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Open tubular capillary electrochromatography using capillaries coated with films of alkanethiol-self-assembled gold nanoparticle layers

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Abstract

We have used alkanethiol self-assembly and dithiol layer-by-layer (LBL) self-assembly processes to prepare an Au nanoparticle (NP)-coated open tubular capillary electrochromatography (OTCEC) column for the separation of three neutral steroid drugs (testosterone, progesterone, and testosterone propionate). The CEC column was fabricated through LBL self-assembly of Au NPs on a 3-aminopropyltrimethoxysilane (APTMS)-modified fused-silica capillary and subsequent surface functionalization of the Au NPs through self-assembly of alkanethiols. We investigated the electrochromatographic properties of the resulting Au NP-coated CEC column using a "reversed phase" test mixture of three steroid drugs. We found that the key factors affecting the separation performance were the number of Au NP layers, the length of the carbon-atom chain of the alkanethiol self-assembled on the Au NPs, the percentage of organic modifier, and the pH of the running electrolyte. This study reveals that the self-assembly of alkanethiols and dithiols onto Au NPs provides stationary phases for CEC separation that are easy to prepare and whose retention behavior is highly controllable and reproducible. We believe that our findings will contribute to further studies of the application of nanotechnology to separation science.
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Keywords: Capillary electrochromatography; Stationary phase; Gold nanoparticles; Self-assembly; Layer-by-layer

1. Introduction

Capillary electrophoresis (CE) is one of the most well-known and powerful separation techniques. Recently, capillary electrochromatography (CEC) emerged as a fairly novel electrokinetic separation technique—it is a hybrid of high-performance liquid chromatography (HPLC) and CE—that has unique potential for high separation efficiency [1]. In CEC, the electroosmotic flow (EOF) drives the mobile phase through the capillary column to effect the essential chromatographic interactions. Because of the flat-plug-like profile of the EOF, CEC offers greatly enhanced separation efficiencies when compared to HPLC. Unlike capillary zone electrophoresis (CZE), CEC is not restricted to charged solutes; indeed, both neutral and charged species can interact with the stationary phase and so become separated by CEC [2]. Thus, the potential for CEC as a separation technique is much wider than that of CZE.

The most common approach for performing CEC involves the use of a fused silica capillary column packed with a stationary phase [3]. An alternative mode of CEC is open tubular capillary electrochromatography (OTCEC), in which the stationary phase is coated on the inner wall of the capillary column. The preparation of the stationary phase is more critical for OTCEC [4,5]. The main methods used to prepare the stationary phase include physical coating [6–8], chemical bonding [9–12], molecular imprinting [13,14], and sol–gel technologies [15–17]. When compared to packed-column CEC, the main advantages of OTCEC are its high efficiency, simple instrumental handling, and short conditioning times. Its evident disadvantage, however, is the low separation capability that arises from the low phase ratio on the limited surface area. More recently, Liu and co-workers

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used an etched capillary strategy and attached various functional groups to increase the surface area of the inner wall to enhance the phase ratio [18].

Nanoparticles (NPs) have received much attention recently as potentially useful materials because they possess novel and fascinating properties [19–21]. Indeed, there is an increasing interest in the synthesis of nanoparticulate micro- and nanostructures [22]. To form NP films, it is necessary for an electrostatic attraction to exist between the layer and the NPs dispersed in the sub-phase. To fabricate nanostructures in a layer, chemical interaction forces, such as those utilized in self-assembly processes, can be utilized. For example, organic molecules containing thiol (SH) or amino (NH_2) groups can adsorb spontaneously onto Au surfaces to form well-organized self-assembled monolayers [23]. The fabrication of structures by means of self-assembly has attracted much attention because of the simplicity and flexibility of this approach [24]. Much work has been undertaken on the self-assembly of Au NPs onto surfaces presenting SH, NH_2, and CN groups because various potential applications are envisaged for them in the fields of optoelectronics [25], microelectronics [26], and bioscience [27,28]. For example, layers of alkanethiols self-assembled on Au behave as model surfaces for studying the interactions of proteins with surfaces [27,28]; indeed, modifications of the self-assembled layer can influence the non-specific interactions between proteins and Au surfaces.

In addition to the self-assembly of monolayers onto NP surfaces, layer-by-layer (LBL) assembly, a simple and new preparation technique, can be utilized for the structural organization of a large variety of NPs into multilayer films [29]. In medical science and in biotechnology, multilayer films of proteins attached to solid surfaces are used widely in the areas of diagnostics, isolation, and localization. For example, multilayer architectures of spatially organized proteins present a high density of biomolecules and, hence, exhibit enhanced sensitivity toward the detection of biomolecular species [30].

Although the applications of such self-assembled films in the biosciences have received intense scrutiny [27,28], the use of these phases in chromatographic science [e.g., liquid chromatography (LC) or CE] has been limited [31,32]. In particular, to the best of our knowledge, the LBL deposition strategy has never been employed for applications in separation science. In electrochromatography, a number of problems—such as column stability and inherent column properties—must be solved before any further separations applications are considered.

In this paper, we describe the preparation of OTCEC columns through the self-assembly of alkanethiols onto Au NP-coated capillaries and our investigations into their use for the separation of neutral steroid drugs. In addition to monolayer-coated Au NPs coated on the capillary surface, we also fabricated a variety of structures of Au NPs films on capillary columns. LBL assembly has been used to fabricate controllable structures of multilayer Au NPs films on glass microscope slides [33]. Therefore, we believed that it would be feasible to use LBL assembly to fabricate multilayer Au NPs films as coatings within capillary columns. Such an LBL strategy would provide us with new insight into understanding the potential of these novel materials for the separation of pharmaceutical drugs. Furthermore, we sought to optimize the separation of steroid drugs by evaluating the effects that various separation parameters—including the acetonitrile (ACN) concentration, the carbon atom chain length of the thiols, and the pH of the running electrolyte—have on the separation performance of the OTCEC column.

2. Experimental

2.1. Apparatus

All CE separations were performed using a Prince capillary electrophoresis system (Prince Technologies B.V., Emmen, The Netherlands). Uncoated fused-silica capillaries (75-μm I.D., 365-μm O.D., and 75-cm length) were obtained from Polymicro Technologies (Phoenix, AZ, USA). A detection window was fabricated ca. 60 cm from the capillary column inlet. Sample injections were performed throughout at a pressure of 30 mbar for 0.03 min. Positive polarity was applied at the capillary column inlet. The pH of the electrolytes was measured using an Orion 420A pH meter (Boston, MA, USA). All CE experiments were performed at a temperature of 25 °C. Separations were performed at 25 kV. A Hitachi (Tokyo, Japan) S-4000 and a JEOL (Tokyo, Japan) JSM-6500F scanning electron microscope (SEM) were employed to characterize the sizes of the Au NPs. In SEM studies, the particle sizes of samples were determined through two-dimensional grain analysis after digitizing the SEM images. The UV–vis spectra of samples were recorded

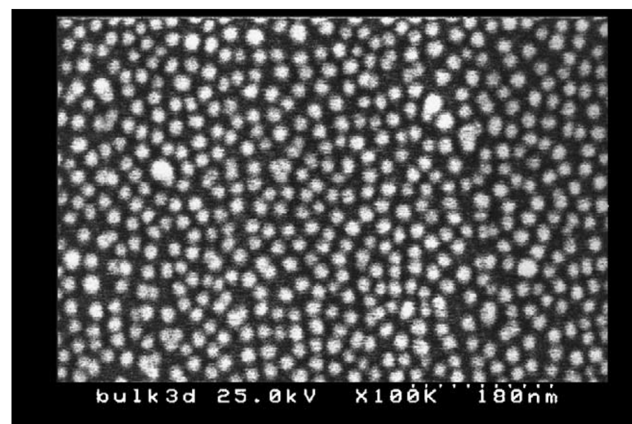


Fig. 1. SEM image of Au NPs prepared by heating an aqueous solution (100 mL) containing 1 mM $HAuCl_4$ to its boiling point under vigorous magnetic stirring, injecting 35 mM sodium citrate solution (10 mL), and then heating the resulting solution under reflux for 30 min.

using a Shimadzu UV 2501PC spectrometer (Shimadzu Corporation, Tokyo, Japan). A Hitachi (Tokyo, Japan) thermal desorption atmospheric-pressure-ionization mass spectrometer (TDS-APIMS) was employed to characterize the surface coverage of carbon on the Au NPs films.

2.2. Chemical reagents

Hydrogen tetrachloroaurate ($HAuCl_4$) and 3-aminopropyltrimethoxysilane (APTMS) were obtained from Acros Organics (Geel, Belgium). 1-Hexanethiol, 1-octanethiol, 1-dodecanethiol, 1-octadecanethiol, 1,9-nonanedithiol, testosterone, progesterone, and testosterone propionate were obtained from Tokyo Chemical Industry (Tokyo, Japan). Sodium citrate, tris(hydroxymethyl)aminomethane (Tris), ACN, and thiourea were obtained from Merck (Darmstadt, Germany). All electrolytes were prepared fresh each day and filtered through a 0.2-μm membrane filter (Alltech Associated, Deerfield, IL, USA) prior to use.

2.3. Running electrolyte preparation

A stock buffer electrolyte was prepared by dissolving a weighed amount of Tris in a 100-mL beaker; the pH of the Tris solution was adjusted in the range 7.0–10.0 through the addition of 0.1 N NaOH and 0.1 N HCl solutions. The pH-adjusted Tris solution was then sonicated for 10 min. The Tris buffer was then added to the appropriate amount of filtered organic solvent, followed by the addition of de-ionized water in an appropriate ratio. The final running electrolyte was sonicated for 10 min and filtered through a 0.2-μm membrane filter prior to use.

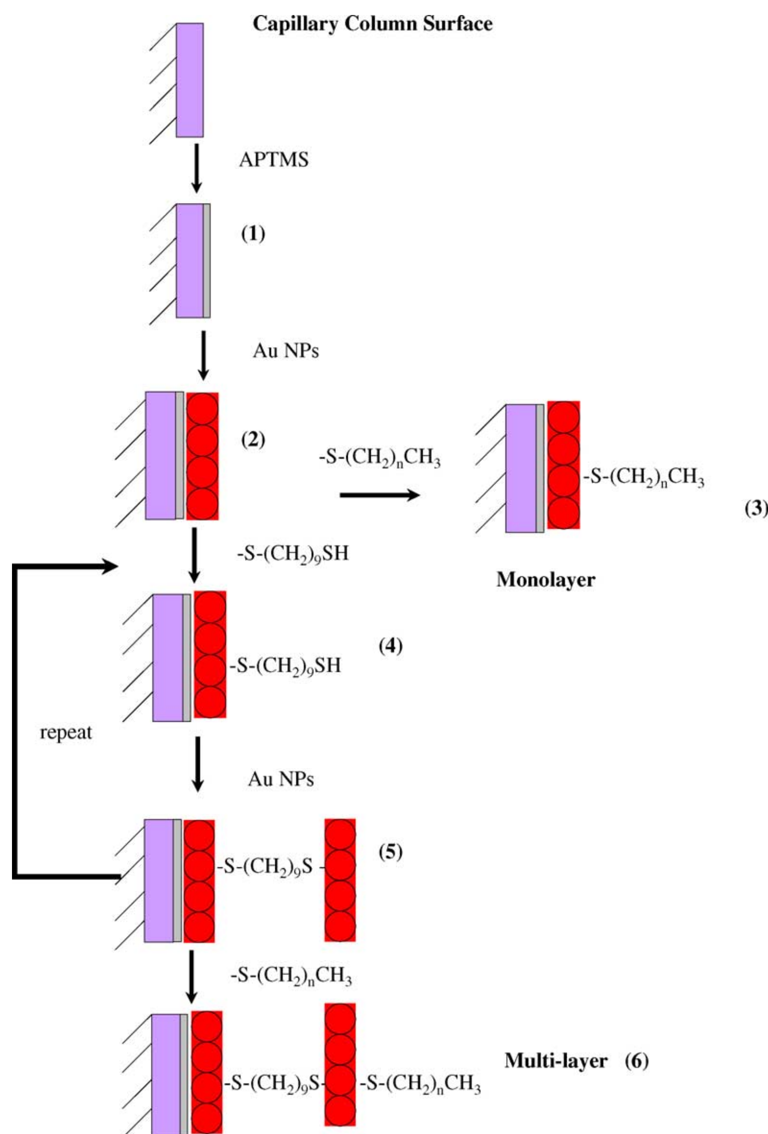


Fig. 2. Schematic representation of the process used to coat capillary columns with films of self-assembled alkanethiols on monolayers and multilayers of Au NPs.

2.4. Sample preparation

Stock solutions of the individual steroid drugs were prepared by dissolving each steroid (1 mg) in ACN (1 mL). The stock solution of the mixture of steroid drugs was prepared by sonicating the individual steroids for 10 min and then mixing an equal aliquot (100 μL) of each solution and diluting them with ACN until the concentration reached ca. 50 ppm. Prior to separation of the steroid drugs, an EOF marker (thiourea) was added into the mixed-steroid sample.

2.5. Homemade Au NPs

The method used for the synthesis of the Au NPs was similar to one described previously [34]. Briefly, 1 mM $HAuCl_4$ (100 mL) was brought to its boiling point (ca. 100 °C) under vigorous magnetic stirring and then 35 mM sodium citrate solution (10 mL) was injected. The resulting solution was heated under reflux for 30 min. The color of the solution changed from yellow to brownish-red upon the chemical reduction of $HAuCl_4$ mediated by citrate; this color change indicates the successful synthesis of the Au NPs. An SEM image (Fig. 1) indicates that the Au NPs obtained were spherical in shape and had estimated particle diameters of 17.8 ± 1.0 nm.

2.6. Preparation of CEC column

Fig. 2 presents the strategy employed to prepare capillary columns coated with monolayers and multilayers of Au NPs films. There are several major steps in these fabrication procedures. For the preparation of the capillary column coated with a monolayer of Au NPs: (1) A capillary column of desired length was placed in the CE instrument. The capillary column was etched at ambient temperature by pressure rinsing (1000 mbar) with 1 M NaOH for 1 h and then with de-ionized water for 10 min at 1000 mbar. Upon rinsing with de-ionized water again, the capillary column was placed in an oven and dried at 100 °C for 24 h to remove all moisture. A ca. 1% APTMS solution was prepared in ethanol. This solution was then pumped (1000 mbar) at ambient temperature through the dried capillary column for 1 h and then left to stand overnight. Finally, the capillary column was sealed and annealed at 100 °C in an oven for 24 h. (2) A solution of Au NPs was pumped (1000 mbar) at ambient temperature through the capillary column and then it was left to stand for 1 h. The excess Au NPs were then removed from the capillary column by pumping with de-ionized water. (3) The capillary column was flush with ethanol at 1000 mbar and then it was left to stand for 1 h. A solution of alkanethiol in hexane solution was pumped through the capillary column at ambient temperature at 1000 mbar and then left to stand for 1 h. Finally, the excess alkanethiol solution was removed from the capillary column by pumping with hexane and then flushing with ethanol. For the preparation of multilayer Au NPs films: (4) The Au NP-coated capillary columns obtained after preparation step (2) were flushed (1000 mbar for 1 h) at ambient temperature with solutions of 1,9-nonanedithiol, which behaved as a cross linker for constructing the multilayer Au NP films. The excess 1,9-nonanedithiol was removed from the capillary column by pumping with hexane and then flushing with ethanol. (5) Treatment with the Au NP solution at ambient temperature led to the adsorption of Au NPs on the 1,9-nonanedithiol layer. By repeating these steps, multilayers of Au NPs films were fabricated in a cyclic manner. (6) Finally, the Au NPs exposed on the capillary surface were subjected to self-assembly with an alkanethiol at ambient temperature. The excess alkanethiol was then removed from the capillary column by pumping with hexane and then flushing with ethanol.

3. Results and discussion

3.1. Preparation and characterization of Au NP films

To examine the feasibility of fabricating multilayers of Au NP films on a capillary column through LBL assembly, we used a method similar to that presented in Fig. 2 to fabricate multilayers of Au NP films on a glass microscope slide and monitored them by using a UV–vis spectrometer. For the deposition of Au NPs, we first applied APTMS onto the surface of the glass microscope slide. The silanol groups at the surface of the glass microscope slide were subjected to condensation reactions at 100 °C to anchor the APTMS moieties onto the surface through the formation of Si–O–Si bonds. After the surfaces of the glass microscope slide had been modified with APTMS units, the Au NPs were then applied onto the APTMS-coated slide through either the deposition of a monolayer of Au NPs or the multilayer assembly of Au NPs films.

Fig. 3 displays the UV–vis spectra of the first seven layers of Au NP films fabricated on a glass microscope slide using the monolayer and multilayer assembly methods. At a low coverage (one layer), a band due to the Mie resonance of the isolated Au NPs appears at ca. 520 nm [35]. Upon increasing the number of depositions, a new broad band appeared as a shoulder in the 610 nm region. The intensity of this new peak displayed an almost linear response to the number of layers. This phenomenon arose from the increase in the average thickness of a film: the average size of the islands increased while the distance between them decreased, causing a redshift of the absorption maximum [36,37]. This trend has been observed previously in the UV–vis spectra of Au NP films [38]. Therefore, our results confirmed the feasibility of fabricating multilayer Au NP films on glass microscope slides and suggested to us that it should also be possible to fabricate multilayer assemblies of Au NPs films within a capillary column.

We evaluated the properties of Au NP-deposited capillary columns. Fig. 4a and c display SEM images of a bare fused silica capillary and an Au NP-coated capillary, respectively.

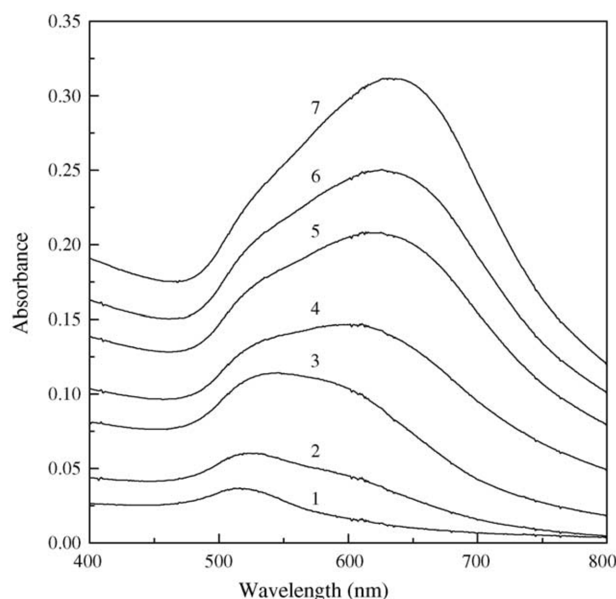


Fig. 3. UV–vis spectra of Au NPs films possessing different numbers of layers. The number associated with each spectrum represents the number of layers of Au NP films.

The smooth surface of the bare capillary is visible in Fig. 4a. We characterized the surface compositions of the products by using energy-dispersive X-ray analysis (EDS). Fig. 4b reveals that no Au signal appeared when we focused the electron beam on the bare capillary column. After coating with four layers of Au NP films, the inner surface of the coated capillary became roughened (Fig. 4c). Fig. 4d indicates that when we focused the electron beam on the column coated with this four-layer film, peaks associated with Au appeared at 9.6 and 11.3 keV. These results demonstrate that we had grown Au NPs successfully onto the capillary column through this self-assembly strategy.

3.2. The effect of Au NPs layers on the electrochromatographic separation

Fig. 5A displays the electrochromatograms obtained for a mixture of testosterone, progesterone, and testosterone propionate on a capillary column coated with a monolayer of Au NPs. The monolayer of Au NPs failed to separate these test mixtures of steroids when using a running electrolyte of 25% (v/v) ACN/75% (v/v) 10 mM Tris at pH 9.5. The reason for this poor separation behavior is probably the low loading density of the hydrophobic component on the surface of the capillary wall; the separation performance of a "reverse phase" system is affected primarily by the amount of material (e.g., hydrocarbon) bonded to the stationary phase [39]. We believed that we could increase the density of hydrophobic components on the capillary wall by incorporating 1,9-nonanedithiol, which possesses a nine-carbon-atom hydrocarbon skeleton, as a cross-linker for the construction of multilayer Au NP films through LBL assembly onto the capillary's inner surface. To verify this hypothesis, we fabricated monolayer, four-layer, and seven-layer Au NPs films on glass microscope slides—by using the method presented in Fig. 2—and then used TDS-APIMS to determine their surface carbon coverages (1.14, 1.77, and 2.56 mmol/m^2, respectively). The trend of increased carbon loading upon increasing the number of Au NP layers is consistent with our expectation. Thus, we next investigated whether the number of Au NP films introduced into a capillary in this manner would display separation selectivity as a function of film thickness.

As indicated in Fig. 5B, we obtained reasonably good electrochromatographic performance when separating the three steroid drugs using a capillary column coated with two layers of Au NPs; the three steroids passed through the capillary within 10 min and were separated at an acceptable resolution. The resolution (R_S) is expressed as

$$R_S = \frac{2(t_{R2} - t_{R1})}{(W_1 + W_2)} \quad (1)$$

where t_{R1} and t_{R2} represent the retention times of the two adjacent peaks, and W_1 and W_2 represent the respective base widths of these peaks. This result confirms that the presence of 1,9-nonanedithiol within the multilayer Au NP films on the capillary surface does have an extreme effect on enhancing the separation resolution and the retention factors for these steroid drugs. Under identical reaction conditions, but with an increase in the number of Au NPs layers, Fig. 5C and D reveal that the retention times of steroid drugs increased even further.

Table 1 summarizes the effect that the number of layers has on the separation of the steroids. According to Table 1, we failed to separate the steroid drugs when using a Au NP monolayer, but with two layers of Au NPs the mixture of steroid drugs began to resolve. Although the resolutions for the mixture of steroid drugs were almost always improved upon increasing the number of Au NPs layers (from one to seven), Fig. 5D (seven layers) indicates that we obtained a decrease in the peak heights of the steroid drugs, and poorer detectability, when the number of Au NPs layers was greater than four. The number of theoretical plates (N) equation is expressed as:

$$N = 16\left(\frac{t_R}{W}\right)^2 \quad (2)$$

where t_R represent the retention time of the peak, and W represents the base width of the peak. The number of theoretical plates for testosterone decreased from 168 100 plates/m (at four Au NP layers) to 22 500 plates/m (at seven Au NP layers). Therefore, the separation efficiency was relatively poorer for layer numbers above four. Based on the results presented in Fig. 5 and Table 1, the use of 1,9-nonanedithiol for the construction of multilayer Au NP films using LBL assembly plays a crucial role in determining the separation resolution of steroid drugs. A four-layer film provides a satisfactory separation and, therefore, we employed such a system in our subsequent studies.

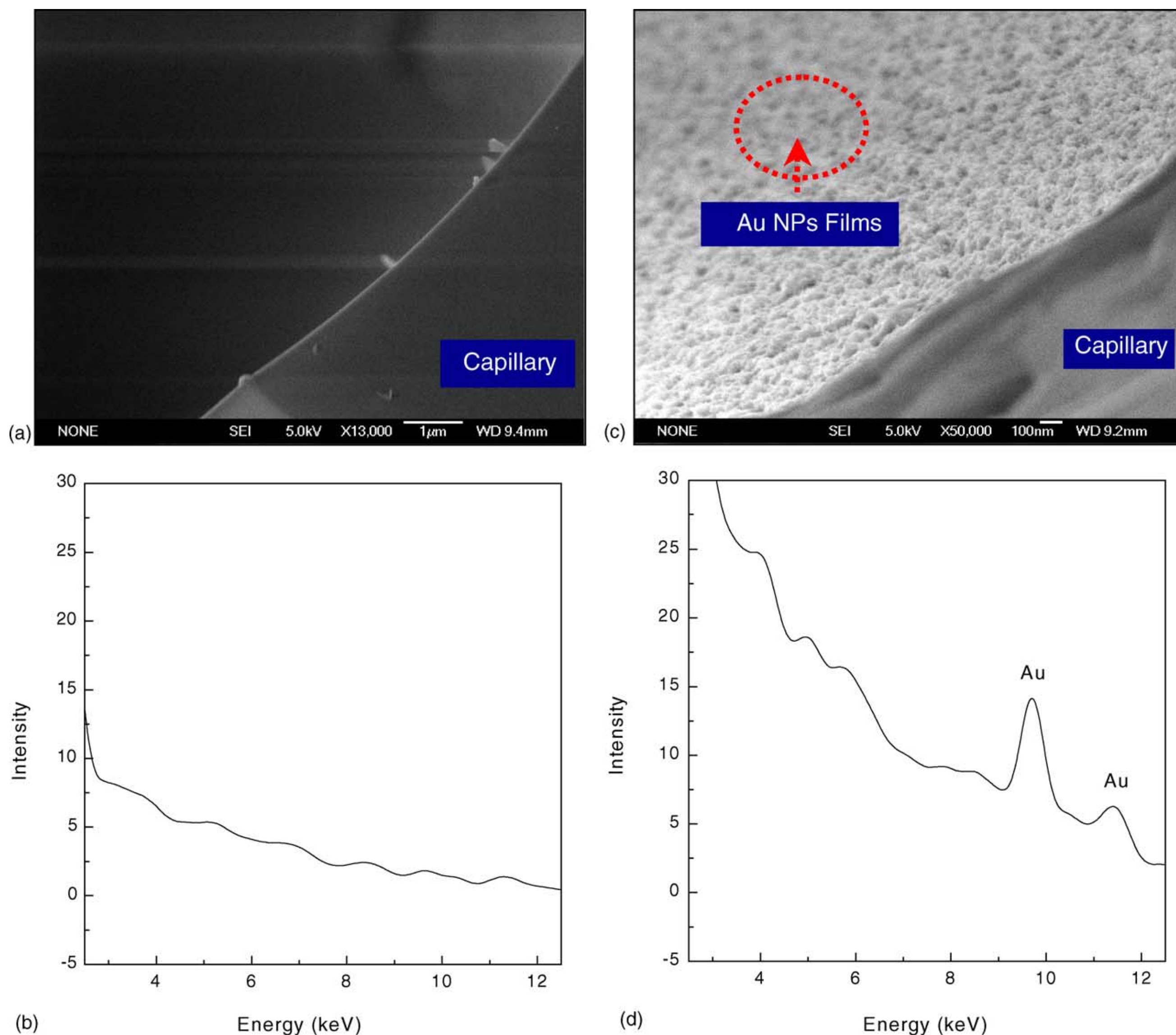


Fig. 4. (a) SEM image of a bare fused-silica capillary, i.e., in the absence of Au NPs film. (b) EDS spectrum of the sample displayed in (a). (c) SEM image of a capillary coated with a four-layer Au NP film. (d) EDS spectrum of the sample displayed in (c).

3.3. The effect that the alkanethiol's chain length has on the electrochromatographic separation

We self-assembled alkanethiols of four chain lengths—1-hexanethiol (C_6), 1-octanethiol (C_8), 1-dodecanethiol (C_{12}), and 1-octadecanethiol (C_{18})—onto capillary columns coated with four-layer Au NP films to observe the effect that the chain length has on the efficiency of separating steroid drugs. We investigated the separations using 25% (v/v) ACN/75% (v/v) 10 mM Tris at pH 9.5 under operating conditions of 25 °C and 25 kV.

Fig. 6A reveals that we achieved the fastest separation of the steroid drugs when using C_6 (i.e., the lowest number of carbon atoms), but the separation resolution was poor. The retention times of each of these neutral solutes increased as the length of the alkyl chain increased. This phenomenon is akin to the trend observed in reverse-phase liquid chromatography [42]. Although the retention times increased, the separation abilities toward steroid drugs of C_8 (Fig. 6B) and C_{12} (Fig. 6C) self-assembled on the Au NP layer structures were similar to that of the C_6 phase. The extremely short retention times obtained when using these phases suggest that they underwent quite weak molecular interactions with the steroid drugs. Overall, when comparing the electropherograms in Fig. 6, we conclude that C_{18} self-assembled on the four-layer Au NP structures provided the best efficiency and reasonable resolution for separating the steroid drugs and, therefore, we employed this phase for our subsequent studies.

The plate height (H) in a capillary tube is determined by the Golay equation (ignoring mass transfer in the mobile phase) [40]:

Table 1
Effects that the number of layers of Au NPs self-assembled on the inner surface of capillary columns have on the retention factor (k'), resolution (R_S), selectivity (α), and number of theoretical plates (N, plates/m) of the steroid drugs

Sample	One layer	Two layers	Four layers	Seven layers
Testosterone				
k'	0	0.023	0.034	0.056
R_S	0	2.45	2.57	1.90
N	7400	300 000	168 100	22 500
Progesterone				
k'	0	0.102	0.159	0.310
$\alpha_{\text{Progesterone/testosterone}}$	0	4.43	4.68	5.54
R_S	0	3.94	4.11	5.57
N	7400	34 700	29 100	15 100
Testosterone propionate				
k'	0	0.467	0.651	1.034
$\alpha_{\text{Testosterone propionate/progesterone}}$	0	4.57	4.09	3.34
R_S	0	6.77	7.40	8.38
N	7400	9600	6700	7900

Running electrolyte: 25% (v/v) ACN/75% (v/v) 10 mM Tris, pH 9.5; UV detection: 247 nm. Separations were performed at 25 kV. Injections were performed throughout at a pressure of 30 mbar for 0.03 min.

$$H = \frac{B}{u} + Cu \tag{3}$$

where u is the velocity of the mobile phase. The term B describes the longitudinal diffusion contribution, resulting from random motion of the solute molecules in the mobile and stationary phases [41]. It is related to the hindrance to the diffusion brought about by column packing, the diffusion factor (γ), and solute diffusion in the mobile phase (D_M):

$$B = 2\gamma D_M \tag{4}$$

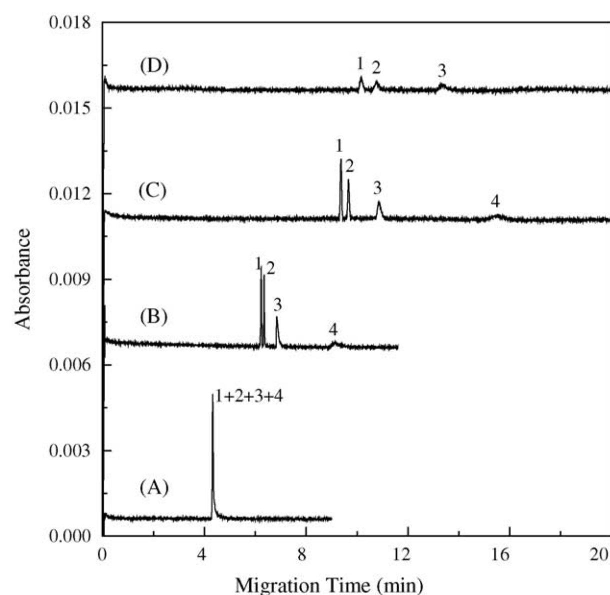


Fig. 5. Electrochromatograms for the CEC separation of the test mixture of steroid drugs when using capillary columns possessing different numbers of Au NP layers. Running electrolyte: 25% (v/v) ACN/75% (v/v) 10 mM Tris, pH 9.5; detection wavelength: 247 nm; sample injection: 30 mbar 0.03 min. (A) One layer; (B) two layers; (C) four layers and (D) seven layers. Samples: (1), EOF; (2), testosterone; (3), progesterone and (4), testosterone propionate.

The term C describes the resistance to the mass transfer occurring at the solute–stationary phase interface. It is dependent on the diffusion coefficient of the solute in the stationary phase (D_S) and the effective film thickness of the stationary phase on the capillary column ($d_{\text{Thickness}}$).

$$C = \frac{(d_{\text{Thickness}})^2}{D_S} \tag{5}$$

According the Eq. (5), thinner stationary phase films produce higher mass transfer rates, but there is an accompanying decrease in the solute retention factor of the column [41]. As indicated in Figs. 5 and 6 and Table 1, the retention fac-

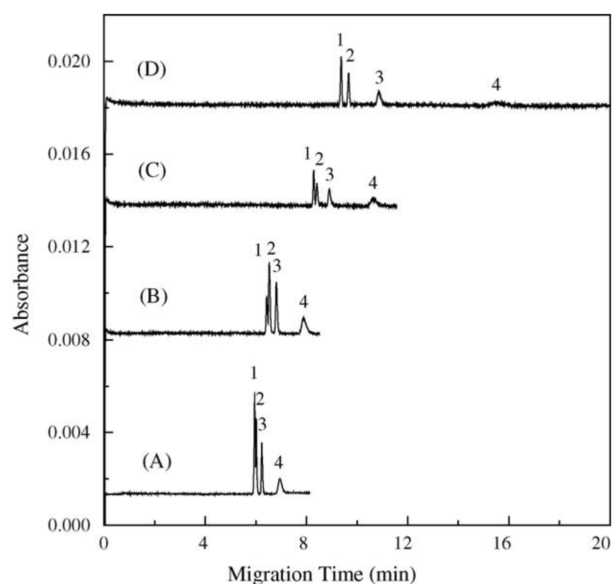


Fig. 6. Electrochromatograms displaying the effect that the length of the carbon chain has on the separation of steroid drugs. Conditions are the same as those described in Fig. 5 but the number of Au NP layers was four. (A) C_6; (B) C_8; (C) C_{12} and (D) C_{18}. Samples: (1), EOF; (2), testosterone; (3), progesterone and (4), testosterone propionate.

tors of the steroid compounds increased upon increasing the thickness of the Au NP stationary phase. The phenomena described in Sections 3.2 and 3.3 are consistent with the *C* term of the Golay equation.

3.4. The effect of ACN on the electrochromatographic separation

In this study, we selected ACN as the organic constituent in the running buffer because of its superior ability to enhance EOF during CEC [43], its UV transparency, and its ability to solubilize the hydrophobic steroid drugs in the running electrolyte. We investigated the effect of ACN on the separation of steroid drugs by using the 1-octadecanethiol-modified capillary column coated with a four-layer Au NP film and adding various fractions of ACN (25–40%, v/v) to the running buffer, while maintaining an aqueous buffer solution of 10 mM Tris at pH 9.5 and operating conditions of 25 °C and 25 kV.

As is apparent in Fig. 7A, the Au NP-coated capillary column was unable to separate the steroid drugs when 40% (v/v) ACN was present in the running electrolyte, but the separation performance increased at 30% ACN (Fig. 7B). These results imply that a partition effect occurred in the separation system because the percentage of ACN in the running electrolyte had an obvious influence on the retention behavior. We obtained good separation when using the organic modifier at contents from 25 to 28%. In this operating range, the resolution of testosterone increased (from 0.84 to 2.57) upon decreasing the percentage of organic modifier (from 28 to 25%). Although the migration times of the steroid drugs became much longer upon decreasing the ACN percentage in the running electrolyte even further (<25%), the peak heights decreased and the peak widths increased. According to these results, it is clear that the elution of these steroid drugs is controlled by a reverse-phase mechanism. Overall, a 25% (v/v) fraction of ACN provided the best trade-off between resolution and analysis time.

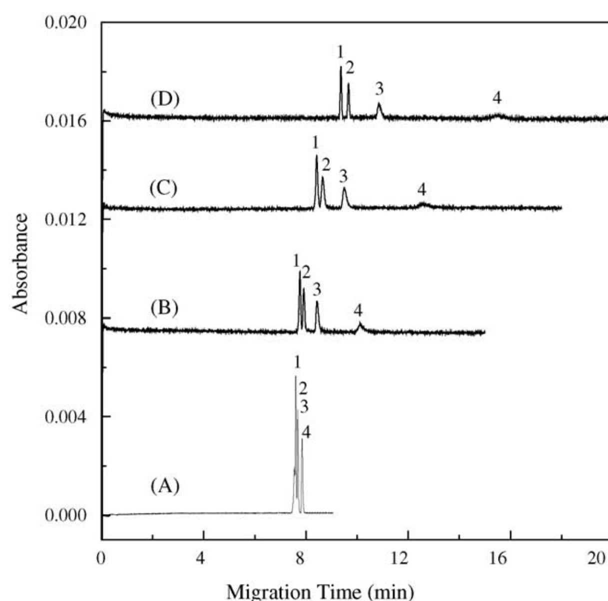
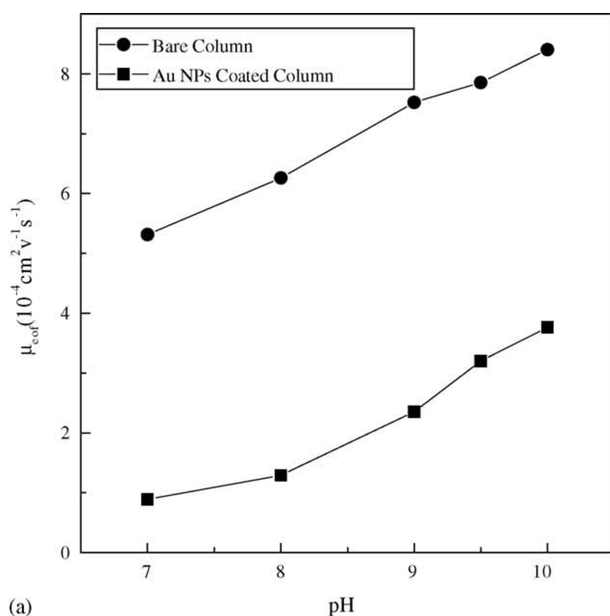


Fig. 7. Electrochromatograms displaying the effect that the volume fraction of ACN has on the separation of the test steroid drugs. The volume fraction [%, v/v] of ACN in the running electrolyte was varied. Other conditions are the same as those described in Fig. 6. (A) 40%; (B) 30%; (C) 28% and (D) 25% ACN. Samples: (1), EOF; (2), testosterone; (3), progesterone; (4), testosterone propionate.



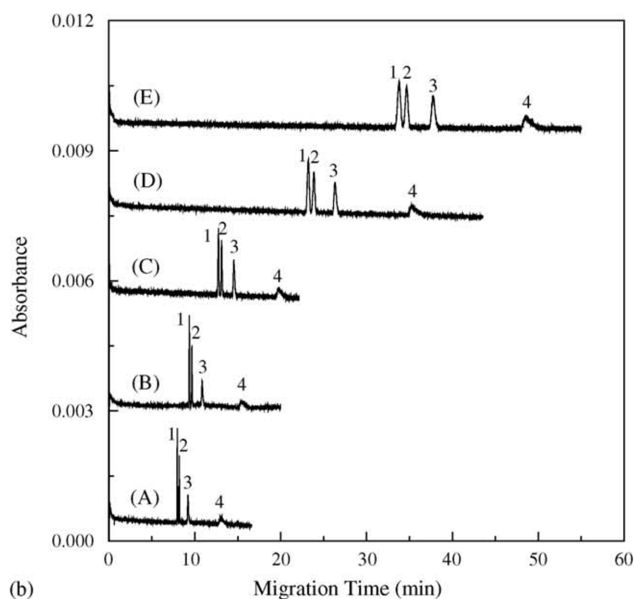


Fig. 8. (a) Effect of pH on the EOF mobility displayed by a bare capillary and a four-layer Au NP-coated capillary. (b) Electrochromatograms displaying the effect that pH has on the separation of steroid drugs. Conditions are the same as those described in Fig. 7 except that the pH was varied. (A) pH 10.0; (B) pH 9.5; (C) pH 9.0; (D) pH 8.0 and (E) pH 7.0. Samples: (1), EOF; (2), testosterone; (3), progesterone (4), testosterone propionate.

3.5. The effect of pH on the electrochromatographic separation

EOF is a wall-generated phenomenon that plays a crucial role in CEC separations [44]. The zeta potential of the electrical double layer on the inner surface is determined directly by its surface charge density. Because EOF is one of the most important parameters directly connected to successful separation, it is necessary to optimize the pH of the running electrolyte.

We have already demonstrated that 25% (v/v) ACN in 10 mM Tris buffer under our standard operating conditions provides a good separation of the steroid drugs and, thus, we used these optimized conditions in the following experiment. We investigated the effect that the pH has on the EOF and separation of steroid drugs by using the 1-octadecanethiol-modified capillary column coated with a four-layer Au NP film and varying the pH of the running electrolyte from 7.0 to 10.0. As indicated in Fig. 8a, the inner surface of the separation channel is negatively charged when using 25% (v/v) ACN/75% (v/v) 10 mM Tris as the running electrolyte at pH 7.0–10.0 because the EOF moves toward the cathode. In addition, the EOF in the CEC channel is slower than that in the bared fused-silica tube, which suggests that a much lower surface charge density exists in the CEC column than that of a bare capillary column. It is very likely that the bonding of APTMS moieties to the channel surface is mainly responsible for the reduction in EOF because some of the potential-determining SiO^- ions on the wall are neutralized by the presence of APTMS. The residual unblocked surface ions of capillary column will result in an EOF, but one that has a much slower speed than that of a bare fused-silica capillary column. In a bare capillary column, more of the SiOH groups become deprotonated when the pH of the running electrolyte increases, which results in increased charge density on the inner wall and, therefore, a dramatic increase in the EOF.

The effect that the separation efficiency of the steroid drugs has on the running electrolyte can be observed in Fig. 8b. When the running electrolyte was used at pH 7.0, the retention time of each steroid drug was very large, which resulted in some loss of chromatographic efficiency. In contrast, increasing the pH of the running electrolyte increased the rates of migration of the test steroid drugs and the EOF marker (thiourea) because of the enhanced EOF flow. According to the results presented in Fig. 8b, the use of the running electrolyte at pH 9.5 provided the best trade-off between resolution and analysis time.

3.6. The chemical stability of the stationary phase with respect to the organic solvent and pH

We tested the stability of the Au NP stationary phase at ambient temperature by passing organic solvent (ACN) or solutions at various values of pH through a column at 1000 mbar for at least 4 h. Prior to each test, we conditioned the column for 30 min using a running electrolyte of 25% (v/v) ACN and 75% (v/v) 10 mM Tris (pH 9.5). Fig. 9 illustrates the stability of the Au NP stationary phase. Fig. 9a reveals that the retention factor of the tested solutes did not change significantly after performing separations for several days; i.e., this stationary phase has the ability to withstand washing with an organic solvent. Likewise, Fig. 9b reveals that the Au NP stationary phase is stable at values of operating pH ranging from 3 to 11 without any noticeable effect on the retention factor of tested solutes. Based on these results, it appears that this stationary phase is very stable toward both organic solvent and extreme values of pH.

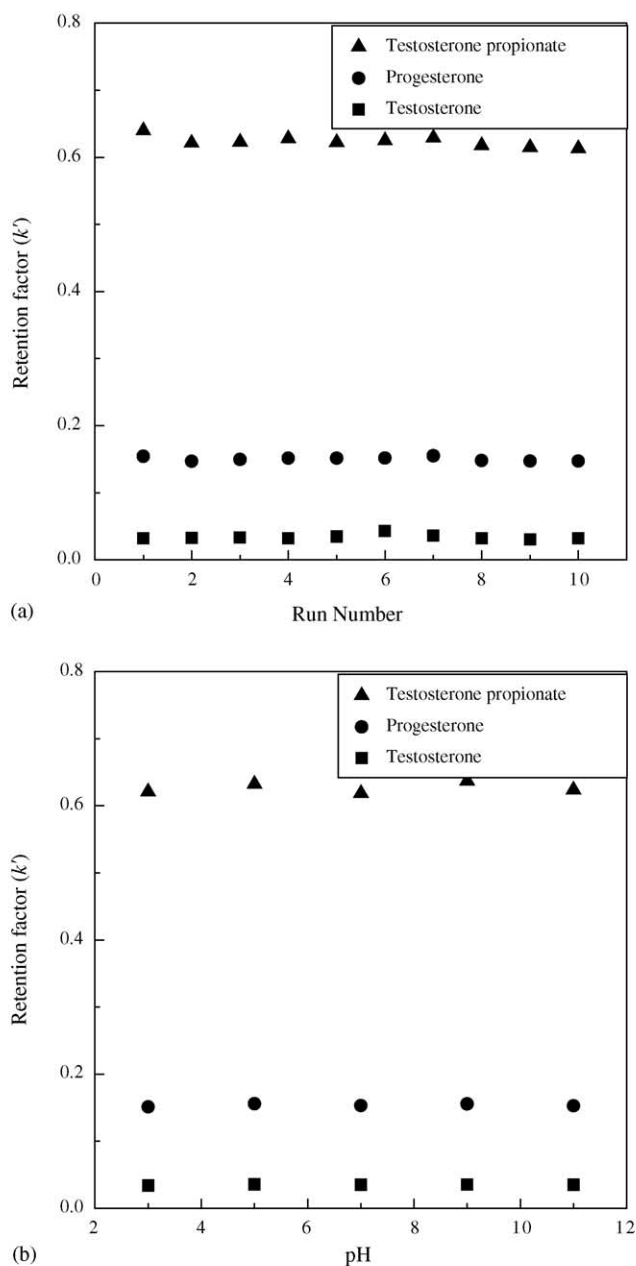


Fig. 9. Stability of the retention factors of steroids on a four-layer Au NP-coated capillary after passing through it, for at least 4 h, (a) ACN and (b) solutions having various values of pH. Separation conditions were the same as those described in Fig. 5.

3.7. The reproducibility of chromatographic retention

If this method is to be used for the routine characterization of steroid drugs, it is necessary to validate the reproducibility of these separations. For this purpose, we evaluated the relative standard deviation of elution times for the steroid drugs over 10 consecutives runs using the 1-octadecanethiol-modified four-layer Au NP-coated capillary column and a running electrolyte of 25% (v/v) ACN/75% (v/v) 10 mM Tris at pH 9.5. The Au NP-coated capillary column exhibits quite reproducible migration times for the test solutes, with values of RSD of 2.5, 2.4, and 2.4% for testosterone, progesterone, and testosterone propionate, respectively. These capillaries appeared to be stable for up to one month when not in use and stored in ACN. In addition, the separation performance did not deteriorate over 100 injections, which indicates that the capillary columns coated with alkanethiols self-assembled on multilayer Au NP films are fairly stable.

4. Conclusions

NPs currently have numerous commercial and technological applications, e.g., in electronic, optical and mechanical devices. As interest in NPs increases because of their unique physical and chemical properties, relative to their bulk materials, so too will the applications of these materials in analytical chemistry. In this study, we confirmed that 1-octadecanethiol self-assembled on Au NP layers provides a novel phase for CEC to be used to separate neutral pharmaceutical steroid drugs. We found that the film thickness on the capillary column surface (through changing the number of Au NP layers and the length of the carbon atom chain of the alkanethiol self-assembled on the Au NPs) plays a crucial role in the separation of these steroid drugs. In addition, our results demonstrate that this system exhibits reproducible retention times and characteristic reverse-phase behavior. Because capillary columns coated with films of 1-octadecanethiol self-assembled on multilayer Au NPs are easy to produce, we believe that this concept will contribute to further studies of the use of nanotechnology in separation science.

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References

[1] M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, Anal. Chem. 73 (2001) 5116.
[2] E. Guihen, J.D. Glennon, J. Chromatogr. A 1044 (2004) 67.
[3] M. Pursch, L.C. Sander, J. Chromatogr. A 887 (2000) 313.
[4] M.J. Xie, Y.Q. Feng, S.L. Da, D.Y. Meng, L.W. Ren, Anal. Chim. Acta 428 (2001) 255.
[5] K. Jinno, H. Sawado, Trends Anal. Chem. 19 (2000) 664.
[6] Z. Liu, H.F. Zou, J.Y. Ni, Y.K. Zhang, Anal. Chim. Acta 378 (1999) 73.
[7] Z. Liu, H.F. Zou, M.L. Ye, J.Y. Ni, Y.K. Zhang, Electrophoresis 20 (1999) 2891.
[8] Z. Liu, K. Otsuka, S. Terabe, J. Sep. Sci. 24 (2001) 17.
[9] S. Mayer, V. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[10] S. Mayer, V. Schurig, J. Liq. Chromatogr. 16 (1993) 915.
[11] D.W. Armstrong, Y.B. Tang, T. Ward, M. Nichols, Anal. Chem. 65 (1993) 1114.
[12] H. Hofstetter, O. Hofstetter, V. Schurig, J. Microcol. Sep. 10 (1998) 287.
[13] O. Bruggemann, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.
[14] Z.X.J. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.
[15] Y. Guo, L.A. Colon, Anal. Chem. 67 (1995) 2511.
[16] P. Narang, L.A. Colon, J. Chromatogr. A 773 (1997) 65.
[17] J.T. Wu, P.Q. Huang, M.X. Li, M.G. Qian, D.M. Lubman, Anal. Chem. 69 (1997) 320.
[18] Z. Liu, K. Otsuka, S. Terabe, J. Chromatogr. A 961 (2002) 285.
[19] C.A. Mirkin, R.L. Letsinger, R.C. Mucic, J.J. Storhoff, Nature 382 (1996) 607.
[20] A. Taleb, C. Petit, M.P. Pileni, Chem. Mater. 9 (1997) 950.
[21] S. Peschel, G. Schmid, Angew. Chem. Int. Ed. Eng. 34 (1995) 1442.
[22] J.H. Fendler, F.C. Meldrum, Adv. Mater. 7 (1995) 607.
[23] A. Ulman, An Introduction to Ultrathin Organic Films, Academic Press, Boston, MA, 1991.
[24] J. Wang, T. Zhu, J.Q. Song, Z.F. Liu, Thin Solid Films 327 (1998) 591.
[25] A.N. Shipway, E. Katz, I. Willner, Chem. Phys. Chem. 1 (2000) 18.
[26] F.K. Liu, Y.C. Chang, F.H. Ko, T.C. Chu, B.T. Dai, Microelectron. Eng. 67–68 (2003) 702.
[27] T.B. Dubrovsky, Z.Z. Hou, P. Stroeve, N.L. Abbott, Anal. Chem. 71 (1999) 327.
[28] M. Mrksich, J.R. Grunwell, G.M. Whitesides, J. Am. Chem. Soc. 117 (1995) 12009.
[29] H.Z. Huang, X.R. Yang, Colloids Surf. A 226 (2003) 77.
[30] F. Caruso, H. Mohwald, J. Am. Chem. Soc. 121 (1999) 6039.
[31] F.K. Liu, G.T. Wei, F.C. Cheng, J. Chin. Chem. Soc. 50 (2003) 931.
[32] T. O'Mahony, V.P. Owens, J.P. Murrihy, E. Guihen, J.D. Holmes, J.D. Glennon, J. Chromatogr. A 1004 (2003) 181.
[33] M. Brust, C.J. Kiely, Colloids Surf. A 202 (2002) 175.
[34] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Anal. Chem. 67 (1995) 735.
[35] U. Kreibig, M. Vollmer, Optical Properties of Metal Clusters, Springer–Verlag, Berlin, 1995.
[36] T.R. Jensen, M.D. Malinsky, C.L. Haynes, R.P. Van Duyne, J. Phys. Chem. B 104 (2000) 10549.
[37] J. Schmitt, P. Machtle, D. Eck, H. Mohwald, C.A. Helm, Langmuir 15 (1999) 3256.
[38] C.Y. Jiang, S. Markutsya, V.V. Tsukruk, Langmuir 20 (2004) 882.
[39] D. Norton, S.A. Shamsi, J. Chromatogr. A 1008 (2003) 217.
[40] R.L. Grob, Modern Practice of Gas Chromatography, Wiley, New York, 1995.
[41] A. Braithwaite, F.J. Smith, Chromatographic Methods, Chapman and Hall, New York, 1985.
[42] L.C. Sander, S.A. Wise, in: K. Jinno (Ed.), Chromatographic Separations Based on Molecular Recognition, Wiley, New York, 1997.
[43] M.G. Cikalo, K.D. Bartle, P. Myers, J. Chromatogr. A 836 (1999) 35.
[44] K.D. Bartle, P. Myers, J. Chromatogr. A 916 (2001) 3.