Qishu Qu[1]
Shengwei Peng[1]
Debby Mangelings[2]
Xiaoya Hu[1]
Chao Yan[3]

[1]College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou, P. R. China
[2]Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel-VUB, Brussels, Belgium
[3]School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P. R. China

Received June 13, 2009
Revised September 7, 2009
Accepted October 1, 2009

Research Article

Silica spheres coated with C18-modified gold nanoparticles for capillary LC and pressurized CEC separations

Nonporous monodispersed silica spheres of 1.3 μm were coated with gold nanoparticles (AuNPs) and subsequently coated with *n*-octadecanethiol. By transmission electron microscopy analysis, the average diameter of the AuNPs on the silica spheres was determined to be 12 nm. The chromatographic and electrochromatographic properties of self-assembled *n*-octadecanethiol AuNP-coated silica microspheres (C18-AuNPs-SiO_2) were investigated using a group of nonpolar PAHs. The stationary phase appears to display a characteristic reversed-phase behavior. Higher separation efficiency and shorter separation times were obtained using pressurized CEC (pCEC) compared with capillary LC (CLC). A maximum column efficiency of about 2.5×10^5 plates *per* meter and less than 18 min separation time for benzene were obtained in pCEC while only 66 507 plates *per* meter and an analysis time of nearly 100 min were observed in CLC mode. A chemical stability test of the C18-AuNPs-SiO_2 stationary phase under extremely high and low pH conditions demonstrated that it is stable at pH 12 and 1 for at least 60 h. The results confirm that C18-AuNPs-SiO_2 possesses a high rigidity to withstand high packing pressures and can be used as a good stationary phase for CLC and pCEC.

Keywords:
Capillary LC / Gold nanoparticles / Pressurized CEC / Silica
DOI 10.1002/elps.200900375

1 Introduction

Columns packed with particle-based stationary phases are the most used columns for chromatographic separation although monolithic and open-tubular columns have gained considerable attention [1]. Among various types of particles used for packings, silica is almost an ideal support since it has some favorable characteristics such as a relatively high specific surface area and pore volume, narrow particle size distribution, and good mechanical stability [2]. However, silica-based stationary phases are limited to a pH range between 2 and 8, since they lack chemical stability at higher and lower pHs [3]. Therefore, some new stationary phases possessing higher pH stability such as organic polymer phases [4], carbon [5], alumina, titania, zirconia [6] were developed as an alternative to silica-based supports. Among these supports, zirconia received more attention from chromatographers due to its chemical and thermal stabilities, unique selectivity, and high efficiency. However, this support has not become a really competitive alternative, since the surface area and pore volume are much lower than with silica [7]. Therefore, many studies still concentrate on the improvement of silica-based supports with various chemical modifications in order to raise their chemical stability [8].

In the various types of nanoparticles, gold nanoparticles (AuNPs) have attracted widespread interest in both material chemistry and biomedical science because of their long-term stability, high surface area-to-volume ratio, and ease of chemical modification [9]. Just like zirconia, AuNPs are very stable under extremely basic and acidic conditions. Moreover, alkanethiols can bind to gold to form highly ordered and densely packed monolayers, while it is difficult to modify zirconia surfaces by any chemical reactions [10]. In spite of having so many merits AuNPs were seldom used to coat the silica surface to enhance its chemical stability. In separation science, self-assembled monolayers (SAMs) of modified AuNPs were generally only used as wall coating materials for open tubular chromatography [11–16] or used

Correspondence: Dr. Qishu Qu, College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, P. R. China
E-mail: quqishu@gmail.com
Fax: +86-514-87975244

Abbreviations: **APTMS,** 3-aminopropyltrimethoxysilane; **AuNPs,** gold nanoparticles; **CLC,** capillary LC; **$HAuCl_4$,** hydrogen tetrachloraurate(III) dehydrate; **MeOH,** methanol; **pCEC,** pressurized CEC; **SAM,** self-assembled monolayer; **TEM,** transmission electron microscopy; **TEOS,** tetraethylorthosilicate; **XRD,** x-ray powder diffraction

as pseudostationary phases to manipulate the selectivity of solutes in capillary electrophoresis [17–20]. To date, only two papers reported using SAM of thiol compounds on AuNP-coated particles as packing materials for HPLC [21, 22].

As pressurized CEC (pCEC) provides distinct advantages for the separation of various mixtures, it has recently attracted interest as an alternative separation mode to CEC and HPLC [23–25]. pCEC is the combination of CEC and HPLC in which both an electrical field and pressure are applied over a capillary column. Consequently, the flow profile of pCEC is changed from plug-like flow in pure CEC to some extent of parabolic-shaped flow. Therefore, the column efficiency of pCEC is somewhat reduced in comparison to pure CEC. However, compared with pure CEC, the application of pressure in CEC provides a new operating parameter to the CEC system, which allows fine-tuning of the selectivity in a separation of charged and uncharged compounds simply by adjusting the ratio of voltage to pressure [23]. Furthermore, the bubble formation in pure CEC separations can be suppressed by applying the pressure in pCEC. On the other hand, compared with HPLC, particles with a diameter smaller than 1.5 μm can be used as stationary phase in pCEC without the need for ultra-high pressures because both pressure flow and EOF can be used to drive the mobile phase through the column [26].

In order to combine the merits of silica and AuNP-based stationary phases, 1.3 μm silica particles were synthesized and coated with AuNPs in this work to serve as a new stationary phase for capillary LC (CLC) and pCEC. The preparation of AuNPs and monodispersed silica spheres and the subsequent self-assembly of *n*-octadecanethiol onto AuNP-coated silica particles were detailed. The chromatographic and electrochromatographic properties of the AuNP-coated silica microspheres (C18-AuNPs-SiO_2) were studied and the influence of experimental conditions on the retention behavior of the test mixture has been investigated.

2 Materials and methods

2.1 Chemicals and materials

Hydrogen tetrachloraurate(III) dehydrate ($HAuCl_4$), tetraethylorthosilicate (TEOS), ammonia (27%), sodium hydroxide, caffeine, uracil, benzene, naphthalene, 2-methylnaphthalene, acenaphthene, aniline, *p*-toluidine, *N,N*-dimethylaniline, benzoic acid, *p*-hydroquinone, and 2-nitrophenol were purchased from Chemical Reagent (Shanghai, China). Chromatographic-grade methanol (MeOH), 3-aminopropyltrimethoxysilane (APTMS), and *n*-octadecanethiol were purchased from Alfa Chemical (St. Louis, MO, USA). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). The 100 μm id and 375 μm od fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2 Equipment

Experiments were carried out on a TriSep™-2010 system (Unimicro Technologies, Pleasanton, CA, USA), on which CLC and pCEC can be performed independently [27]. For the CLC and pCEC separations, a pressure of 124 bar (1800 psi) was applied on the column inlet corresponding with a pump flow of 0.04 mL/min. Solvent from the pump and samples from the loop were split before they were introduced into the column with a 1540:1 ratio. For pCEC separations, normal polarity was used; thus the EOF goes from the anode (inlet) to the cathode (outlet). A sample loop of 2 μL was used for all experiments. With a split ratio of 1540:1, the amount of sample injected into the column was estimated to be 1.3 nL [24]. Transmission electron microscopy (TEM) observation was conducted on a TECNAI-12 instrument (Philips, the Netherlands), operated at an accelerating voltage of 120 kV. X-ray powder diffraction (XRD) data were taken with a graphite monochromator and Cu Kα radiation ($\lambda = 0.1541$ nm) on a D8 Advance Superspeed powder diffractometer (Bruker AXS, Germany), operated in the θ–2θ mode primarily in the 20–80° (2θ) range and at a step scan of $2\theta = 0.04°$. Fourier transmission infrared spectra of the samples were collected in the transmission mode on a Nicolet 740 FT-IR spectrometer.

2.3 Synthesis of AuNPs

All glassware was cleaned in aqua regia (3:1, HCl/HNO_3) for at least 1 h, thoroughly rinsed with deionized water and acetone, and then dried in an oven at ~100°C prior to use. Synthesis of monodispersed spherical AuNPs used in this study was similar to that reported by Grabar *et al.* [28]. Briefly, 100 mL 1 mM $HAuCl_4$ was heated to its boiling point under vigorous magnetic stirring and 10 mL 35 mM sodium citrate solution was added, which resulted in reduction of the gold. The mixture was stirred and heated under reflux for *ca.* 10 min. The AuNPs were formed during this time and witnessed by a color change from yellow to red-violet. TEM data indicated an average diameter of 4.2 nm (Fig. 1B).

2.4 Synthesis of monodispersed nonporous silica spheres

Nonporous silica microspheres were prepared according to Stöber *et al.* [29] with slight modifications. Aliquots of 7 M of H_2O and 3.0 M ammonia were dissolved in ethanol solution. Then, 0.3 M of TEOS solution was added to this solution with moderate stirring. The mixture was stirred in a closed vessel for about 30 min, followed by the introduction of 3.9 mL of H_2O and TEOS (in a TEOS/H_2O molar ratio of 2:1). The above mixture was stirred at 30°C for 10 h, and then it was aged for 10 h. The products were collected by filtration, washed with water, and dried at 100°C for 4 h. Spherical silica particles with a size of about 1.3 μm were obtained from the above experimental procedure (Fig. 1A).

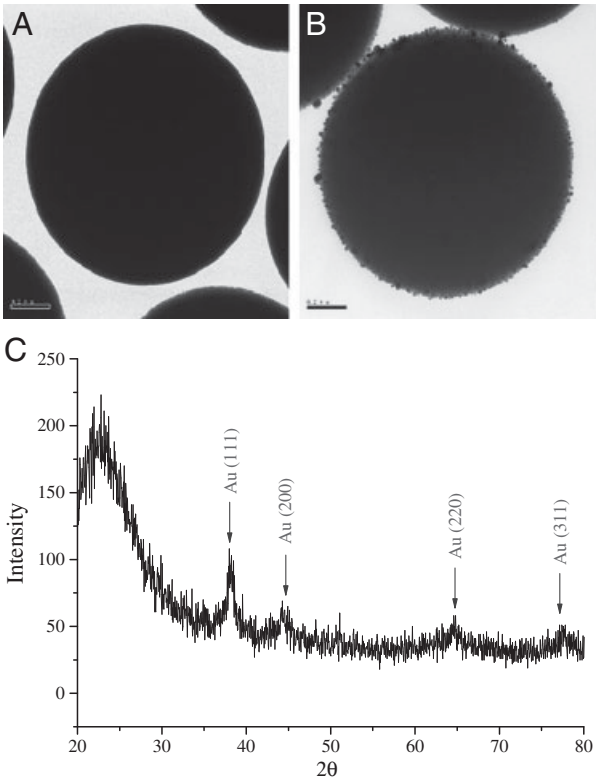

Figure 1. (A) TEM image of APTMS-derivatized silica microspheres before AuNP coating. (B) TEM image of silica microspheres after coating with AuNPs. (C) XRD pattern for a layer of AuNPs deposited on APTMS-derivatized silica microspheres.

2.5 Preparation of SAM of *n*-octadecanethiol on AuNP-coated silica particles

The preparation process has three major steps. First, the above prepared spherical silica particles were added into a solution containing 1% APTMS for 10 h. The modified silica particles were filtered out, washed with ethanol, and dried at 100°C for 24 h. Second, the dried silica particles from Section 2.4 were added into the solution of AuNPs with moderate stirring. The stirring was continued for 1 h. Then the silica particles were filtered out and washed with ethanol again. Third, the silica particles were soaked in an ethanol solution containing 20 mM *n*-octadecanethiol at 25°C for 24 h. The resulting C18 modified AuNP-coated silica microspheres were washed with ethanol and distilled water, respectively. Finally, the obtained particles were dried in vacuum at 60°C for 4 h.

2.6 Column preparation

A 20 cm section of a total length of 45 cm capillary with 100 μm id was packed electrokinetically [30], and frits (each about 1.5 mm in length) were made by sintering the packed particles at both ends of the packed section. A pressure of 138 bar was applied to the column during the sintering of the frits. A detection window was created at about 1 mm to the outlet frit on the empty section of the capillary by burning off the polyimide coating.

3 Results and discussion

3.1 Characterization of C18 - AuNPs - SiO_2

TEM and XRD were used to characterize the AuNP-coated silica microspheres. TEM analysis of the uncoated silica microspheres (Fig. 1A) shows that they are smooth and uniform prior to gold deposition. After deposition (Fig. 1B), individual AuNPs and small aggregates of AuNPs can be seen on the surface of individual silica microspheres. These data are consistent with the results of previous work showing mostly isolated and a few partially aggregated gold seeds attached to APTMS-modified silica surfaces [31]. The deposition of a layer of AuNPs on APTMS-derivatized silica microspheres was also studied by XRD (Fig. 1C). The Bragg angles 38.1, 44.3, 64.5, and 77.5° correspond to Au(111), Au(200), Au(220), and Au(311), respectively, of the face-centered cubic phase of metallic gold. As the mean diameter of AuNPs is 4.2 nm and 2 g silica microspheres were coated with 1200 mL 1.25×10^{-4} M $HAuCl_4$, taking the gold core as a sphere with density $\rho_{Au} = 58.01$ atoms/nm^3 [32], the maximum area of the AuNPs covered onto the silica surface can be calculated as 0.46 m^2. The surface area of 2 g nonporous silica microspheres is about 4.6 m^2 [33]. Thus the coverage of AuNPs on the silica surface is estimated to be 10%. The low coverage of the AuNPs indicates that the surface of the silica was not well protected by the AuNPs. When the stationary phase was rinsed with the mobile phase with high pH value for a long time, it would dissolve. Therefore, in order to protect the surface of the silica well from the erosion of basic solutions, silica particles with a dense AuNP layer should be prepared. Future work on preparing this type stationary phase is under way. The IR spectra of C18-AuNPs-SiO_2 display a characteristic signal from the alkyl chains in the region from 2800 to 3000 cm^{-1} while no such signal appeared in the IR spectra of bare AuNP-coated silica microspheres. It demonstrated that *n*-octadecanethiol has been successfully introduced onto the surface of the AuNP-coated silica microspheres.

3.2 Stability of AuNP-coated silica modified with C18

The most widely used method to deposit AuNPs on silica is to graft the AuNPs onto the silica after capturing the AuNPs by an amine-terminated coupling agent [34]. No obvious loss of AuNPs from the surface of silica particles was found after the C18-AuNPs-SiO_2 were packed into the capillary using a pressure up to 517 bar. This was confirmed by comparing the TEM micrographs of the gold-coated silica particles before and after packing. During the CLC and pCEC separations, no loss of AuNPs was observed either since

no sharp signal enhancement was found during analysis. The RSD of repeatability of retention time for benzene of a gold-coated silica particles packed column was below 1% (ten consecutive runs using pCEC separation mode), which also proves that C18-AuNPs-SiO_2 stationary phase is very stable.

3.3 Chromatographic properties

Figure 2 shows an example of a CLC separation using the C18-AuNPs-SiO_2 column. The theoretical plate numbers *per* meter for this experiment were 61 189 for benzene, 56 248 for naphthalene, 42 131 for 2-methylnaphthalene, and 20 240 for acenaphthene. These values are below those normally obtained in ultra-performance LC with sub-2 μm particles [35–37]. The separation time is as long as 50 min because a pressure of only 124 bar was used, which is far below the pressure (normally >1000 bar in ultra-performance LC) needed to obtain a optimum linear velocity when sub-2 μm spherical particles are used as stationary phase. Using a shorter column would allow faster separations, but unfortunately the commercial instrument used in this work requires a minimal column length of 20 cm. The linear dependence of log k as a function of the MeOH content (% v/v) in the mobile phase indicates that the C18-AuNPs-SiO_2 stationary phase shows a reversed phase chromatographic retention mechanism.

Since the plug-like flow profile generated by EOF can improve the column efficiency compared with CLC and the existing EOF can speed up the flow rate inside the column, voltages varying between 0 and 20 kV (2 μA at 20 kV) with a 124 bar supplementary pressure were used for the pCEC experiments. Faster separations were achieved using higher voltages (Fig. 3). The five solutes could be resolved within

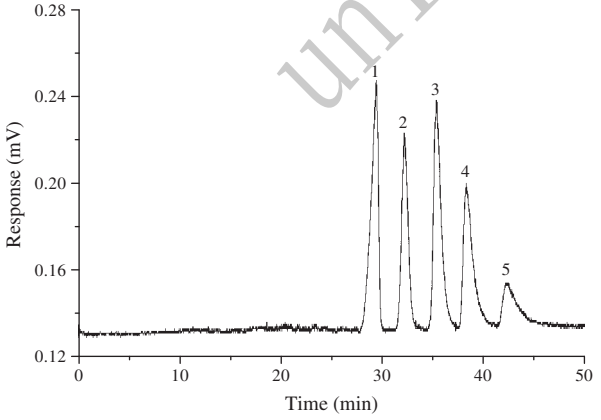

Figure 2. Chromatogram of a mixture of PAH compounds. Column 45 cm (packed length 20 cm) × 100 μm id capillary packed with 1.3 μm *n*-octadecanethiol-modified AuNP-coated silica microspheres. Mobile phase: MeOH/H_2O (70:30, v/v). Flow rate: 52 nL/min. Detection: UV 254 nm. Peak identification: (1) uracil, (2) benzene, (3) naphthalene, (4) 2-methylnaphthalene, (5) acenaphthene.

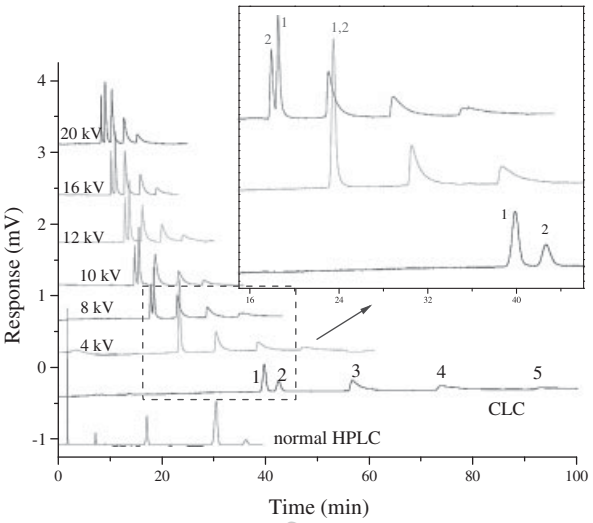

Figure 3. Effect of applied voltage on the separation of five analytes. Mobile phase: 60% v/v MeOH and 40% v/v 10 mM phosphate buffer solution (pH 7.0). For normal HPLC a 15 cm × 4.6 mm id column packed with 5 μm ODS was used. Other conditions were as in Fig. 2.

18 min when 20 kV was applied, while the separation time was about 100 min when the CLC mode was used and about 38 min when normal HPLC mode was used. The same elution order was obtained when the mixtures were separated by 5 μm porous ODS particles using normal HPLC mode. It demonstrates again that C18 was modified onto the surface of AuNPs. The rapidly decreasing retention time of the solutes with increasing voltage demonstrated that the AuNPs covered on the surface of silica spheres are negatively charged. Thus the direction of EOF is from the anode to cathode. Different elution order of uracil and benzene was found when a voltage higher than 4 kV was applied. This reversal of elution order was attributed to the unique characteristic of pCEC that the EOF can be combined with pressure and the separation mechanism is therefore based on both electrophoretic mobility and chromatographic partitioning. The retention factor (k) in a pCEC system can be written as follows [38]:

$$k = \frac{ck'P + (\mu_{eo}k' - \mu_{em})E}{cP + (\mu_{eo} + \mu_{em})E} \quad (1)$$

where c is a constant, P is the supplementary pressure, k' is the retention factor of the compound in the same column operated in the HPLC mode, μ_{eo} is electroosmotic mobility, μ_{em} is electrophoretic mobility, and E is the electric field. Uracil is slightly positive charged when it is dissolved in a pH 7 solution, while benzene is neutral. When a voltage is applied, the electrophoretic mobility of uracil contributes to its retention factor, while this is not the case for benzene. This can lead to a difference in elution order in pCEC mode. By varying the magnitude of the electric field, the elution order of uracil and benzene hence can be changed in pCEC while this cannot be achieved in a pure electro-driven or pressure-driven system [39].

The voltage applied on the capillary also affects the separation performance. High voltage improves the separation efficiency for benzene, where a maximum column efficiency of about 2.5×10^5 plates *per* meter was generated when 12 kV (1 μA) was applied over the column while only 66507 plates *per* meter was obtained in CLC. The column efficiency slightly decreased with the gradual increase of the voltage when the voltage was higher than 12 kV due to the reasons we do not yet understand. For other compounds, however, the column efficiencies increased only a little while the analysis times were greatly reduced.

The C18-AuNPs-SiO_2 stationary phase was also tested for the separation of various types of compounds using CLC and pCEC mode. As shown in Fig. 4, four basic compounds can be well separated using CLC or pCEC mode. Compared with CLC, the analysis time is greatly reduced in pCEC mode. However, in both of these two separation modes, especially in CLC mode, peak tailing is observed. This may be attributed to the negatively charged surface of C18-AuNPs-SiO_2 stationary phase, which causes secondary interactions with the basic compounds. These phenomena can be eliminated using AuNPs stabilized by a positively charged substance such as 4-dimethyl-aminopyridine instead of citrate [40]. The C18-AuNPs-SiO_2 stationary phase could also separate three acidic compounds without any observable peak tailing (Fig. 5).

3.4 Chemical stability of C18 - AuNPs - SiO_2 stationary phase

In order to test the chemical stability of C18-AuNPs-SiO_2 stationary phase, the column was rinsed with a mobile phase of MeOH/NaOH (pH = 12) (70:30, v/v) or MeOH/HCl (pH = 1) (70:30, v/v) at 0.11 mm/sec (52 nL/min) linear velocity for 60 h. Chromatograms of a mixture of uracil, benzene, naphthalene, 2-methylnaphalene, and acenaphthene were obtained periodically with the mobile phase MeOH/H_2O (70:30, v/v) in CLC mode during the rinse. Prior to each test, the column was conditioned with this mobile phase for 60 min. The results in Fig. 6 demonstrate that the C18-Au capillary column was stable under pH 1 and 12 for 60 h without any noticeable deterioration of performance according to the retention factor of test solutes. The RSD of retention factor (k) for the test mixtures over seven consecutives runs during the basic stability test of the column were 2.70, 3.26, 6.81, and 3.89% for benzene, naphthalene, 2-methylnaphalene, and acenaphthene, respectively. In acidic conditions, RSDs of k were 2.70, 5.00, 7.80, and 9.76% for benzene, naphthalene, 2-methylnaphalene, and acenaphthene, respectively. In addition, the column appeared to be stable for up to 1 month when stored in MeOH. The above results indicate that the stationary phase had a good stability towards extreme pH values.

4 Concluding remarks

AuNPs can be bond strongly onto the surface of silica spheres and the prepared stationary phase can withstand a pressure up to 517 bar. After self-assembly of *n*-octadecanethiol onto the surface of the AuNP-coated silica spheres, the obtained C18-AuNPs-SiO_2 stationary phase shows basically a reversed phase behavior and was very stable in a wide pH range (from 1–12). Compared with the pure gold-based particles for which only the HPLC mode can be used because of the conductivity of gold [41], the developed

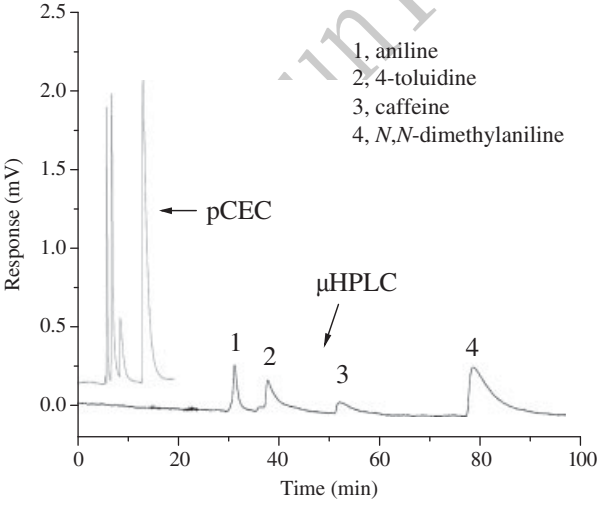

Figure 4. Comparison of separation of basic compounds using CLC and pCEC mode. Mobile phase: 5% v/v MeOH and 95% v/v 2.5 mM phosphate buffer solution (pH 7.0). Applied voltage for the pCEC separation was 20 kV. Other conditions were as in Fig. 2.

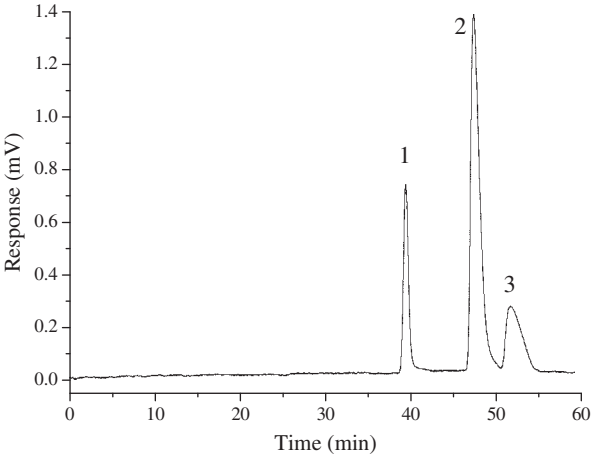

Figure 5. Electrochromatogram of a mixture of acidic solutes. Mobile phase: 60% v/v MeOH and 40% v/v 10 mM phosphate buffer solution (pH 7.0). Applied voltage was 1 kV. Peak identification: (1) *p*-hydroquinone, (2) 2-nitrophenol, (3) benzoic acid. Other conditions were the same as in Fig. 2.

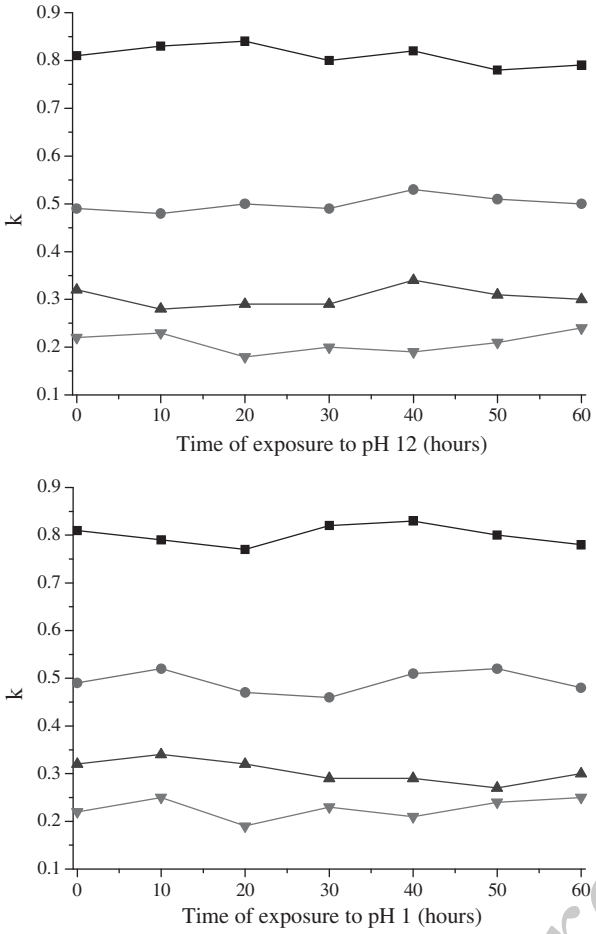

Figure 6. Stability test of C18-AuNP column in strong alkaline (pH 12) and acid (pH 1) mobile phase in CLC. Mobile phase: 75% MeOH in water. Solutes: (▼) benzene; (▲) naphthalene; (●) 2-methylnaphthalene; (■) acenaphthene. Other conditions were the same as in Fig. 2.

C18-AuNPs-SiO_2 stationary phase can be used in pCEC separation mode. Various types of compounds including acidic and basic solutes were efficiently separated. In pCEC, higher separation efficiency and shorter analysis time were obtained using the particles with a core diameter of only 1.3 μm. The results presented in this work demonstrate that C18-AuNPs-SiO_2 is a valuable alternative to be used as packing material for CLC and pCEC applications.

This work was supported by the National Natural Science Foundation of China (20975090, 20675071), the Qing Lan Project, the foundation of Jiangsu Provincial Nature Foundation of China (BK2006065), and by a Bilateral Project between China and Belgium (BWS07/03).

D. Mangelings is a postdoctoral fellow of the Research Foundation Flanders (FWO).

The authors have declared no conflict of interest.

5 References

[1] Unger, K. K., Skudas, R., Schulte, M. M., *J. Chromatogr. A* 2008, *1184*, 393–415.

[2] Nawrocki, J., Dunlap, C., McCormick, A., Carr, P. W., *J. Chromatogr. A* 2004, *1028*, 1–30.

[3] Kirkland, J. J., Adams, J. B., van Straten, M. A., Claessens, H. A., *Anal. Chem.* 1998, *70*, 4344–4352.

[4] Perrier-Cornet, R., Héroguez, V., Thienpont, A., Babot, O., Toupance, T., *J. Chromatogr. A* 2008, *1179*, 2–8.

[5] Knox, J. H., Kaur, B., Millard, G. R., *J. Chromatogr.* 1986, *352*, 3–25.

[6] Nawrocki, J., Dunlap, C., McCormick, A., Carr, P. W., *J. Chromatogr. A* 2004, *1028*, 1–30.

[7] Zhang, Z. P., Zheng, Y. J., Zhang, J. X., Chen, J. P., Liang, X. M., *J. Chromatogr. A* 2007, *1165*, 116–121.

[8] Claessens, H. A., van Straten, M. A., *J. Chromatogr. A.* 2004, *1060*, 23–41.

[9] Daniel, M. C., Astruc, D., *Chem. Rev.* 2004, *104*, 293–346.

[10] Nawrocki, J., Dunlap, C., Li, J., Zhao, J., McNeff, C. V., McCormick, A., Carr, P. W., *J. Chromatogr. A* 2004, *1028*, 31–62.

[11] Pumera, M., Wang, J., Grushka, E., Polsky, R., *Anal. Chem.* 2001, *73*, 5625–5628.

[12] Lin, Y. W., Huang, M. J., Chang, H. T., *J. Chromatogr. A.* 2003, *1014*, 47–55.

[13] Liu, F. K., Hsu, Y. T., Wu, C. H., *J. Chromatogr. A* 2005, *1083*, 205–214.

[14] Yang, L., Guihen, E., Holmes, J. D., Loughran, M., O'Sullivan, G. P., Glennon, J. D., *Anal. Chem.* 2005, *77*, 1840–1846.

[15] Gross, G. M., Grate, J. W., Synovec, R. E., *J. Chromatogr. A* 2004, *1060*, 225–236.

[16] Qu, Q. S., Shen, F., Shen, M., Hu, X. Y., Yang, G. J., Wang, C. Y., Yan, C., Zhang, Y. K., *Anal. Chim. Acta* 2008, *609*, 76–81.

[17] Tseng, W. L., Huang, M. F., Huang, Y. F., Chang, H. T., *Electrophoresis* 2005, *26*, 3069–3075.

[18] Neiman, B., Grushka, E., Lev, O., *Anal. Chem.* 2001, *73*, 5220–5227.

[19] Yu, C. J., Su, C. L., Tseng, W. L., *Anal. Chem.* 2006, *78*, 8004–8010.

[20] Nilsson, C., Nilsson, S., *Electrophoresis* 2006, *27*, 76–83.

[21] Ortiz, Y., Cintron, J. M., Colón, L. A., Abstracts of Papers, 221st ACS National Meeting, San Diego, CA, 1–5 April 2001.

[22] Kobayashi, K., Kitagawa, S., Ohtani, H., *J. Chromatogr. A* 2006, *1110*, 95–101.

[23] Eimer, T., Unger, K. K., *Trend Analyt. Chem.* 1996, *15*, 463–468.

[24] Qu, Q. S., Lu, X., Huang, X. J., Hu, X. Y., Zhang, Y. K., Yan, C., *Electrophoresis* 2006, *27*, 3981–3987.

[25] Wu, X. P., Wang, L., Xie, Z. H., Lu, J. S., Yan, C., Yang, P. Y., Chen, G. N., *Electrophoresis* 2006, *27*, 768–777.

[26] Patel, K. D., Jerkovich, A. D., Link, J. C., Jorgenson, J. W., *Anal. Chem.* 2004, *76*, 5777–5786.

[27] Zhang, K., Jiang, Z. J., Yao, C. Y., Zhang, Z. C., Wang, Q. S., Gao, R. Y., Yan, C., *J. Chromatogr. A* 2003, *987*, 453–458.

[28] Grabar, K. C., Freeman, R. G., Hommer, M. B., Natan, M. J., *Anal. Chem.* 1995, *67*, 735–743.

[29] Stöber, W., Fink, A., Bohn, E., *J. Colloid Interf. Sci.* 1968, *26*, 62–69.

[30] Yan, C., Electrokinetic Packing of Capillary Columns 1995. US patent 5453163.

[31] Westscott, S. L., Oldenburg, S. J., Lee, T. R., Halas, N. J., *Langmuir* 1998, *14*, 5396–5401.

[32] Terrill, R. H., Postlethwaite, T. A., Chen, C., Poon, C., Terzis, A., Chen, A., Hutchison, J. E. *et al.*, *J. Am. Chem. Soc.* 1995, *117*, 12537–12548.

[33] Unger, K. K., Jilge, G., Janzen, R., Giesche, H., *Chromatographia* 1986, *22*, 379–380.

[34] Freeman, R. G., Grabar, K. C., Allison, K. J., Bright, R. M., Davis, J. A., Guthrie, A. P., Hommer, M. B. *et al.*, *Science* 1995, *267*, 1629–1631.

[35] Patel, K. D., Jerkovich, A. D., Link, J. C., Jorgenson, J. W., *Anal. Chem.* 2004, *76*, 5777–5786.

[36] de Villiers, A., Lestremau, F., Szucs, R., Célébart, S., David, F., Sandra, P., *J. Chromatogr. A* 2006, *1127*, 60–69.

[37] Cintrón, J. M., Colón, L. A., *J. Chromatogr. A* 2006, *1106*, 131–138.

[38] Wu, J. T., Huang, P. Q., Li, M. X., Lubman, D. M., *Anal. Chem.* 1997, *69*, 2908–2913.

[39] Dittmann, M. M., Masuch, K., Rozing, G. P., *J. Chromatogr. A* 2000, *887*, 209–221.

[40] Gittins, D. I., Caruso, F., *Angew. Chem. Int. Ed.* 2001, *40*, 3001–3004.

[41] Qu, Q. S., Zhang, X. X., Zhao, Z. Z., Hu, X. Y., Yan, C., *J. Chromatogr. A* 2008, *1198–1199*, 95–100.

Address：489 Songtao Road, C01, Zhangjiang High-tech Park, Pudong, Shanghai 201203, P.R. China
E-Mail：info@unimicrotech.com，lemonyu@unimicrotech.com
Website：www.unimicrotech.com