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Short Communication

Application of bare gold nanoparticles in open-tubular CEC separations of polycyclic aromatic hydrocarbons and peptides

In this study, bare gold nanoparticles (GNPs) immobilized in the sol-gel-pretreated fused-silica (FS) capillary as a stationary phase for open-tubular capillary electrochromatography (OT-CEC) are for the first time shown to be able to separate both hydrophobic polycyclic aromatic hydrocarbons (PAHs) as well as hydrophilic cationic antimicrobial peptides. Model mixture of four PAHs, naphthalene, fluorene, phenanthrene, and anthracene, was resolved by OT-CEC in the GNP-modified FS capillaries using the hydro-organic background electrolyte (BGE) composed of 20 mmol/L sodium phosphate buffer, pH 7, modified with ACN at 8:2 v/v ratio. On the other hand, three synthetic analogues of an antimicrobial peptide mastoparan PDD-B, basic tetradecapeptides INWKLLGKKILGAL-NH₂, INSLKLGKKILGAL-NH₂ and NWLRLGRRILGAL-NH₂, were separated in aqueous acidic BGEs, pH 2.1–3.1, composed of weak acids (formic and acetic) or amphoteric amino or imino acids (aspartic or iminodiacetic), utilizing the advantage of a slow reversed (anodic) EOF and slightly positive charge of the GNP-modified FS capillary suppressing the adsorption of cationic peptides on the inner capillary wall and improving their resolution.

Keywords: CE / Gold nanoparticles / Peptides / Polycyclic aromatic hydrocarbons / Stationary phase
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1 Introduction

Nanoparticles (NPs) are objects of variable shapes with the dimensions in the range of units to hundreds of nanometers possessing unique physical and chemical properties [1–4]. Typical features of NPs are a large surface-to-volume ratio and other fascinating properties derived from the “quantum size effect” [3]. NPs have significantly influenced many fields of science including analytical separation and preconcentration of a variety of analytes. The potential of gold NPs (GNPs) in separation science has been recognized at the beginning of this century and since then a bunch of interesting papers on this topic has been published as further documented by several cited papers. In capillary electromigration techniques, the GNPs can serve either as permanent or dynamic inner surface coatings in open-tubular capillary electrochromatography (OT-CEC) [5–7] or

as pseudostationary phases in partial filling or continuous filling mode in micellar electrokinetic chromatography (MEKC) [8, 9]. Application of GNPs is popular also in separations on microchips [10, 11]. Besides, surface-modified GNPs have found applications as stationary phase modifiers also in gas chromatography (GC) [12, 13] and high-performance liquid chromatography (HPLC) [14]. Moreover, derivatized gold microspheres [15] and gold-coated polystyrene [16] and silica [17] particles as the capillary column packing have been employed in HPLC mode. Lately, also the potential of the GNPs for sample preconcentration has been distinguished and this area undergoes a dynamic progress [18, 19]. Recently, several detailed review papers devoted specifically to the applications of GNPs in separation science have been published [20, 21].

In OT-CEC mode, GNPs modified with alkylthiols of various chain lengths are used in applications where hydrophobic compounds are separated in reversed-phase (RP) mode [5–8, 22, 23]. Here, we demonstrate for the first time the utilization of bare (citrate stabilized) GNPs immobilized on the sol-gel-pretreated fused-silica (FS) capillary wall in the RP mode for the separation of both hydrophobic polycyclic aromatic hydrocarbons (PAH) (naphthalene, fluorene, phenanthrene, and anthracene) and hydrophilic biologically active cationic peptides, analogues of antimicrobial peptide mastoparan PDD-B (tetradecapeptide

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Abbreviations: FS, fused-silica; GNP, gold nanoparticle; NP, nanoparticle; OT-CEC, open-tubular CEC; PAH, polycyclic aromatic hydrocarbon; TP, theoretical plate

INWLKLGKKILGAL-NH₂) isolated from the venom of social wasp *Polistes dorsalis dorsalis*.

While the use of bare GNPs for the preconcentration of various compounds has been successfully demonstrated elsewhere [11, 18, 19], to the best of our knowledge, the separations utilizing bare GNPs immobilized on pretreated sol-gel FS capillary as the stationary phase in OT-CEC mode is described for the first time in this study.

2 Materials and methods

2.1 Chemicals and materials

Trisodium citrate dihydrate (99%, p.a., Penta, Czech Republic), hydrochloric acid (30%), hydrofluoric acid (40%), and nitric acid (65%) (all Suprapur, Merck, Germany), sodium hydroxide (Tripur, Merck), acetonitrile (99.8%, LiChrosolv, Merck), and potassium tetrachloroaurate (III) (98%), ortho-phosphoric acid (50%), thiourea, fluorene, anthracene, phenanthrene, naphthalene, and (3-mercaptopropyl)trimethoxysilane (MPTMS, 95%), aspartic acid and iminodiacetic acid (all Sigma-Aldrich, Czech Republic), DMSO, acetic acid, formic acid and sodium hydroxide (all of analytical grade, Lachema Brno, Czech Republic), and ultrapure water (Milli-Q grade, Millipore, France) were used. Analogues of antimicrobial peptide mastoparan PDD-B (INWLKLGKKILGAL-NH₂), three tetradecapeptides, INWKLGKKILGAL-NH₂, INSLKLGKKILGAL-NH₂, and NWLRLGRRILGAL-NH₂, were prepared by the solid-phase synthesis in the group of Dr. V. Čeřovský, IOCB ASCR, Prague [24].

2.2 Instrumentation

OT-CEC separations of PAHs were performed with an Agilent CE instrument (Agilent 3D HPCE, Germany) equipped with a UV-Vis diode-array detector. The bare FS capillaries of 50/375 µm id/od and 50/41.5 cm total/effective length (Polymicro Technologies, USA) were pretreated by a sol-gel procedure and then modified by immobilized GNPs as described below.

OT-CEC separations of peptides were carried out in a home-made CE device equipped with a UV photometric detector monitoring absorbance at 206 nm [25] and GNP-modified sol-gel-pretreated FS capillary (id/od 50/375 µm, total/effective length 30.8/20.0 cm).

For details on other instrumentation and equipment used, see Supporting Information.

2.3 Preparation of the GNPs

The GNPs were prepared as previously described [26]. Briefly, 1 mL of 1% m/m aqueous solution of the potassium tetrachloroaurate (III) and 2.5 mL of 1% m/m 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.

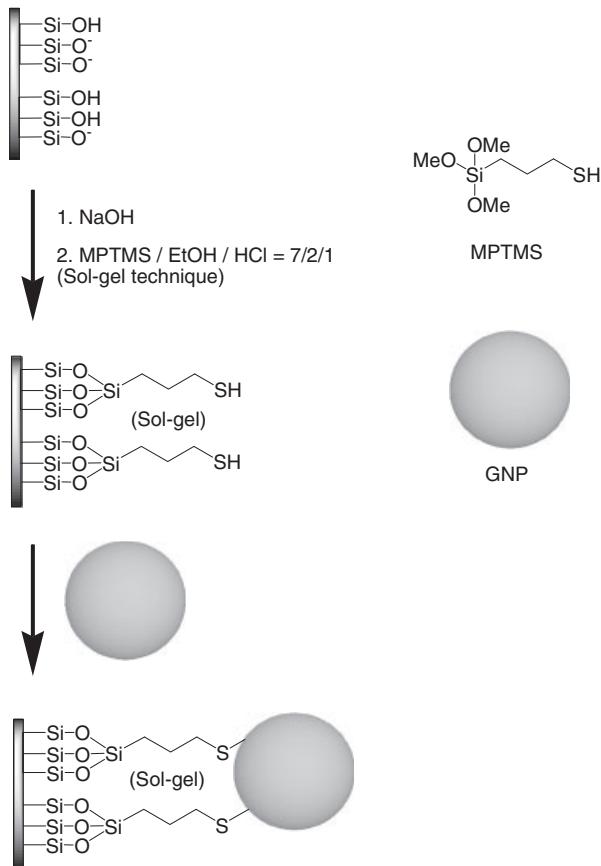
2.4 Preparation of the sol-gel-treated FS capillary with immobilized GNPs

A sol-gel-treated FS capillary with immobilized GNPs was prepared according to the following procedure. Bare FS capillary with outer polyimide coating (50 cm long) was rinsed (16.6 µL/min) with 1 mL NaOH (1 mol/L) followed by 1 mL of ultrapure water (16.6 µL/min) and dried at 180°C overnight. The activated capillary was then rinsed (20 µL/min) with 100 µL 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. The excess of the 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 µL/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. Totally, 2 mL of freshly prepared GNPs solution was pumped (16.6 µL/min) through the capillary and allowed to stand for 2 days with both ends submerged in this solution. Finally, 1 mL of ultrapure water was pumped (20 µL/min) through the capillary. The complete synthetic protocol is depicted in Scheme 1.

2.5 OT-CEC separation conditions

2.5.1 Separations of PAHs

Naphthalene, fluorene, phenanthrene, and anthracene were dissolved in ACN, each of them at 1 mg/mL concentration. Thiourea used as electroosmotic flow (EOF) marker was dissolved in 20 mmol/L sodium phosphate, pH 7, at 2 mg/mL concentration. The test mixture was made up in 20 mmol/L sodium phosphate/ACN, 8:2, v/v at final concentration 0.1 mg/mL of each PAH as well as thiourea. The background electrolyte (BGE) was composed of 20 mmol/L sodium phosphate buffer, pH 7, modified with ACN at 8:2 v/v ratio. GNP-modified FS capillaries were conditioned in the Agilent CE instrument by pressure rinsing with the BGE for 15 min before the first run. Between individual OT-CEC analyses, the capillary was rinsed for 2 min with the BGE. Prior to storing the capillaries when not in use, 5 min rinsing with water was applied. Samples were hydrodynamically injected by pressure of 1 kPa for 10 s. Analytes were detected at several wavelengths as specified in the electrochromatograms. All separations were performed at 25 kV (anode at the injection



Scheme 1. Preparation of sol-gel pretreated GNPs modified FS capillary.

capillary end) with a voltage ramp time of 0.5 s, the capillary cartridge was held at constant temperature 25°C. For data acquisition and evaluation Chemstation (Agilent, Germany) software was used.

2.5.2 Separations of peptides

The antimicrobial peptides and DMSO (EOF marker) were dissolved in deionized water at concentrations of 0.3–0.5 mg/mL. EOF marker and mixture of peptides were introduced into the capillary hydrodynamically (1.1 kPa pressure), first DMSO from cathodic capillary end for 6 s and then peptides from anodic capillary end for 2 s. The composition and pH of three BGEs used, applied separation voltage and mobility of EOF marker in these BGEs are given in the legend of Fig. 2. GNP-modified FS capillaries were conditioned in the home-made CE analyzer by pressure rinsing with the BGE for 15 min before the first run. Between the analyses in the different BGEs, the capillary was conditioned by subsequent rinsing with water, 0.1 mol/L NaOH, water and BGE, each rinsing for 2 min at 100 kPa pressure. Between the analyses in the same BGE, the capillary was rinsed only with the BGE for 2 min at 100 kPa pressure. Programs Clarity (DataApex, Czech

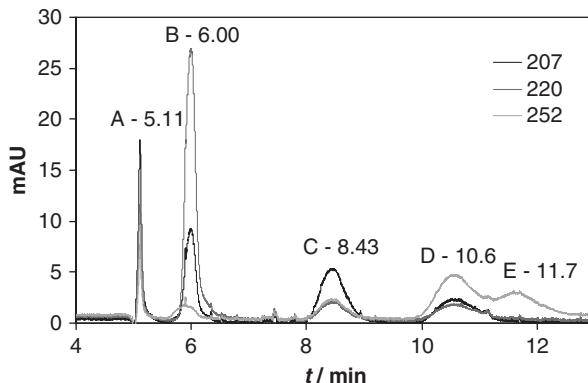


Figure 1. Electrochromatogram of the test mixture consisted of EOF marker thiourea (A) and four PAHs, naphthalene (B), fluorene (C), phenanthrene (D), and anthracene (E) in the sol-gel-pretreated GNP-modified FS capillary (id/od 50/375 µm; total/effective length 50.0/41.5 cm); BGE: 20 mmol/L sodium phosphate, pH 7/ACN, 8:2, v/v, voltage 25 kV, temperature 25°C.

Republic) and Origin 6.1 (OriginLab, USA) were used for data acquisition and evaluation.

3 Results and discussion

3.1 Characterization of the GNPs and GNP-modified capillaries

The preparation procedure for the GNPs resulted in spherical nanoparticles with diameter of 15 nm at concentration 2.75 nmol/L, which exhibited plasmon resonance band at 518 nm (see TEM image and UV-Vis spectrum of the freshly prepared GNPs in Fig. S1 in Supporting Information). The amount of gold bonded onto the capillary inner wall determined by ICP-MS was found to be equal to 80 ng for 50 cm-long capillary, for more details on ICP-MS measurement see Supporting Information.

3.2 OT-CEC separation of polyaromatic hydrocarbons

It is well known from the literature that hydrophobic compounds can be successfully separated by OT-CEC mode with immobilized GNPs onto the inner capillary wall when the GNPs are modified with a suitable thioalkane of variable chain length [5–8, 22, 23]. If the sole citrate stabilized GNPs are applied under the same separation conditions, no retention of hydrophobic compounds is observed.

However, we have found out that if the sol-gel procedure is first used for the FS capillary inner wall pretreatment followed by the subsequent immobilization of the GNPs, the behavior of such capillary column is quite different. Figure 1 shows a typical electrochromatogram obtained by OT-CEC separation of thiourea (EOF marker) and four polyaromatic

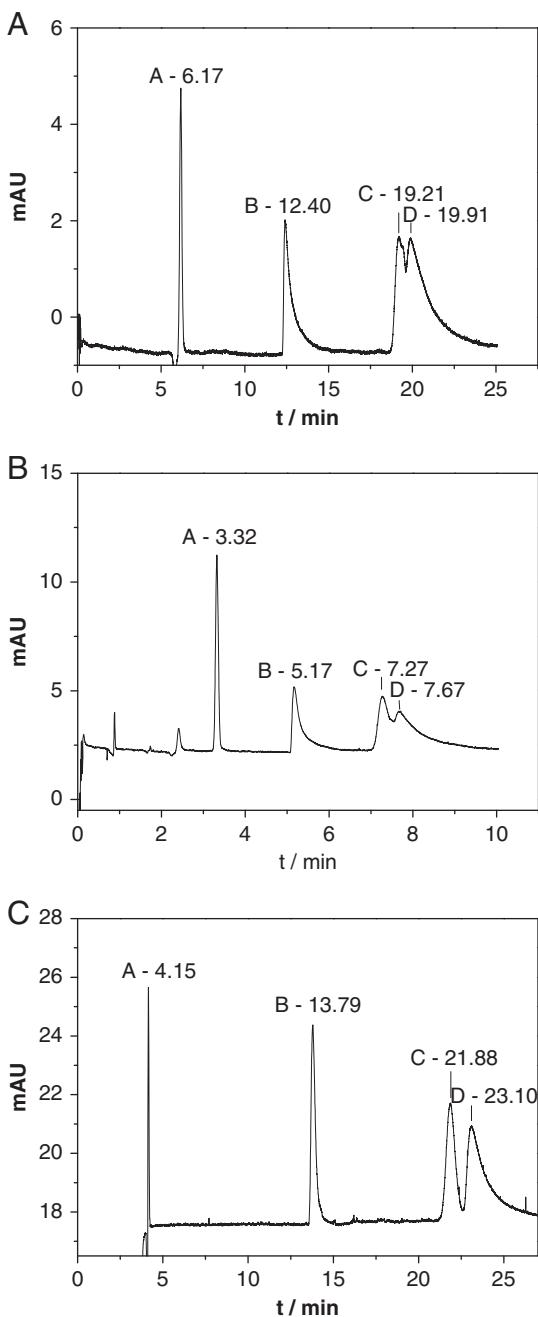


Figure 2. OT-CEC separations of the mixture of analogues of antimicrobial mastoparan PDD-B peptide in the sol-gel-pre-treated FS capillary modified by the bare GNPs in three acidic BGEs. (A) BGE I: 200 mmol/L acetic acid, 200 mmol/L formic acid, pH 2.1, separation voltage +5 kV, EOF mobility $m_{EOF} = -18.0$ [$10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$]; (B) BGE II: 30 mmol/L aspartic acid, pH 3.1, separation voltage +10 kV, EOF mobility $m_{EOF} = -16.7$ [$10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$]; (C) BGE III: 200 mmol/L iminodiacetic acid, pH 2.3, separation voltage +7 kV, EOF mobility $m_{EOF} = -19.1$ [$10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$]. (A) DMSO; (B) INWKKLGKKILGAL-NH₂; (C) INSLKLGKKILGAL-NH₂; (D) NWLRLGRRILGAL-NH₂; Capillary: id/od 50/375 μm , total/effective length 30.8/20.0 cm.

hydrocarbons (PAHs), naphthalene, fluorene, phenanthrene, and anthracene, in the GNP-modified sol-gel-pretreated FS capillary. Significant retention of PAHs is observed and the separation selectivity is similar to that obtained with “classical” thioalkyl-modified GNP-immobilized FS capillaries [5–8, 22, 23]. The separation efficiency for thiourea was high, 119 000 theoretical plates (TP) per meter. For the PAHs it was one order lower decreasing from 12 500 TP/m for naphthalene to 4500 TP/m for fluorene, 2250 TP/m for phenanthrene, and 3890 TP/m for anthracene. 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 analytes [27].

Finally, a control (blank) experiment with a sol-gel capillary unmodified with the GNPs was carried out with the goal to find out if there was retention of the PAHs even in the case of the absence of GNPs on the capillary walls. Some retention of the test compounds was registered indeed but the peak shape was very bad and the separation efficiency low (data not shown). Hence, the benefit of the GNPs for the PAHs separation was evident.

3.3 OT-CEC separation of antimicrobial peptides

Three analogues of antimicrobial peptide mastoparan PDD-B (INWLKLGKKILGAL-NH₂, isolated from the venom of social wasp *Polistes dorsalis dorsalis*), tetradecapeptides with the sequences INWKKLGKKILGAL-NH₂, INSLKLGKKILGAL-NH₂, and NWLRLGRRILGAL-NH₂ were analyzed and separated in three aqueous acidic BGEs in the pH range 2.1–3.1. All three BGEs employed in this case exhibited anodic (reversed) EOF; the determined EOF mobilities are given in the legend of Fig. 2. By comparing these EOF mobilities (being in the range from -16.7 to -19.1 [$10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$] with the effective electrophoretic mobilities of peptides in Table 1 (achieving values from $+25.4$ to $+36.6$ [$10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$]) one can see that the absolute values of the latter mobilities are almost two times higher. Thus, highly charged peptides are migrating against relatively slow anodic EOF toward the cathode. In order to detect peptides and the EOF marker within a single run, the EOF marker was introduced into the capillary at the cathodic end, while the analytes were injected from the anodic end. OT-CEC separations of antimicrobial peptide mixtures in the FS capillary coated by GNPs in three acidic BGEs are presented in Fig. 2. Despite relatively long analysis time, the peptides were only partially separated in the BGE I (Fig. 2A). The fastest separation was achieved in BGE II but the separation efficiencies and resolution of two more slowly migrating peptides were rather poor in this BGE (Fig. 2B). Longer migration times and remarkably higher separation efficiencies and the best resolution were attained employing isoelectric BGE III, 200 mmol/L iminodiacetic acid, pH 2.3 (Fig. 2C). The effective electrophoretic mobilities and the numbers of theoretical plates of OT-CEC

Table 1. Effective electrophoretic mobilities (m_{eff}) and number of theoretical plates (N) of analogues of mastoparan PDD-B peptide separated by OT-CEC in the GNP-modified FS capillary in the background electrolytes BGE I–III, the compositions of which is given in the legend of the Fig. 2

| Peptide | $m_{\text{eff}} [10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}]^{\text{a)}$ | | | $N (\text{TP/m})^{\text{a)}$ | | |
|--------------------------------|---------------------------------------------------------------------------------|--------|---------|------------------------------|--------|---------|
| | BGE I | BGE II | BGE III | BGE I | BGE II | BGE III |
| INWKKLGKKILGAL-NH ₂ | 34.5 | 36.6 | 29.7 | 104 800 | 84 900 | 282 200 |
| INSLKLGGKILGAL-NH ₂ | 28.7 | 30.8 | 25.8 | 99 800 | 42 000 | 165 200 |
| INWLRLGRRILGAL-NH ₂ | 28.3 | 30.1 | 25.4 | 28 900 | 29 100 | 69 900 |

a) Average values from two to three measurements are presented, which did not differ more than 2–3% in the case of effective mobilities and 5–8% in the case of number of theoretical plates.

separations of the above antimicrobial peptides in GNP-modified FS capillary in three BGEs used are given in Table 1. A great advantage of the GNP-modified FS capillary for analysis of cationic peptides is the slow anodic EOF and the slightly positive charge of the inner capillary wall, which suppresses adsorption of strongly basic peptides on the inner capillary wall and results in higher separation efficiency and better resolution. GNP modification of the FS capillary thus offers an alternative to commonly used approach, where for the CE and CEC analyses of strongly cationic peptides, cationic detergents are added to the BGEs [28–30]. It is worth to note that in the bare FS capillary, the polycationic peptides were strongly adsorbed to the capillary wall and their CZE analyses ruined.

4 Concluding remarks

In this study we have for the first time demonstrated ability of bare GNPs anchored into the sol-gel-pretreated FS capillary to perform effectively as a stationary phase for the OT-CEC separation of hydrophobic PAHs in the hydro-organic solvent-based BGE, as well as for polar hydrophilic analytes, cationic antimicrobial peptides, in the aqueous acidic BGEs. In the latter case, a slightly positive charge of the GNP-modified FS capillary reverses the EOF, suppresses the adsorption of cationic peptides on the inner capillary wall and improves their separation. Currently, we work on further improvement of the separation efficiency and resolution of peptides and other analytes on the sol-gel-pretreated GNP-modified FS capillaries.

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The authors have declared no conflict of interest.

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