

Cyclodextrin modified gold nanoparticles-based open-tubular capillary electrochromatographic separations of polyaromatic hydrocarbons

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Abstract In this study, spherical gold nanoparticles (GNPs) of 14.7 nm diameter, prepared by citrate reduction of a gold(III) salt and characterized by UV–Vis absorption spectrometry and transmission electron microscopy, were modified by a covalent attachment of 6^l-O-(3-mercaptopropyl) β -cyclodextrin (β -CD-SH) or per-6-deoxy-per-6-mercapto- β -cyclodextrin (β -CD-SH7). Subsequently, via three alternative approaches, β -CD-modified GNPs were immobilized onto the inner wall of the fused-silica (FS) capillaries and applied as special stationary phases for open-tubular capillary electrochromatography (OT-CEC). The first immobilization procedure was based on pre-derivatization of a FS capillary with (3-mercaptopropyl)trimethoxysilane (MPTMS) followed by subsequent reactions with GNPs and

β -CD-SH or β -CD-SH7. The other two preparation protocols took advantage of sol–gel approach gaining a significant increase in the interaction surface for solutes. In both instances, the sol–gel created 3D structure was further covalently modified with GNPs. Serving that purpose, either β -CD-SH7 modified GNPs were used for the immobilization into the sol–gel matrix (“one-step sol–gel technique”) or native GNPs were immobilized first into the sol–gel matrix and subsequently modified with β -CD-SH7 (“two-step sol–gel technique”). The separation performance of CD-GNPs modified FS capillaries was tested by OT-CEC in reversed-phase mode applied to separation of a model mixture of five polyaromatic hydrocarbons. The highest separation efficiencies were obtained with the capillaries prepared by two-step sol–gel technique. However, with respect to the relatively low reproducibility of this method, the first of the above preparation procedures, i.e., a simple pre-derivatization of the FS capillary with MPTMS ensued with β -CD-SH7-GNPs immobilization seems to be more feasible approach providing decent separation efficiency.

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Introduction

Nanoparticles (NPs) recently attract an extensive attention in various fields of chemistry due to their

unique physical and chemical properties. NPs usually refer to a kind of material with a spherical-like appearance with a large surface-to-volume ratio and other fascinating properties derived from the “quantum size effect” (Daniel and Astruc 2004). The potential of nanostructured materials in separation sciences has gradually been recognized in recent years and significant advances have been achieved in capillary electrophoresis (CE), capillary electrochromatography (CEC), microchip CE, high-performance liquid chromatography, and gas chromatography (GC) separations of both low- and high-molecular mass substances employing NPs as components of the separation media. The NPs serve either as permanent or as dynamic capillary inner surface coatings in CE, as stationary phases in CEC, as pseudostationary phases in partial filling or continuous filling mode in electrokinetic chromatography (EKC) and as modifiers of stationary phases in LC and GC. The recent advances in application of NPs in separation science have been summarized in several review papers (Dios and Díaz-García 2010; Guihen and Glennon 2003; Sekhon 2010; Sýkora et al. 2010). The successful utilization of gold nanoparticles (GNPs) in the separation science stems from a facile covalent immobilization of SH- group containing compounds on the gold surface. This allows for the preparation of a wide variety of new modified GNPs potentially possessing very distant properties. Moreover, immobilization of GNPs on the capillary walls leads to an increase in the inner capillary surface and thus, conditions for more interactions with analytes are imposed.

Fused-silica (FS) capillaries modified with GNPs have been introduced for open-tubular CEC (OT-CEC) by Glennon's group in 2003 (O'Mahony et al. 2003). Dodecanethiol-GNPs have been physically or covalently immobilized on the inner walls of capillaries and these have been utilized for the separation of neutral compounds, namely thiourea, benzophenone, biphenyl, and two pesticides in reversed-phase (RP) mode. To improve the separation efficiency and the selectivity of the method, a sol-gel-based approach (Yang et al. 2005a) and a capillary etching (Yang et al. 2005b) have been successfully implemented in the preparation protocol. Liu et al. has prepared capillaries coated with films of alkanethiol self-assembled GNP layers (Liu et al. 2005). Three neutral steroid drugs have been

separated and the influence of the number of GNP layers and several other parameters on the separation performance has been investigated. Further, a hydrophobic coating of GNPs with octadecylamine (ODA) has been achieved by flushing a capillary with a solution of ODA-modified GNPs (Qu et al. 2008). As in all the above mentioned instances, the resulting stationary phases have been tested by separation of hydrophobic solutes in RP separation mode. Recently, a separation of naphthalene and biphenyl based on permanent GNP coatings on polyelectrolyte multilayer modified capillaries in OT-CEC has been published (Qu et al. 2010)

Cyclodextrins (CDs) have been used for many years for separation purposes extensively because of their capability to discriminate positional isomers, homologue compounds, compounds differing in functional groups, and enantiomers of chiral analytes. This makes them one of the most effective agents for a wide variety of separations. The most frequently applied CDs are α -, β -, and γ -CD, containing six, seven, and eight glucose units, respectively. CDs show unique properties originating from their structure. In addition to a hydrophilic outer-shell, they contain a hydrophobic cavity. This enables CDs to entrap hydrophobic solutes and create inclusion non-covalent complexes with many compounds. The native CD rims are lined with primary hydroxyls on one side and secondary hydroxyl groups on the other side of the cavity. Both types of hydroxyls can be chemically modified to further enhance complex forming capability and selectivity toward specific analytes.

There are several studies describing applications of CDs and CD derivatives in CEC. In general, the CEC experiments can be performed with particle packed capillaries, monolithic capillary columns, and open-tubular capillaries.

In the case of capillaries packed with CD-modified particles, the selector (CD) is either chemically bonded to or dynamically adsorbed onto solid particles, which are packed in a capillary. Such approach has been demonstrated by Zhang and El Rassi for enantiomeric separation of dansyl-amino acids and several pesticides on diol-silica dynamically coated with 2-hydroxypropyl- β -CD (Zhang and El Rassi 2000a) and sulfonated silica having surface-bound 2-hydroxypropyl- β -CD (Zhang and El Rassi 2000b). For the separation of negatively charged

dansyl-amino acids, also commercial native β - and γ -CD bound silica particles have been successfully employed as a packing in nonaqueous and aqueous CEC mode (Wistuba et al. 2001). Permethy-CD and perphenylcarbamoylated β -CD bonded silica particles have been utilized in pressure-assisted CEC (Lin et al. 2006; Wistuba and Schurig 1999; Zhou et al. 2005) as well as in “true” CEC (Wang et al. 2009).

Silica-based permethylated β -CD-modified monoliths have been prepared by Wistuba et al. (2005; Wistuba and Schurig 2000) and for various chiral compounds in average about twofold higher separation efficiency in CEC mode than in LC mode has been found. Recently, also polymeric monoliths modified with β -CD derivatives have been introduced. Racemic mixtures of eight amino acids and several chiral drugs have been separated on poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) modified monoliths (Li et al. 2010; Tian et al. 2009).

Open-tubular CEC has several advantages, simple instrumental handling, short conditioning times and high separation efficiency. Its drawback consists mainly in a lower separation capacity, as compared to the two foregoing CEC arrangements. A bunch of papers on OT-CEC with Chirasil-Dex-coated capillaries have been published by Mayer et al. (1994; Jung et al. 1994; Mayer and Schurig 1992, 1993, 1994; Schurig and Mayer 2001). Also a sol-gel technique leading to 2,6-dibutyl- β -CD-modified inner walls of FS capillaries has been utilized for the separation of positional isomers of aminophenols, dihydroxybenzenes, and nitrophenols (Wang et al. 2001). Very recently, open-tubular capillaries based on sulfated β -CD intercalated in layered double hydroxides have been described and a chiral separation of 1-phenyl-1,2-ethanediol has been demonstrated (Hongjun et al. 2009).

In 2010, Yang et al. have utilized CD-modified GNPs as pseudostationary phase for enantioseparation of amino acids and drugs by CEC (Yang et al. 2010).

However, to the best of our knowledge, OT-CEC with CD-modified GNPs-based stationary phase has never been shown yet. Here, we describe the influence of amount of thiol groups in β -CD immobilized via GNPs onto the capillary wall and the influence of the stationary phase preparation procedure on the separation efficiency and resolution of the

OT-CEC separation of a model mixture of polyaromatic hydrocarbons.

Experimental section

Chemicals

Potassium tetrachloroaurate(III) (98%, Sigma-Aldrich, Czech Republic), trisodium citrate dihydrate (99%, p.a., Penta, Czech Republic), sodium borate (p.a., Lachema, Czech Republic), hydrochloric acid (30%, Suprapur, Merck, Germany), sodium hydroxide (Tripur, Merck), acetonitrile (ACN, 99.8%, Li Chrosolv, Merck), and thiourea (99%), fluorene (98%), anthracene (97%), phenanthrene (98%), naphthalene (99%), 1,2-benzanthracene (99%), and (3-mercaptopropyl)trimethoxysilane (MPTMS, 95%) (all Sigma-Aldrich), and deionized water (Milli-Q grade, Millipore, France) were used. 6¹-*O*-(3-mercaptopropyl)- β -cyclodextrin (β -CD-SH) and per-6-deoxy-per-6-mercapto- β -cyclodextrin (β -CD-SH7) were prepared according to the published procedure (Rojas et al. 1995).

Equipment

For characterization of GNPs and capillaries UV-Vis spectra were measured using a Cary 400 SCAN UV-Vis spectrophotometer (Varian, USA), transmission electron microscopy (TEM) images were obtained from a JEM-3010 microscope (Jeol, Japan), thermogravimetric analysis was measured with a TG 750 thermo gravimetric analyzer (Stanton Redcroft, USA), and energy dispersive X-ray fluorescence spectra were measured using a Spectro iQ II (Spectro Analytical Instruments, Germany).

All OT-CEC experiments were performed with Agilent capillary electrophoresis instrument (Agilent 3D HPCE, Germany). The FS capillaries used were 375 μ m o.d. \times 50 μ m i.d. (Polymicro Technologies, Phoenix, AZ, USA).

Mobile phase and sample preparation

Thiourea (2 mg/mL) was dissolved in 20 mmol/L sodium borate, pH 9.2. Fluorene (1 mg/mL), anthracene (1 mg/mL), phenanthrene (1 mg/mL), naphthalene (1 mg/mL), and 1,2-benzanthracene (1 mg/mL)

were dissolved in ACN. The test mixture was made up in 20 mmol/L sodium borate/ACN (7/3 or 1/1; v/v, final concentration 0.1 mg/mL of each component) with thiourea (final concentration 0.1 mg/mL) as the electroosmotic flow (EOF) marker.

Preparation of the GNPs

GNPs were prepared as previously described (Řezanka et al. 2008). Shortly, 1 mL of 1% aqueous solution of the potassium tetrachloroaurate(III) and 2.5 mL of 1% aqueous solution of the trisodium citrate dihydrate were added to 100 mL of boiling water (under reflux). Boiling was continued for 10 min. During that time the solution color changed from pale yellow to gray-blue, then to purple, and finally to wine-red. Reaction vessel was then allowed to cool to room temperature.

Preparation of the pseudostationary phase

β -CD-SH7 (6 mg) was added to the solution of freshly prepared GNPs (4 mL) and pH was adjusted to 12 with NaOH (10 mol/L). After 4 days, unbound β -CD-SH7 species were removed by centrifugation followed by redispersion of the modified GNPs in the background electrolyte (BGE) (20 mM sodium borate, pH 9.2/ACN, 7/3 or 1/1, v/v).

Preparation of the modified capillaries

The preparation procedures are shown in Fig. 1 and described below in detail.

Method 1

A modified capillary was prepared according adjusted procedure (O'Mahony et al. 2003). A 50-cm long capillary was rinsed (20 μ L/min) with 0.9 mL NaOH (1 mol/L) followed with 0.4 mL deionized water (20 μ L/min) and then with 0.9 mL HCl (0.1 mol/L; 20 μ L/min). Upon rinsing with water again, the capillary was placed in an oven and dried at 180 °C for 1 h. 3 mL of MPTMS/ACN (1/12, v/v) solution was then pumped (20 μ L/min) through the dried capillary and allowed to stand overnight with both ends submerged in this solution. The modified capillary was then rinsed (20 μ L/min) with 1 mL ACN followed by drying at 100 °C for

1 h. Then 4 mL of freshly prepared GNPs solution was pumped (20 μ L/min) through the dried capillary and allowed to stand overnight with both ends submerged in this solution. Next, the modified capillary was rinsed (20 μ L/min) with 1 mL of deionized water followed by 3 mL of alkaline β -CD-SH or β -CD-SH7 solution (5.0 mg/4 mL) (20 μ L/min) and allowed to stand for over 3 days with both ends submerged in this solution. Finally, 1 mL of deionized water was pumped (20 μ L/min) through the capillary.

Method 2: two-step sol-gel technique

A capillary was prepared according modified procedure (Yang et al. 2005a). A 50-cm long capillary was rinsed (16.6 μ L/min) with 1 mL NaOH (1 mol/L) followed by 1 mL of deionized water (16.6 μ L/min) and drying at 180 °C overnight. The modified capillary was then rinsed (20 μ L/min) with 100 μ L of MPTMS/EtOH(96%)/HCl (0.01 mol/L) solution (7/2/1, v/v/v), which was previously stirred for 24 h. The capillary filled with this solution was allowed to stay for 2 h at room temperature. Excess solution was forced out of the capillary under argon pressure (500 kPa) for 10 min followed by drying at 120 °C overnight. After that, the capillary was rinsed (20 μ L/min) with 0.5 mL acetone and 0.5 mL methanol followed by drying at room temperature by argon flow (500 kPa) for 15 min. Then 3 mL of freshly prepared GNPs solution was pumped (16.6 μ L/min) through the dried capillary and allowed to stand for 2 days with both ends submerged in this solution. The modified capillary was then rinsed (20 μ L/min) with 2 mL of alkaline β -CD-SH7 solution (12.5 mg/4 mL) and allowed to stand for over 2 days with both ends submerged in this solution. Finally, 1 mL of deionized water was pumped (20 μ L/min) through the capillary.

Method 3: one-step sol-gel technique

In this procedure, the solution of GNPs (4.0 mL) modified with β -CD-SH7 (5.0 mg) was prepared and allowed to stay for 3 days. Then a 50-cm long capillary was modified as described in the previous paragraph for two-step procedure with the exception that GNPs modified by β -CD-SH7 were used instead unmodified GNPs.

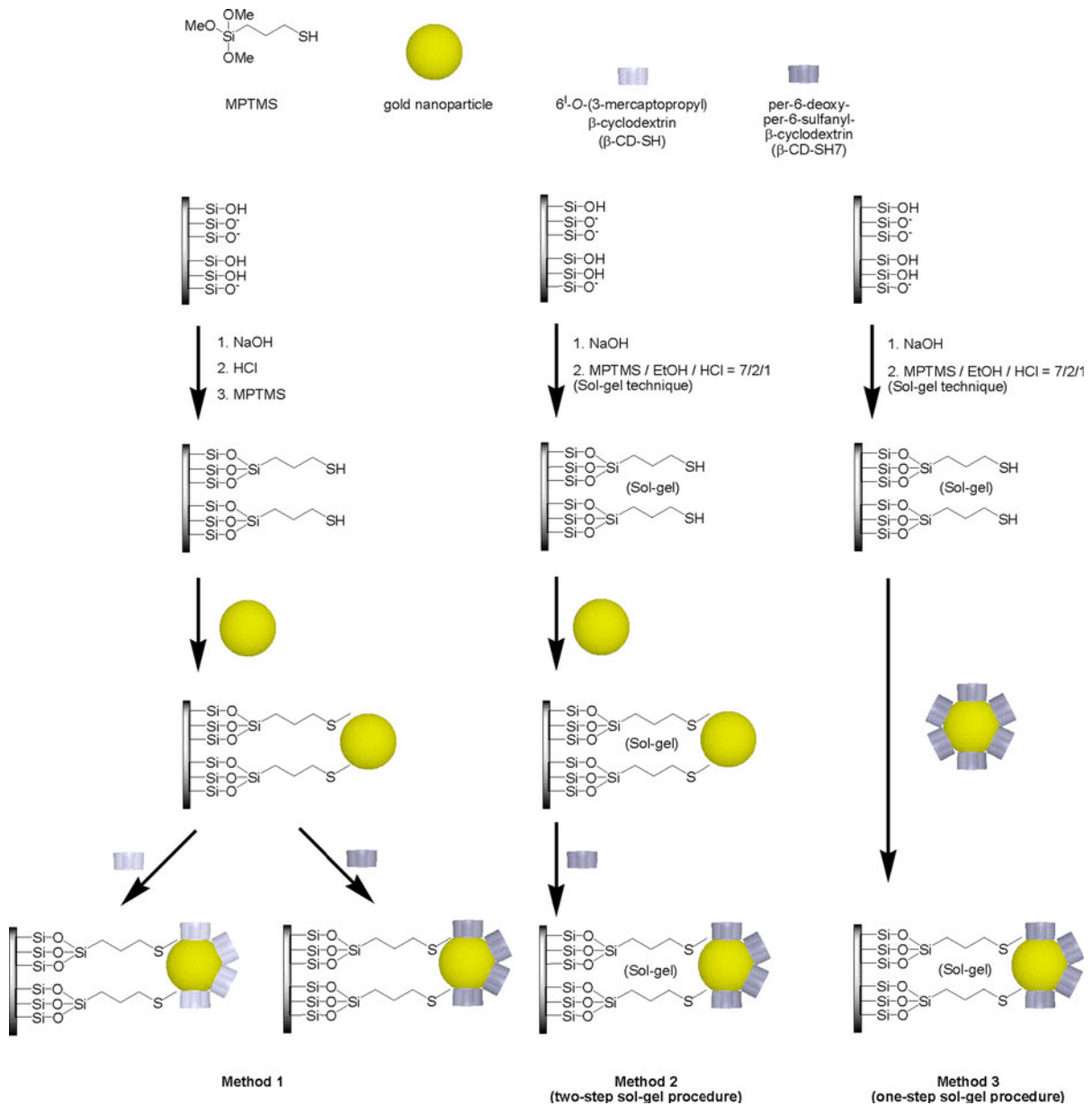


Fig. 1 Scheme of the capillary preparation procedures according to method 1, 2, and 3

OT-CEC conditions

All capillaries were conditioned first in the Agilent CE instrument by pressure rinsing with the BGE (mobile phase) for 15 min before the first run. Between runs, the capillaries were rinsed for 2 min with the BGE. To store the capillaries when not in use 2 min rinsing with water was applied. Samples

were injected by pressure 1 kPa for 10 s. Analytes were detected at several particular wavelengths specified in the presented electrochromatograms. Thiourea was used as an EOF marker. All separations were performed at 20 kV (anode at the injection capillary end) with a voltage ramp time of 0.5 s and the temperature was kept constant at 20 °C.

Results and discussion

Characterization of the modified GNPs and the capillaries

The preparation procedure for the GNPs (characterized elsewhere (Řezanka et al. 2008)) resulted in spherical nanoparticles with diameters of 14.7 nm at concentration 2.75 nmol/L, which exhibited plasmon resonance band at 518 nm (Fig. 2).

Based on the data obtained from the thermogravimetric analysis (TGA) (Fig. 3), the number of immobilized molecules of β -CD-SH7 per one GNP was approximately 775. This estimation was based on the observed loss of mass of the GNPs modified with β -CD-SH7 derivative, assuming that the size of the GNPs was of 14.7 nm. Based on the theoretical

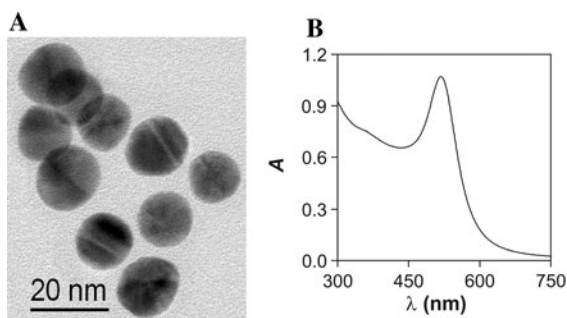


Fig. 2 Transmission electron microscopy image (a) and UV-Vis spectrum (b) of the freshly prepared GNPs

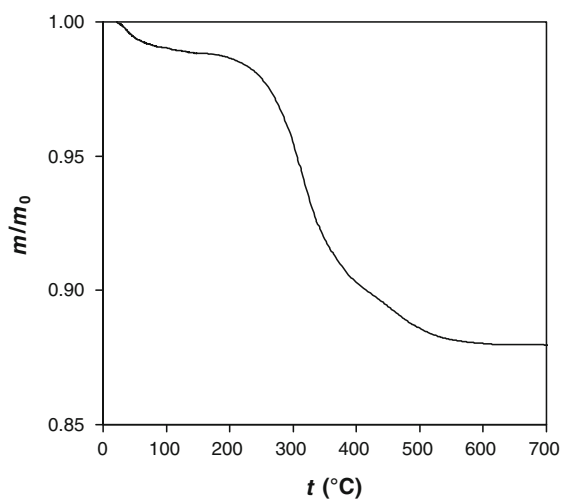


Fig. 3 Thermogravimetric analysis of the GNPs modified with β -CD-SH7 derivative

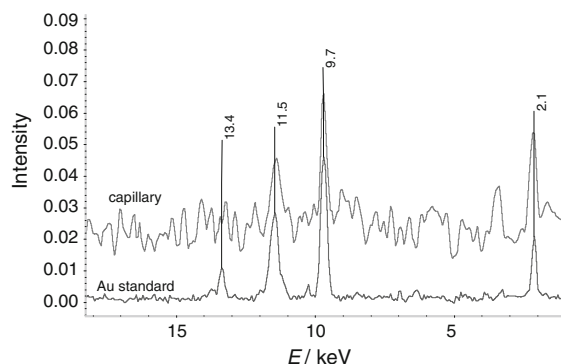


Fig. 4 Energy dispersive X-ray fluorescence spectra of Au standard (10 ppm) and the capillary prepared according to the method 1 with β -CD-SH7; spectra were shifted in y axis

calculation considering the size of GNPs and β -CD-SH7, i.e., 14.7 and 1.35 nm, respectively) the number of β -CD units to form a monomolecular coverage was approximately 408. Thus, the experimental results indicated the formation of more than monomolecular layer of β -CD-SH7 on GNPs.

An energy dispersive X-ray fluorescence (ED-XRF) spectrometer was used for quantification of gold bonded to the capillary synthesized by the method 1 with β -CD-SH7 (Fig. 4). Based on calibration curve, the amount of gold was estimated to be 10 ppm, which is approximately ten times less than maximum expected value for covering the capillary with monolayer of gold nanoparticles (108 ppm). In conclusion, there is approximately 1.0 μ g of GNPs and 85 ng of β -CD-SH7 in 50-cm length of capillary.

The effect of the type of cyclodextrin derivative on the OT-CEC separation

First, the effect of the two different mercapto derivatives of β -CD (β -CD-SH and β -CD-SH7) on the OT-CEC separation of five polyaromatic compounds (naphthalene, fluorene, phenanthrene, anthracene, and 1,2-benzanthracene) was studied using thiourea as EOF marker. The main difference between the above two β -CD derivatives consists in their different immobilization and orientation on the GNPs surface. β -CD-SH is bonded only by one covalent bond, so its orientation after immobilization is relatively random and not well defined. On the other hand, β -CD-SH7 is bonded by up seven covalent bonds (S–Au) on the GNPs surface, hence the CD derivative is more strongly bound to the

GNPs and the CD cavity has a better defined orientation, presumably perpendicular to the GNPs surface.

Figure 5 displays the electrochromatogram obtained for OT-CEC separation of thiourea and five above mentioned aromatic compounds on the capillary coated by GNPs modified by β -CD-SH and prepared according to the method 1. As can be seen, the peaks of the analytes are rather broad and one of the analyte (1,2-benzanthracene) was not detected at all due to a high dispersion of its zone (peak).

On the other hand, the OT-CEC separation of the same test mixture in the FS capillary coated with GNPs modified with β -CD-SH7 derivative provided narrower peaks for the analytes and allowed for their improved separation (Fig. 6). The separation efficiency, represented by theoretical plate numbers calculated according the equation $N = 5.54 t_R^2/w_{1/2}^2$, where t_R is the retention time and $w_{1/2}$ is the peak width at its half height, is much better on the β -CD-SH7 capillary than for the β -CD-SH capillary (Table 1). For instance, for thiourea 40,000 plates/m on the β -CD-SH7 capillary was obtained versus 22,400 plates/m reached on the β -CD-SH capillary). The increased separation efficiency may be possibly explained by a better accessibility of the suitably oriented CD cavity of the β -CD-SH7 capillary for the analytes with respect to the β -CD-SH capillary. Hence, in the all subsequent experiments the β -CD-SH7 derivative was used

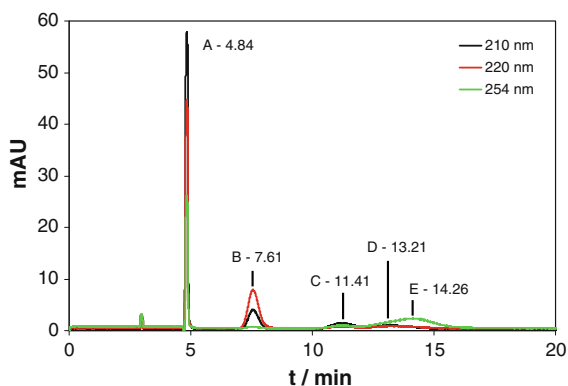


Fig. 5 Electrochromatogram of the test mixture on the capillary prepared by the method 1 using β -CD-SH; the test mixture consisted of thiourea (A), naphthalene (B), fluorene (C), phenanthrene (D), anthracene (E), and 1,2-benzanthracene (F) (not detected due to high dispersion); capillary size $L_{\text{tot}} = 50$ cm, $L_{\text{eff}} = 41.5$ cm, i.d. = 50 μm ; separation conditions: 20 mmol/L sodium borate, pH 9.2/ACN, 7/3, v/v

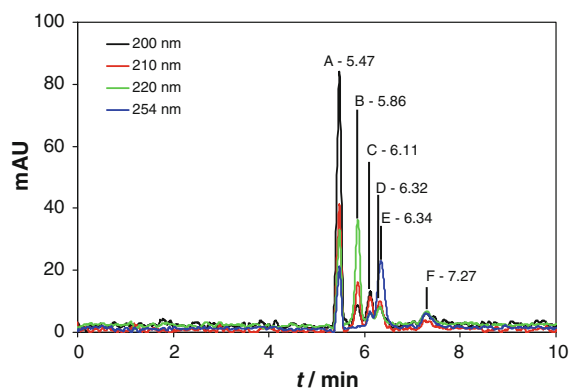


Fig. 6 Electrochromatogram of the test mixture on the capillary prepared by the method 1 using β -CD-SH7; the test mixture, capillary size, and separation conditions as in Fig. 5

exclusively. Nevertheless, in the Fig. 6, it is also evident a relatively fast decrease in the number of theoretical plates with the increasing retention times of the analytes demonstrated by a pronounced peak broadening. The separation efficiency of 40,000 plates/m for thiourea decreased to only 3,300 plates/m for 1,2-benzanthracene. This phenomenon might be explained by Golay equation as a consequence of the operating the capillary far away from the optimum of mobile phase velocity for the last eluting analyte (Schurig et al. 1995).

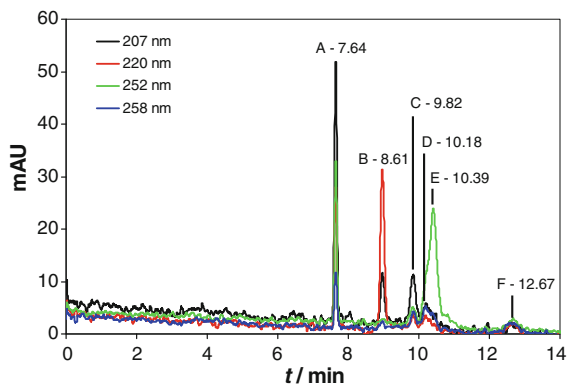
The effect of the capillary preparation procedure on the OT-CEC separation

An alternative approach to the preparation of OT-CEC capillaries with GNPs modified by CDs is based on the utilization of a sol-gel procedure. In this approach, the thin polymer film of MPTMS is formed on the inner wall of capillary. Two alternative versions of the sol-gel method were used here, two-step (method 2) and one-step (method 3) technique, the difference in the synthetic protocols was explained above (in “Experimental section” and Fig. 1).

The two-step sol-gel technique enabled to separate all five aromatic compounds (Fig. 7). Separation was good except for a pair anthracene–phenanthrene, and the capillary performance, expressed as number of theoretical plates, was better than for the capillaries prepared by the method 1. For example, the number of theoretical plates for EOF marker, thiourea, increased to 104,000 plates/m (Table 1).

Table 1 Retention times (t_R) and number of theoretical plates (N) of thiourea, naphthalene, and fluorene for the respective capillary preparation method

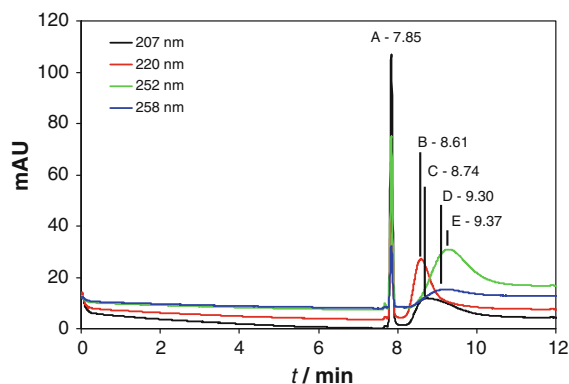
Capillary	Thiourea (EOF marker)		Naphthalene		Fluorene	
	t_R (min)	N (plates/m)	t_R (min)	N (plates/m)	t_R (min)	N (plates/m)
Method 1 (β -CD-SH)	4.84	22,400	7.56	3 370	11.22	1,340
Method 1 (β -CD-SH7)	5.47	40,000	5.86	40 200	6.12	28,200
Method 2 (two-step sol–gel technique)	7.64	104,000	8.67	83 600	9.85	46,700
Method 3 (one-step sol–gel technique)	7.85	153,000	8.59	4 290	8.73	810

**Fig. 7** Electrochromatogram of the test mixture on the capillary prepared by the method 2 (two-step sol–gel technique); the test mixture and capillary size as in Fig. 5; separation conditions: 20 mmol/L sodium borate, pH 9.2/ACN, 1/1, v/v

For the capillary prepared by one-step sol–gel technique, the number of theoretical plates for thiourea increased even further up to 153,000 plates/m but the separation of other analytes was much worse compared to the results obtained for the two-step sol gel capillary (Fig. 8; Table 1).

At this point, we have to mention that in our repetitive experiments we found the sol–gel preparation procedure for the capillary coating very sensitive to the temperature and air-moisture. It was rather difficult to prepare the capillaries reproducibly, i.e., to obtain the sol–gel capillaries with the reproducible separation properties.

To summarize, the number of theoretical plates for thiourea was the highest for one-step sol–gel procedure, but considering the overall separation performance for the aromatic compounds, the best results were obtained with the capillaries prepared with two-step sol–gel technique. It is also evident that use of β -CD-SH7 provided much better results than for β -CD-SH. However, taking into account the overall

**Fig. 8** Electrochromatogram of the test mixture on the capillary prepared by the method 3 (one-step sol–gel technique); the test mixture and capillary size as in Fig. 5; separation conditions: 20 mmol/L sodium borate, pH 9.2/ACN, 1/1, v/v

separation efficiency and the reproducibility of the capillary preparation, the best results were obtained with the capillary prepared by the method 1 using β -CD-SH7, as in this case the peak shapes, separation efficiency, and selectivity were reasonable and reproducible (Table 1).

For completeness, it should be added that for comparative purposes, GNPs were also used as pseudostationary phase in EKC mode, when the β -CD-SH7 capped GNPs were added directly to the BGE. Unfortunately, the GNPs aggregated fast in the used BGE, hence no separations could be achieved in this separation mode.

Separation of the test mixture with MEKC

For comparative purposes, we have tried to separate the components of our test mixture also by an independent and well-established method, MEKC, with anionic detergent sodium dodecylsulfate

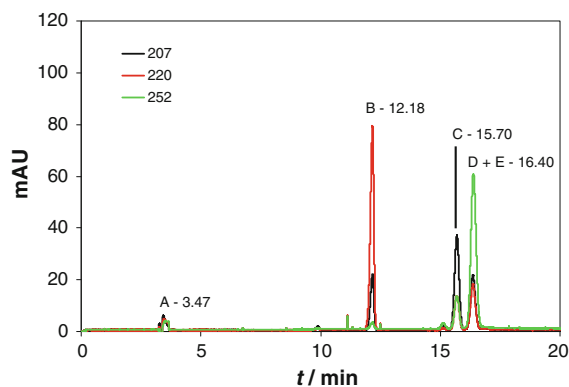


Fig. 9 Electrochromatogram of the test mixture on the unmodified capillary in MEKC mode; the test mixture consisted of thiourea (A), naphthalene (B), fluorene (C), phenanthrene (D), and anthracene (E), and 1,2-benzanthracene (F) (not detected due to high dispersion); capillary size $L_{\text{tot}} = 50$ cm, $L_{\text{eff}} = 41.5$ cm, i.d. = 50 μm ; separation conditions: 50 mmol/L sodium phosphate and 20 mmol/L SDS, pH 7.0

(SDS)-based pseudostationary phase. As can be seen in Fig. 9 in MEKC mode, phenanthrene and anthracene could not be separated, whereas on the capillaries prepared according to the methods 1 and 2 with capillaries modified with β -CD-SH7-GNPs (Figs. 6, 7) at least a partial separation of these analytes was achieved. The number of theoretical plates for naphthalene and fluorene in MEKC mode were 61,600 and 23,800 plates/m, respectively. Our capillary prepared according to the method 2 provided similar efficiencies (Table 1).

Conclusion

FS capillaries covalently coated with β -CD-SH or β -CD-SH7 modified GNPs were used for OT-CEC separation of the model mixture of aromatic compounds. It was found that the use of β -CD derivative with seven thiol groups led to better separation of the individual compounds than that obtained with a single thiol group containing β -CD derivative.

The benefit of the utilization of the covalently bonded GNPs to the inner wall of capillaries lies in more freedom in the selection of composition of the BGE (mobile phase) for the separations. The application of GNPs and other types of NPs as pseudostationary phase in CEC, i.e., the addition of NPs to the BGE, is restricted to BGEs, in which the NPs are

stable; therefore, many organic solvents and low and high pH BGEs cannot be often used.

The other positive feature brought into OT-CEC technique by GNPs immobilization on the inner capillary wall consists in the enlargement of the modifiable surface as compared to the bare capillary wall. Thus, a larger amount of ligand can be immobilized in the capillary and a higher separation capacity can be obtained.

For further enhancement of the separation capabilities, a sol-gel technique was used for the preparation of the capillaries coatings. In the first preparation procedure, the method 2, the bare GNPs were immobilized onto the capillaries walls and then a covalent attachment with β -CD-SH7 followed. In the other coating mode, the method 3, β -CD-SH7 derivative capped GNPs were used in the sol-gel procedure.

The comparison of OT-CEC separations of the selected polyaromatic hydrocarbons using the GNPs coated capillaries prepared by different procedures revealed that in terms of reproducibility and separation capability, the best results were achieved with the capillaries prepared by a simple covalent modification of FS capillary walls with MPTMS followed by subsequent immobilization of GNPs and β -CD-SH7. The application of the two-step sol-gel technique also led to the capillaries possessing a good separation efficiency, however, the synthetic procedure was found to be sensitive to the preparation conditions and it was difficult to prepare the capillaries with reproducible separation parameters.

In our forthcoming experiments, we will focus our research to chiral separations using capillaries with β -CD derivatives modified GNPs. Our aim will be to separate enantiomers of biologically important compounds, such as amino acids, peptides, drugs, and saccharides.

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