol–gel capillary, C –sol–gel + PB-conjugate capillary.
Graph of effective mobilities: A-uncoated capillary (n = 4), **B**-sol–gel capillary (n = 3), **C**-sol–gel + PB-conjugate capillary (n = 2).
⋆ – $p < 0.05$ (modified capillaries compared to uncoated capillary); ☆ – $p < 0.05$ (PB conjugate capillary C compared to sol–gel B capillary). Calculation performed according to T-test, n = number of repeated analyses in particular capillary.

particularly in the porphyrin-brucine modified capillary. Graphical description of obtained results are shown at Fig. 7.

The separation of various nucleotides is shown at Fig. 8. From the first point of view is obvious that the nucleotides migrate in a different order in modified capillaries (line B and C) then in the unmodified one (line A)... Using sol–gel modified capillaries, it was necessary to run experiments with nucleotides with reversed polarity of the apparatus, because at normal polarity it was impossible to see both EOF and migrating nucleotides under the same polarity in one run. The assumption of the inner wall modification and its consequent influence on the electrophoretic analysis (effective mobility) of analytes in the alkaline buffer is as follows: The inner wall of the capillary became positively charged even in the alkaline medium, i.e. the pH 9.0 of the background electrolyte, due to the condensation using APTES at the end of the sol–gel process. Thus, the EOF is slowed down and allows no or minimal pulling of nucleotides toward the cathode.

The calculation of effective mobilities is usually as follows:

$\mu_{eff} = \mu_{app} - \mu_{EOF}$; where μ_{eff} – effective mobility; μ_{app} – apparent mobility; μ_{EOF} – mobility of electroosmotic flow.

Bearing in mind that these two motions are in opposite directions and due to the slowing down of the EOF, the following equation was used for the final calculation of the effective mobilities of nucleotides:

$$\mu_{eff} = -\mu_{app} - (\mu_{EOF}),$$

$-\mu_{app}$ has the minus sign (−) because of the mobility of an anion back to the anode, and the mobility of the EOF marker towards cathode is still (+). The electrophoretic mobility of EOF was measured separately and repeatedly under normal polarity (using thiourea as a marker) and the final obtained value was used for calculation of the effective mobilities of nucleotides.

The best separation of nucleotides in alkaline buffer was observed in uncoated capillary (line A) at the expense of time.

Shortening of migration time in modified capillaries (B, C) caused a slight deterioration of the separation of nucleotides, particularly nucleotide di- and triphosphates. This could be explained by the slowing down of the electrophoretic mobility of EOF thus enabling increased mobility of nucleotides toward the anode. Nevertheless, the separation of nucleotides in porphyrin-brucine modified capillary (C) was better than in pure sol–gel (B) even in shorter time and specially for nucleotide di- and triphosphates. A comparison of the effective mobilities obtained for each analyte in different capillaries can be seen in graph at Fig. 8.

4. Concluding remarks

This paper first deals with the application of a sol–gel for the preparation of the stationary phase for open tubular capillary chromatography, then uses PB- conjugate incorporated into the sol–gel and then applies both modified capillaries in the separation of biological compounds. The combination of two types of these widely developing branches of electrophoresis/electrochromatography (e.g. a sol–gel stationary phase and the incorporation of an appropriate modifier) seems to be a promising direction for analytical chemistry. The experiments showed not only dramatic changes in the effective mobilities of the tested biological compounds and several changes in migration sequences, but also a possible application for a specific type of compounds (especially with the aromatic substituent, specific functional group and larger/broader molecule structure) due to the non-covalent type of interactions, e.g. π–π stacking, hydrogen bonds and possibly ionic interactions. Last but not least, a long-term stability and repeatability of analyses was observed, as well as minimal (if any) leaking of PB-conjugate, even with different pH values of the background electrolyte. Finally, the presence of the modified inner capillary surface was confirmed by SEM images, even though it is not the only overall method that can be used for this purpose. Application of other methods, i.e. UV–vis, infrared spectroscopy and fluorescence spectroscopy to prove the presence of various porphyrins in sol–gel is the subject of further research.

Acknowledgements

Financial support from the Czech Science Foundation, grant No 15-01948S is gratefully appreciated.

The authors gratefully acknowledge the access to the electron microscopy facility, supported by the project LO1509 of the Ministry of Education, Youth and Sports of the Czech Republic and by Operational Program Prague-Competitiveness project (CZ.2.16/3.1.00/24023), supported by the EU.

References

[1] A. Malik, Advances in sol–gel based columns for capillary electrochromatography: sol–gel open-tubular columns, Electrophoresis 23 (2002) 3973–3992.
[2] W. Li, D.P. Fries, A. Malik, Sol–gel sationary phases for capillary elecrochromatography, J. Chromatogr. A 1044 (2004) 23–52.
[3] R. Gupta, N.K. Chaudhury, Entrapment of biomolecules in sol–gel matrix for applications in biosensors: problems and future prospects, Biosens. Bioelectron. 22 (2007) 2387–2399.
[4] V.S. Tripathi, V.B. Kandimalla, H. Ju, Preparation of ormosil and its applications in the immobilizing biomolecules, Sens. Actuators B 114 (2006) 1071–1082.
[5] B.C. Dave, B. Dunn, J.S. Valentine, J.I. Zink, Sol–gel encapsulation methods for biosensors, Anal. Chem. 66 (22) (1994) 1120A–1127A.
[6] R. Ciriminna, M. Sciortino, G. Alonzo, A. de Schrijver, M. Pagliaro, From molecules to systems: sol–gel microencapsulation in silica-based materials, Chem. Rev. 111 (2011) 765–789.
[7] M.A. García-Sánchez, F. Rojas-Gonzáles, E.C. Menchaca-Campos, S.R. Tello-Solís, R.I.Y. Quiroz-Segoviano, L.A. Diaz-Alejo, E. Salas-Bañales, A. Campero, Crossed and linked histories of tetrapyrrolic macrocycles and their use for engineering pores within sol–gel matrices, Molecules 18 (2013) 588–653.
[8] M. Biesaga, K. Pyrzyńska, M. Trojanowicz, Porphyrins in analytical chemistry. A review, Talanta 51 (2000) 209–224.
[9] M.A. García-Sánchez, V. de la Luz, M.I. Coahuila-Hernández, F. Rojas-Gonzáles, S.R. Tello-Solís, A. Campero, Effect of the structure of entrapped substituted porphyrins on the textural characteristics of silica networks, J. Photochem. Photobiol. A: Chem. 223 (2011) 172–181.
[10] V. De la Luz, M.A. García-Sánchez, A. Campero, Luminescent porphyrinosilica obtained by the sol–gel method, J. Non-Crystaline Solids 353 (2007) 2143–2149.
[11] M.A. García-Sánchéz, V. De la Luz, M.L. Estrada-Rico, M.M. Murillo-Martínez, M.I. Coahuila-Hernández, R. Sosa-Fonseca, S.R. Tello-Solís, F. Rojas, A. Campero, Fluorescent porphyrins covalently bound to silica xerogel matrices, J. Non Crystaline Solids 355 (2009) 120–125.
[12] H. Tanaka, T. Yamada, S. Sugiyama, H. Shiratori, R. Hino, Synthesis of porphyrin introduced silica gels by sol–gel process, J. Colloid Interface Sci. 286 (2005) 812–815.
[13] M.A. García Sánchez, S.R. Tello, R. Sosa F, A. Campero, Fluorescent porphyrins trapped in monolithic SiO_2 gels, J. Sol–Gel Sci. Technol. 37 (2006) 93–97.
[14] E. Fagadar-Cosma, C. Enache, D. Vlascici, G. Fagadar-Cosma, M. Vasile, G. Bazylak, Novel nanomaterials based on 5,10,15,20-tetrakis(3,4-dimethoxyphenyl)-21H,23H-porphyrin entrapped in silica matrices, Mater. Res. Bull. 44 (2009) 2186–2193.
[15] R.I.Y. Quiroz-Segoviano, F. Rojas-Gonzáles, M.A. García-Sánchez, Cobalt porphyrin covalently bonded to organo modified silica xerogels, J. Non-Crystaline Solids 358 (2012) 2868–2876.
[16] Y. Yang, Y. Long, Z. Li, N. Li, K. Li, F. Liu, Real-time molecular recognition between protein and photosensitizer of photodynamic therapy by quartz crystal microbalance sensor, Anal. Biochem. (2009) 22–27.
[17] A. Yone, R.R. Carballo, D.A. Grela, I.N. Rezzano, N.M. Vizoli, Study of peptide-ligand interactions in open-tubular capillary columns covalently modified with porphyrins, Electrophoresis 32 (20) (2011) 2840–2847.
[18] A. Yone, R.R. Carballo, I.N. Rezzano, N.M. Vizioli, Separation of peptides by open-tubular capillary electrochromatography using Fe(III)-deuteroporphyrin as a covalently attached stationary phase, Electrophoresis 30 (2009) 2293–2299.
[19] Z. Deyl, I. Mikšík, A. Eckhardt, V. Kašička, V. Král, Porphyrin based affinity interactions: analytical applications with special reference to open tubular capillary electrochromatography, Current Anal. Chem 1 (2005) 103–119.
[20] K. Záruba, V. Setnička, J. Charvátová, O. Rusin, Z. Tománková, J. Hrdlička, D. Sýkora, V. Král, Analytical application of oligopyrrole macrocycles, Collect. Czech. Chem. Commun 66 (2001) 693–769.
[21] P. Oudhia, in: G.H. Schmelzer, A. Gurib-Fakim (Eds.), Medicinal Plants 1, PROTA Foundation, Vageninghen, Netherland, 2008, pp. 575–577.
[22] K. Záruba, V. Král, Quaternized brucine as a novel chiral receptor, Tetrahedron: Asymmetry 13 (2002) 2567–2570.
[23] L. Veverková, K. Záruba, J. Koukolová, V. Král, Oxoanion binding: a change of selectivity for porphyrin-alkaloid conjugates as a result of substitution pattern, New J. Chem. 34 (2010) 117–122.
[24] L. Veverková, K. Záruba, V. Král, Study of receptor mediated selective anion transmembrane transport using parallel artificial membrane permeability assay, Analyst 138 (2013) 2804–2807.
[25] L. Veverková, P. Žvátora, K. Záruba, V. Král, Receptor modified gold and silver nanoparticles: effect of interactions with oxoanions, Analyst 138 (2013) 333–338.
[26] K. Záruba, J. Králová, P. Řezanka, P. Poučková, L. Veverková, V. Král, Modified porphyrin-brucine conjugated to gold nanoparitcles and their application in photodynamic therapy, Org. Biomol. Chem 8 (2010) 3202–3206.