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# Alkylthiol gold nanoparticles in open-tubular capillary electrochromatography

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**Abstract**

Open-tubular columns for capillary electrochromatography (CEC) were formed by immobilising dodecanethiol gold nanoparticles on prederivatised 3-aminopropyl-trimethoxysilane (APTMS) or 3-mercaptopropyl-trimethoxysilane (MPTMS) fused-silica capillaries. The initial stage of this research involved the synthesis and characterisation of dodecanethiol gold nanoparticles, with tunnelling electron microscopy analysis of the dispersed phase of the gold nanoparticles dispersion in $CHCl_3$ revealing spherical particles. The surface features of an Au-MPTMS coated capillary column were determined using scanning electron microscopy. The electroosmotic flow characteristics of Au-APTMS and Au-MPTMS capillary columns were then determined, by varying the pH and the voltage. The electrochromatographic properties of the gold nanoparticles CEC capillaries were investigated using a ''reversed-phase'' test mixture of thiourea, benzophenone and biphenyl and selected pyrethroid pesticides. Efficient separations of benzophenone and biphenyl solutes on Au-MPTMS and Au-APTMS capillary columns were obtained, as were linear plots of logarithm capacity factor versus % MeOH. A study of the reproducibility of retention for these solutes on Au-APTMS, Au-MPTMS and on a loosely coated capillary demonstrated the necessity of a coupling agent to prevent the gold nanoparticles from washing-off. These dodecanethiol gold capillary columns are easier to produce and operate than packed capillary columns. The research work confirms the use of gold nanoparticles as a novel phase for open-tubular CEC, demonstrating reproducible retention and characteristic reversed-phase behaviour.


*Keywords:* Electrochromatography; Stationary phases, electrochromatography; Alkylthiol gold nanoparticles; Alkyltriethoxysilanes

## 1. Introduction

In recent years, there has been increasing interest in template-directed synthesis of nano-particulate micro/nano structures. However, it is difficult to fabricate a monolayer of regular nanoparticles in a given region. Chemical interaction forces can be utilised for the fabrication of self-assembled monolayers; for example, organic molecules containing thiol groups (–SH) can be spontaneously adsorbed on gold surfaces to form well-organised self-assembled monolayers [1]. In 1983, Nuzzo and Allara first reported the self-assembly of dialkyldisulfides on gold surfaces [2]. Since then, much work has been carried out on the self-assembly of alkanethiols on gold surfaces through S–Au covalent bonding [1]. In recent years, the reverse process has been achieved through specific interaction between gold nanoparti-

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cles and surface terminating groups such as –SH, $–NH_2$ and –CN, leading to well defined, stable two-dimensional arrays of gold nanoparticles [3].

Extension of these principles to a wider variety of surface functional and solid substrates is the subject of current research. Zhu et al. [4] created a pyridyl-terminated organic surface by self-assembling *p*-mercaptopyridine (p-Mpy) onto a sputtered gold substrate, onto which gold nanoparticulate films were formed.

Although alkyltriethoxysilanes have been used to form monolayers bearing various functional groups ($–NH_2$, –SH), the structures of these monolayers also depend strongly on the method of deposition and the reaction time [5].

### *1.1. Development and uses of nanoparticles in biological assays*

Silica gel is widely used as a stationary phase in analytical chemistry and biotechnology. However, the limitations of such phases in protein-based assays has been realised for some time. As the surface of silica is formed from silanol (Si–OH) ($pK_a > 6.25$) and siloxane (Si–O–Si) groups, the former are deprotonated under physiological conditions, with the surface of the silica gel presenting a net negative charge. It is the electrostatic interactions between the surface-bound charges and proteins that are the principal reason for non-specific adsorption of proteins onto silica. Most protein-based uses of silica therefore require treatment to eliminate the presence of these charges or suppress their influence on proteins (high ionic strength).

Thickness of films formed from 3-aminopropyl-triethoxysilane (APTMS) on the surface of silica are reported to range from 50 to 100 Å, depending on the reaction time, thus suggesting that APTMS forms multilayers under the reported conditions. Recently Park et al. [6] demonstrated that the rate of formation of the first monolayer with 16 nm gold nanoparticles is faster than that of the multilayers, whereas for larger nanoparticles the multilayer formation rate is much faster. Park et al. [6] also demonstrated that the adsorption kinetic behaviour of gold colloid of different sizes on a 3-mercaptopropyl-trimethoxysilane (MPTMS) modified surface would closely resemble that on an APTMS surface.

In medicine, methods are required for depositing highly biocompatible and bioactive surface coatings that can, for example, promote and stabilise cell attachment. These coatings are made by first depositing thin films of materials, such as metals, including gold and platinum [7]. These surfaces are further altered to either promote or inhibit cell growth by an additional over-coat of biological materials. The biospecific adsorption of proteins at inert interfaces that present appropriate ligands, is important for anchorage-dependent cell culture, screening of combinatorial libraries and biosensing [8].

Self-assembled monolayers (SAMs) formed from ω-substituted alkanethiols on the surface of gold have been used as model surfaces in a number of past studies on the interaction of proteins with surfaces [9,10]. Prime and Whitesides [11,12] reported a study of non-specific adsorption of fibrinogen, lysozyme, pyruvate kinase and RNAse to mixed SAMs. The results demonstrate that SAMs formed on gold can be used to influence non-specific interactions between proteins and surfaces of gold.

While the applicability of SAMs to protein and DNA assay has received intense focus, especially in bioscience, the emergence of such phases in LC or electrochromatography has been limited to-date. In electrochromatography questions such as column stability, flow-rate generation and inherent column properties have to be examined before protein analysis is considered. Just recently, Neiman et al. [13] have shown that the use of gold nanoparticles in the run buffer in capillary electrophoresis modifies the electroosmotic mobility and can affect significantly apparent mobilities, resulting in selectivity alterations. Pumera et al., in conjunction with the same author, have also recently extended the use of gold-based nanoparticles to chip-based capillary electrophoresis devices for selectivity modification [14].

In this paper, the synthesis of dodecanethiol gold nanoparticles and the subsequent immobilisation of the nanoparticles on prederivatised fused-silica capillaries are detailed. Scanning electron microscopy (SEM) images are shown, characterising the surface features of the wall coated open tubular capillaries. A study of the electroosmotic properties of the columns is also reported through variation of the pH and voltage. The electrochromatographic properties of the gold capillaries were also investigated using a

''reversed-phase'' test mixture and using selected pyrethroid pesticides.

## 2. Materials and methods

### 2.1. Materials

HPLC-grade methanol, chloroform and propan-2-ol were purchased from Labscan (Dublin, Ireland). HPLC-grade hexane, toluene, ethanol and benzene were purchased from Aldrich (Poole, UK). The buffers were prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate, obtained from Sigma–Aldrich (Poole, UK). The pH was adjusted to the desired value using either HCl or NaOH from E. Merck, Darmstadt, Germany. Thiourea, gold trichloride, tetraoctylammonium bromide, 1-dodecanethiol and sodium borohydride were obtained from Aldrich (Steinheim, Germany). Both benzophenone and biphenyl were purchased from BDH (Poole, UK). (*R*,*S*)-Fenpropathrin (98.5%) and (*R*,*S*)-fenvalerate (99.9%) were purchased from Riedel-de Haën (Seelze, Germany). (*R*,*S*)-Fluvalinate (77.8%) was kindly donated by Novartis (Basle, Switzerland). APTMS and MPTMS were obtained from Aldrich (Steinheim, Germany). All water used was Milli-Q grade with a resistivity of 18.2 mΩ cm. Disposable plastic syringes were purchased from Becton Dickinson (Dublin, Ireland). Microtight tubing sleeves, microtight unions, adapters and peek tubing, were also purchased from Sigma–Aldrich. Aqueous filters membranes (0.45 μm) were purchased from Millipore (Cork, Ireland)

### 2.2. Instrumentation

All capillary electrochromatography (CEC) separations were obtained using a Beckman P/ACE MDQ instrument (Beckman, Fullerton, CA, USA). The system comprised a 0–30-kV high-voltage power supply, a diode array detector, and the P/ACE software (version 1.6) for system control and data processing. Fused silica capillary of varying diameters (50 μm, 30 and 20 μm I.D.) having a length of 30 cm, 21 cm to detector, were obtained from Composite Metals (Worcester, UK). The temperature was controlled at 25 °C using a fluorocarbon-based cooling system. Both normal and reverse polarities were employed depending on the capillary and the working pH value used.

SEM was carried out in the National Microelectronics Research Centre (Cork, Ireland) using a Hitachi S4000 SEM microscope. Tunnelling electron microscopy (TEM) was performed using a JEM 1200 EX TEMSCAN located in the Plant Science Department, U.C.C.

### 2.3. Procedure for SEM photographs

Cleanly cut sections (1–4 mm) of Au-MPTMS coated capillary were initially placed on a dirt-free sample holder and sputtered with gold for 20 min. The sputtered sample was then placed in the microscope's vacuum chamber, where an electron gun emitted a beam of high-energy electrons onto a defined area, leading to three-dimensional high magnification images of the inner capillary wall. Magnification values from 10 kV×6.00 K to 20 kV×50.0 K were used to depict the surface topography.

### 2.4. Mobile phase and sample preparation

Test mixtures containing benzophenone and biphenyl were made up in methanol–water (70:30) (0.1 g $l^{-1}$ of each component) with thiourea (3 m*M*) used as the dead volume marker. Fenpropathrin, fenvalerate and fluvalinate were dissolved in 100% methanol. All eluents were filtered using an aqueous 0.45 μm Millipore filter membrane and sonicated (ULTRAsonik NEY) for 10 min to remove dissolved air prior to use. Depending on the pH value required (3.1, 4.5, 7.0 and 8.7), phosphate buffer solution (0.025 *M*) was prepared by dissolving either 3.0 g of sodium dihydrogen phosphate or 3.55 g of disodium hydrogen phosphate in 1 l deionised water and the pH values recorded (Expandable IonAnalyser pH meter EA 920).

### 2.5. Synthesis of gold nanoparticles

Dodecanethiol gold nanoparticles were synthesised according to the following procedure: $AuCl_3$ (0.1212 g) was dissolved in 13 ml of distilled water. A phase transfer catalyst tetraoctylammonium bromide

(TOAB) (0.9645 g) was dissolved in 8.8 ml of $CHCl_3$. The resulting $AuCl_3$ and TOAB solutions were added together and stirred for 1 h at room temperature. This mixture was placed in a 25-ml separating funnel and the $CHCl_3$ layer collected. Dodecanethiol (86 μl) was then added to the stirring $CHCl_3$ solution and stirred further for 5 min. $NaBH_4$ (0.1786 g) was then dissolved in 11 ml of distilled water. This aqueous solution was added to the organic solution and the mixture allowed to stir overnight in a 25-ml separating funnel. The resulting $CHCl_3$ phase was then collected and a polar solvent (EtOH) added. The solution was then centrifuged (MSE MISTRAL 1000) at 4500 rpm for 30 min. Centrifuging the $CHCl_3$ phase in the presence of a polar solvent resulted in the precipitation of the dodecanethiol gold nanoparticles from the liquid phase. The resulting supernatant was then removed from the centrifuge tube and the nanoparticles reconstituted in hexane, toluene or $CHCl_3$.

TEM of the gold particles was used to determine the average nanoparticle size. A single drop of the dodecanethiol gold nanoparticles suspended in $CHCl_3$ was placed on a Carbon Film 400 Mesh copper grid (Agar Scientific) and positioned in the TEM instrument (JEM 1200 EX TEMSCAN). The results obtained showed that the dispersed phase of the gold nanoparticles in $CHCl_3$ consisted of spherical particles (1–2 nm size range).

### 2.6. Open-tubular capillary column preparation

Two major steps are involved in the preparation of capillary columns. The first entailed the derivatisation of the inner capillary wall with an organosilane-coupling agent by using a modified version of a previously published method for the preparation of such on a glass slide by Park et al. [6]. The second step involved the immobilisation of the gold nanoparticles to the coupling agent.

### 2.7. Coating procedure

A 30-cm length of capillary (50 μm, 30 μm, 20 μm I.D.) was placed in the CEC instrument. To expose the maximum number of surface silanol groups on the inner walls of the fused-silica capillary, the capillary was first etched by pressure rinsing (20 p.s.i.) with 1 *M* NaOH for 30 min, followed with deionised water for 10 min at 10 p.s.i., and then with 0.1 *M* HCl for 30 min at 20 p.s.i. (p.s.i. = 6894.76 Pa). Upon rinsing with water again, the capillary was placed in an oven (Gilson 831 Temperature Regulator) and dried at 100 °C for 1 h to remove all moisture. An APTMS (or MPTMS) solution was prepared by adding 2 ml APTMS (or MPTMS) to 25 ml of propan-2-ol. This solution was then pumped with a plastic syringe through the dried capillary for 1 h and allowed to stand overnight. The modified capillary was then rinsed with propan-2-ol and annealed at 110 °C in the oven for 10 min.

A solution of dodecanethiol gold nanoparticles in hexane was pumped through the capillary with a hand-held plastic syringe and allowed to stand for 1 h. The excess gold solution was then removed from the capillary by pumping with a water-filled syringe and stored in water until use. A schematic diagram of the processes involved in the formation of the metal film preparation is illustrated in Fig. 1.

The term ''loosely coated'' refers to capillary columns formed from the passing of gold particles through a NaOH/HCl treated capillary that was not derivatised with APTMS or MPTMS.

### 2.8. Preparation of the detection window

A small (2–3 mm) section of the outer polyimide coating of the capillary was burned off using an InnovaTech capillary frit former (InnovaTech, Hertfordshire, UK). The capillaries were cut to have a length (inlet to outlet) of 30 cm with an effective length (inlet to detection window) of 21 cm. Even though it was not possible to prevent the window area from being coated, the detectability was not adversely affected.

### 2.9. Electrophoretic conditions

Capillaries coated with either APTMS- or MPTMS-gold nanoparticles were conditioned in the CEC instrument by pressure rinsing at 10 p.s.i. (forward direction) with the running electrolytes for 15 min prior to use. If the pH of the running buffer was changed, the capillaries were pressure rinsed with the new running buffer for at least 30 min before commencing new runs. The capillaries were

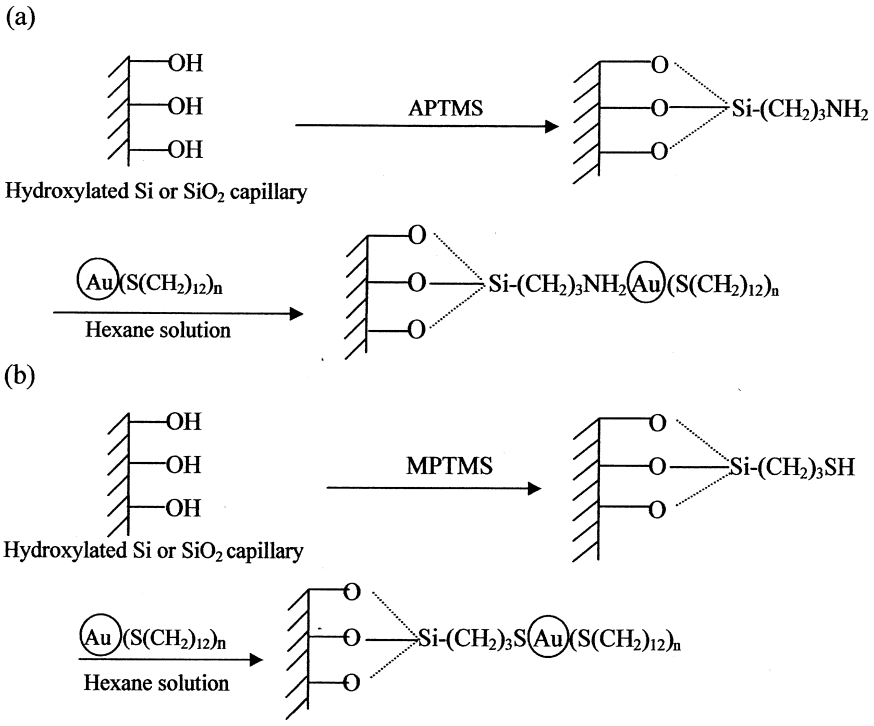


Fig. 1. Schematic diagram for the reaction steps involved between alkylthiol gold nanoparticles and fused-silica capillary prederivatised with (a) APTMS and (b) MPTMS.

rinsed for 2 min between runs with the running electrolyte, and were stored in water when not in use. Samples were injected by pressure (0.5 p.s.i. for 4 s).

Separations were performed at 25 kV with a ramp time (time taken for voltage to rise from 0 to 25 kV) of 0.17 s unless stated otherwise. Normal polarity conditions (electroosmotic flow towards the cathode) were used for work involving MPTMS derivatised capillaries over all pH ranges and for APTMS coated capillaries above pH 6.3. However, at pH values $<$6.3 for APTMS coated capillaries, reverse polarity was employed.

## 3. Results and discussion

The surface features of the Au-MPTMS coated capillary are shown in the SEM photographs in Fig. 2. Fig. 2a shows a TEM micrograph of the synthesised spherical dodecanethiol gold nanoparticles, alongside SEM images of a blank capillary (10 kV$\times$6.0 K magnification) and a gold nanoparticle coated capillary (20.0 kV$\times$5.0 K magnification) in Fig. 2b and d. Greater magnification (20 kV$\times$50.0 K) of the gold nanoparticle coated surface is given in Fig. 2f, with the surface features resembling a series of hills or mounds of varying depth, width and shape, relative to that of an untreated capillary wall.

### *3.1. Effect of pH and applied field strength on electroosmotic flow (EOF)*

With regard to APTMS/MPTMS derivatised capillaries, the presence of amino/thiol groups on the capillary wall surface can be demonstrated by observing the EOF as a function of pH. Since the time taken for a solute to migrate through a capillary under an applied voltage is given by Eq. (1), the EOF velocity is easily determined using an unretained dead volume marker in CEC.

$$t = L/\nu_{eo} \quad (1)$$

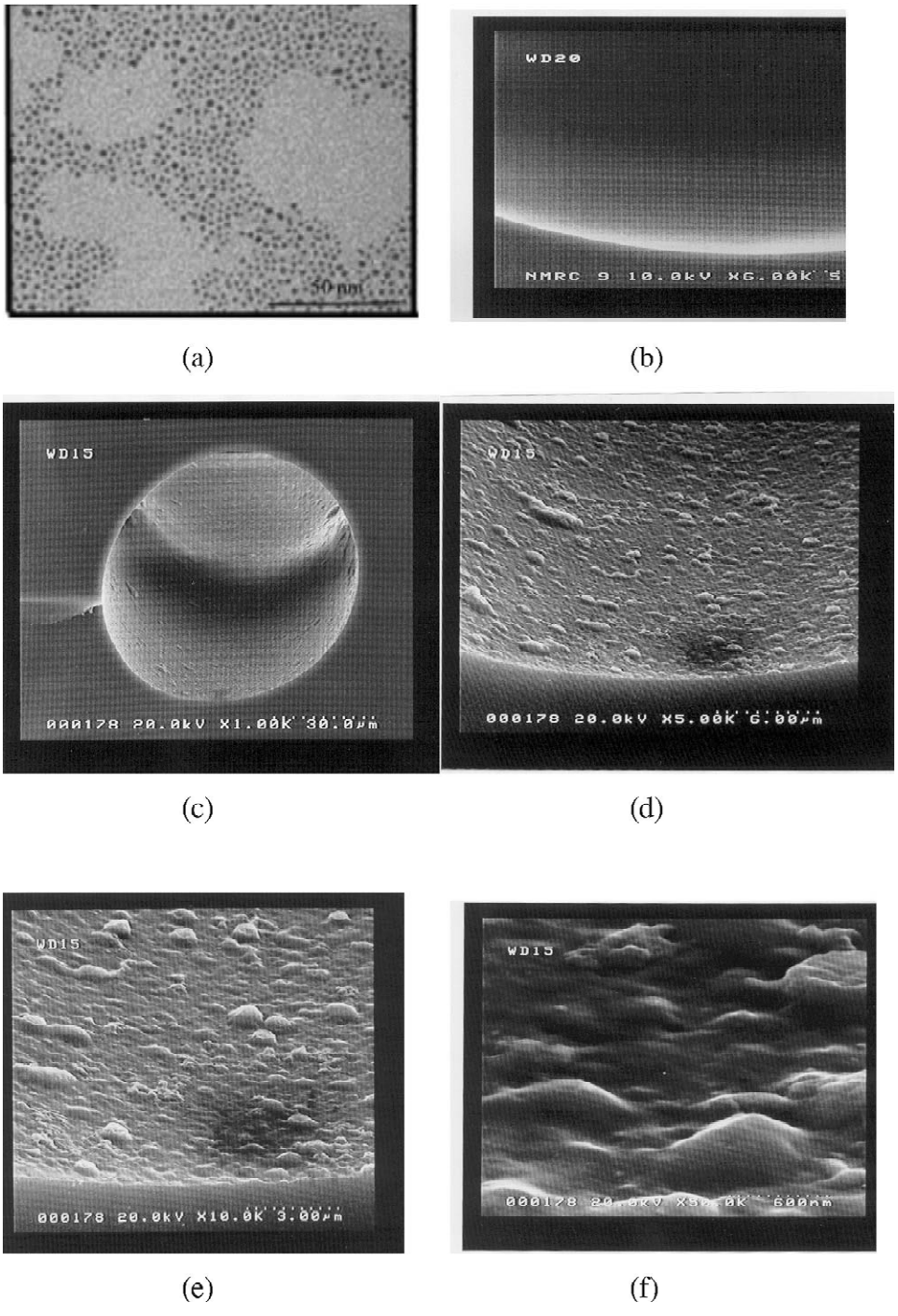


Fig. 2. (a) TEM micrograph of dodecanethiol gold nanoparticles, on a carbon film 400 mesh copper grid and (b) SEM of blank capillary at 10.0 kV×6.0 K magnification. The SEM images in (c) at 20.0 kV×1.0 K, (d) at 20.0 kV×5.0 K, (e) at 20.0 kV×10.0 K and (f) at 20 kV×50.0 K are photographs of the fused-silica capillary pretreated with MPTMS and coated with gold nanoparticles.

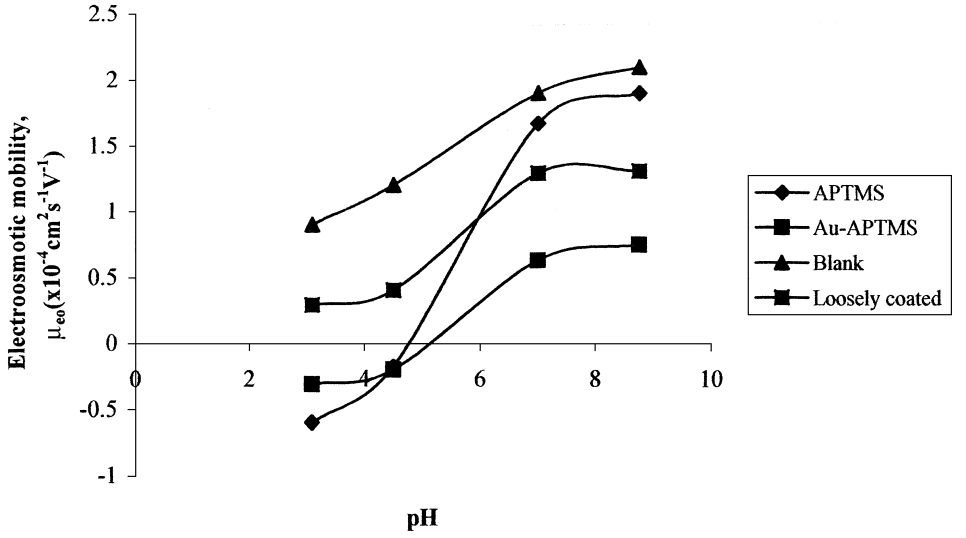


Fig. 3. The effect of pH on the electroosmotic flow mobility of thiourea in APTMS, Au-APTMS, blank and loosely coated capillary. Conditions: 30 cm×30 μm I.D., MeOH–25 m*M* sodium phosphate (45:55). Sample: thiourea (3 m*M* in methanol–water 70:30). Injection: 0.5 p.s.i. for 4 s.

where $t$ = time (s), $L$ = effective length of capillary (cm) and $\nu_{eo}$ = electroosmotic velocity (cm $s^{-1}$)

Assuming that there is no electrical double layer overlap, the EOF mobility can be expressed as

$$\mu_{eo} = \nu_{eo}/E \quad (2)$$

where $E$ = applied electric field strength (V/cm).

It was initially thought that thiourea might be retained on gold-coated capillaries through interaction of the thio moiety of the marker with the gold particle (i.e., adsorption). Acetone was therefore tested as an EOF marker to observe if thiourea was being retained. Under identical conditions both markers eluted at approximately the same time, 4.39

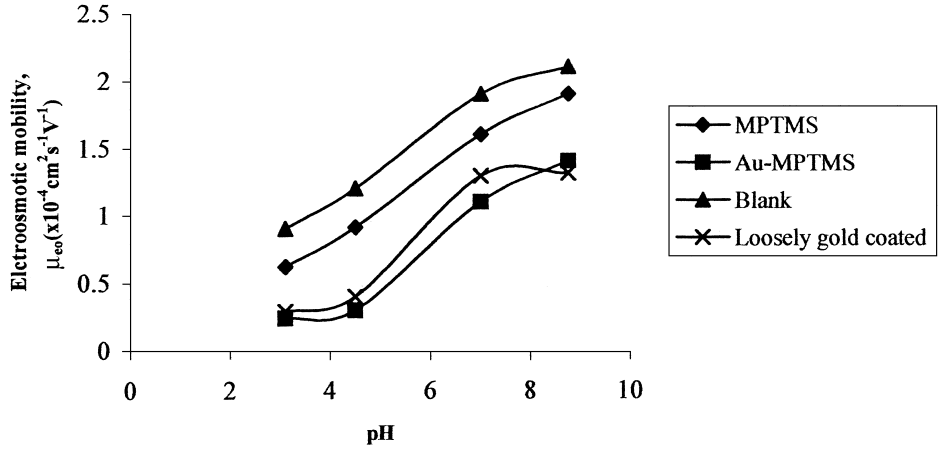


Fig. 4. The effect of pH on the electroosmotic flow mobility of thiourea in MPTMS, Au-MPTMS, blank and loosely coated capillary. Conditions: 30 cm×30 μm I.D., MeOH–25 m*M* sodium phosphate (45:55). Sample: thiourea (3 m*M* in methanol–water 70:30). Injection: 0.5 p.s.i. for 4 s.

min for thiourea and 4.40 min for acetone, confirming thiourea as an acceptable EOF marker in gold-coated capillaries (conditions: 30 cm×30 μm I.D. Au-APTMS capillary column, MeOH–25 m*M* $NaH_2PO_4$ pH 3.1 (30:70), UV detection 270 nm, voltage: −25 kV. Injection: 0.5 p.s.i. for 4 s)

Electrochromatograms of thiourea as the EOF marker were also recorded over the pH range 3–9 on Au-APTMS and Au-MPTMS capillary columns. Figs. 3 and 4 depict the electroosmotic mobility obtained with 25 m*M* sodium phosphate buffer over the pH range from 3 to 9, for both APTMS and MPTMS functionalised capillaries.

In Fig. 3, the blank fused-silica capillary displays a characteristic profile for pH variation, with the maximum EOF occurring at high pH. At low pH values (<6.25), protonation of the silanol groups increases with a corresponding reduction in EOF. However, for APTMS derivatised capillaries, it is apparent that both the magnitude and direction of the EOF change with the pH of the running electrolyte. The p$K_a$ of propylamino groups is ~10.7 [15]. Thus at pH values <8.7, all the amino groups are fully protonated. However, the EOF's direction is still towards the cathode i.e., dominated by the deprotonated silanol groups of the capillary wall. Reducing the pH, as expected, leads to EOF suppression. As the pH decreases further (<5.0) and the silanol groups are protonated, the coating of the wall bears a net positive charge due to the surface amino groups, resulting in an electroosmotic flow from the cathode to the anode. The Au-APTMS coated capillary displays similar electroosmotic behaviour over the pH range, but with reduced electroosmotic mobility due to possible shielding of the residual silanol and amino groups by the gold particles. As the loosely coated capillary is not prederivatised with APTMS (or MPTMS), its EOF is generated by the silanol groups on the silica wall. Hence, it displays a mobility profile with pH variation similar to that of the blank capillary. Again, the magnitude of its electroosmotic mobility is reduced due to probable shielding of the silanol groups by the gold nanoparticles.

In Fig. 4, the MPTMS coated capillaries display a similar mobility profile to that of an untreated capillary. As the p$K_a$ of a thiol group is ~10.6 [14], they do not contribute to the EOF's behaviour, hence the flow is dominated by the residual silanol groups. As expected the $\mu_{eo}$ values of MPTMS coated capillaries are lower than that of an untreated capillary due to silanol blocking. Similarly, the Au-MPTMS displays an EOF profile that is greatly suppressed relative to MPTMS.

Fig. 5 shows plots of the EOF velocity versus the applied electric field strength, when using 45% methanol aqueous mobile phase containing 25 m*M* phosphate buffer at pH 7.0 and thiourea as the EOF marker. The electroosmotic flow increased linearly with an increase in applied electric field strength, as predicted from Eq. (2), for all derivatised capillaries over the range of 166–1000 V/cm. The differing

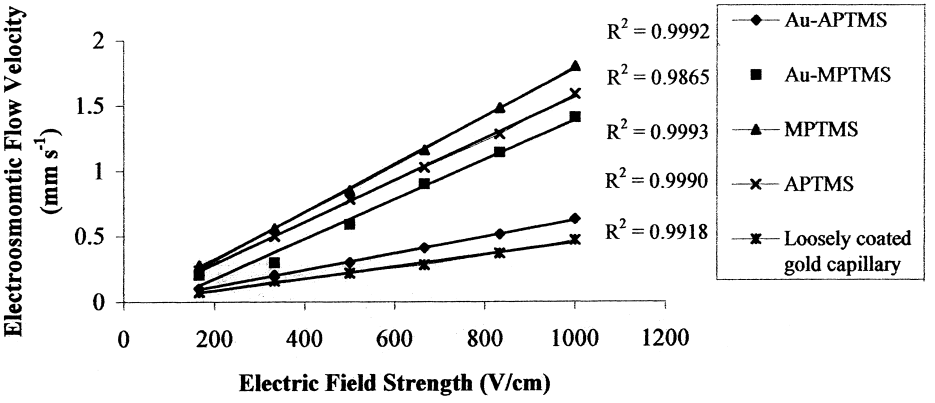


Fig. 5. The effect of applied electric field strength on the electroosmotic flow velocity in various capillaries for thiourea EOF marker. Conditions: 30 cm×30 μm I.D., MeOH–25 m*M* $Na_2HPO_4$ pH 7.0 (45:55). Sample: thiourea (3 m*M* in methanol–water, 70:30). Injection: 0.5 p.s.i. for 4 s.

slopes obtained for the coated capillaries are related to the percentage of residual silanol groups (after derivatisation) that generate the EOF and, in the case of the gold coated columns, the extent of surface coverage by the gold nanoparticles. The loosely coated capillary displays the smallest increase in electroosmotic velocity with electric field strength. In contrast, greatest electroosmotic velocity with electric field variation is achieved for the MPTMS capillary.

### *3.2. Effect of organic modifier on chromatographic retention*

Fig. 6 displays the chromatograms obtained for a test-mixture, containing thiourea, benzophenone and

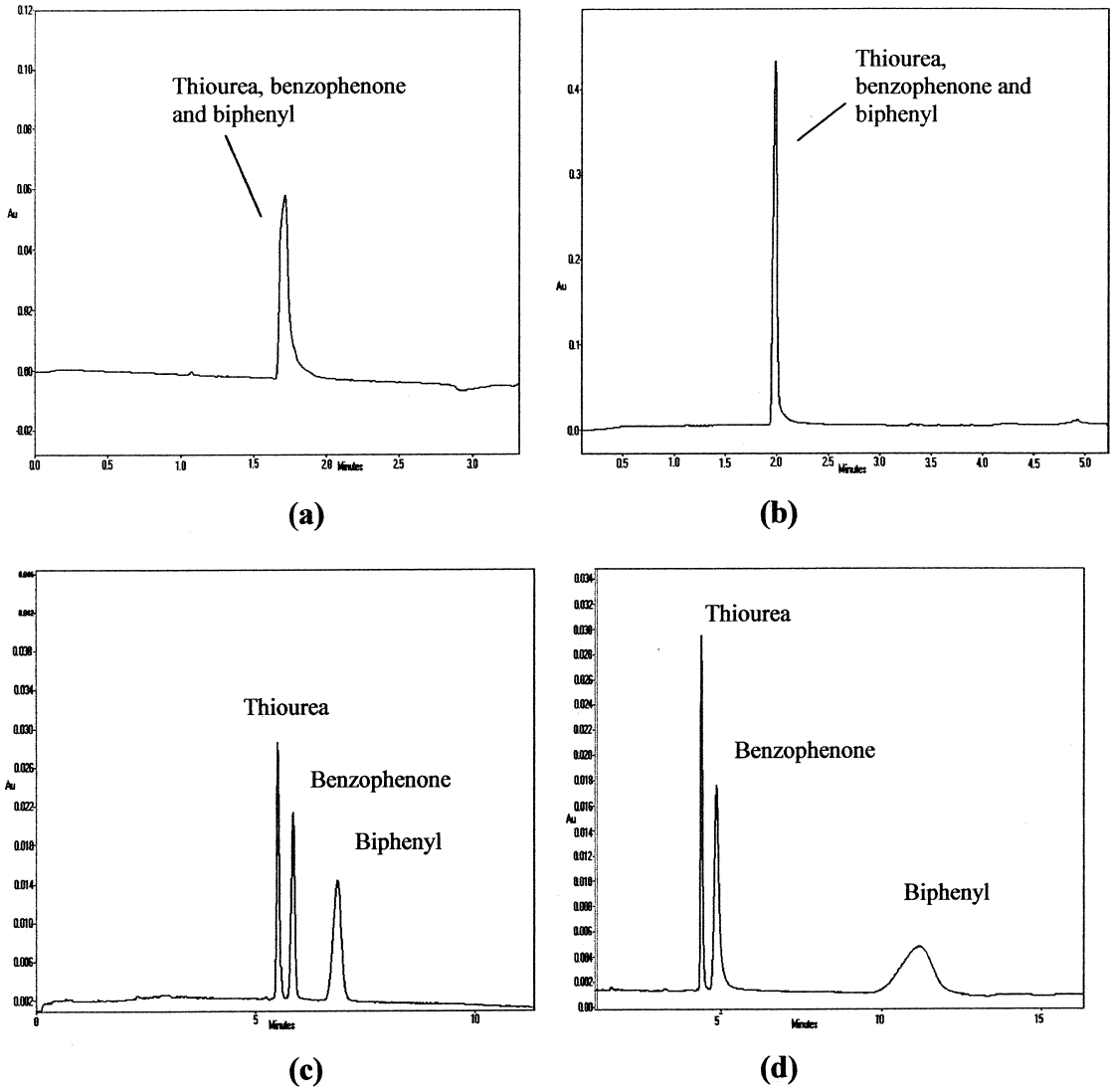


Fig. 6. Electrochromatograms representing test-mixture separations on various capillaries: (a) MPTMS coated capillary, 45% MeOH, (b) APTMS coated capillary, 45% MeOH, (c) Au-MPTMS coated capillary, 70% MeOH and (d) Au-APTMS coated capillary, 35% MeOH. Conditions: capillaries 30 cm×30 μm I.D.; mobile phase MeOH–25 m*M* $Na_2HPO_4$ pH 7.0; voltage 30 kV. Sample: thiourea, benzophenone and biphenyl in methanol–water (70:30). Injection: 0.5 p.s.i. for 4 s.

biphenyl, on APTMS/MPTMS- and Au-APTMS/Au-MPTMS-coated capillaries. APTMS and MPTMS functionalised capillaries failed to separate the test solutes at 45% MeOH, as shown in Fig. 6a and b. However, upon coating with dodecanethiol gold nanoparticles, the resulting Au-APTMS and Au-MPTMS capillaries proved capable of separating test solutes, even at higher organic modifier percentages. Theoretical plates per metre for the solutes increased with decreasing internal diameter capillaries from 13 000 and 6 374 plates/m for benzophenone and biphenyl on a 50-μm I.D. capillary to 81 407 and 20 960, respectively, on a 30-μm I.D. capillary (Fig. 6c).

Plots of the logarithm of the capacity factor $k'$, for benzophenone and biphenyl, versus the methanol content on Au-MPTMS, Au-APTMS and Au-loosely coated capillaries were constructed and exhibited a linear relationship, with negative slopes, typifying reversed-phase behaviour. The regression coefficients, $r^2$, were 0.9997 and 0.9998 for Au-MPTMS, 0.9954 and 0.9972 for Au-APTMS, and 0.9854 and 0.9801 for Au-loosely coated columns, for biphenyl and benzophenone respectively (conditions: 30 cm × 30 μm I.D., 25 m*M* aqueous $Na_2HPO_4$ pH 8.7). However, under identical conditions, the Au-MPTMS coated capillary yielded greater log $k'$ values than the corresponding Au-APMTS column, indicating greater hydrophobicity in Au-MPTMS derivatised capillaries.

### 3.3. Reproducibility of chromatographic retention

Figs. 7–9 display the run-to-run reproducibility of capacity factor $k'$ for benzophenone and biphenyl compounds on Au-MPTMS, Au-APTMS and Au-loosely coated capillaries, using 55% methanol aqueous mobile phase with 5 m*M* phosphate buffer at pH 7.0. In a short study, both Au-MPTMS and Au-APTMS coated capillaries demonstrate reproducible $k'$ values for the test solutes, with an RSD of $<4\%$ for 15 consecutive runs under the same conditions (Table 1). These capillaries appeared to be stable for up to 2 months when not in use and stored in water. Even with extensive washing/rinsing of these narrow bore capillaries when in use, the separation performance did not deteriorate for 35–40 injections. However, the Au-loosely coated capillary suffers

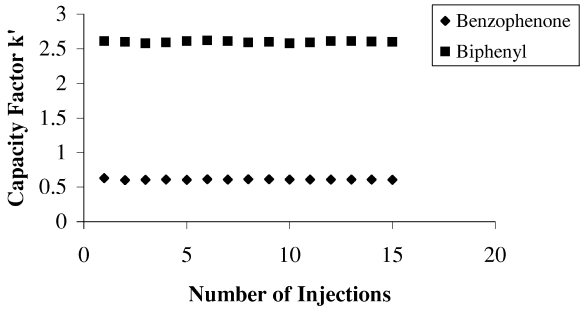


Fig. 7. Capacity factor measured as a function of number of consecutive injections for Au-MPTMS coated capillary. Conditions: 30 cm × 30 μm I.D., MeOH–5 m*M* $Na_2HPO_4$ pH 7.0 (60:40), voltage: 30 kV.

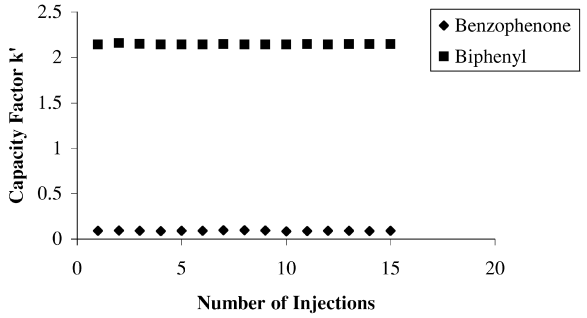


Fig. 8. Capacity factor measured as a function of number of consecutive injections for Au-APTMS coated capillary. Conditions: 30 cm × 30 μm I.D., MeOH–5 m*M* $Na_2HPO_4$ pH 7.0 (45:55), and voltage: 30 kV.

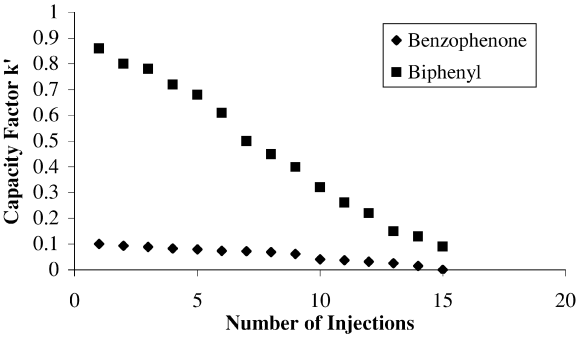


Fig. 9. Capacity factor, $k'$, measured as a function of the number of consecutive injections for Au-loosely coated capillary. Conditions: 30 cm × 30 μm I.D., MeOH–5 m*M* $Na_2HPO_4$ pH 7.0 (45:55), and voltage: 30 kV.

Table 1
The mean $k'$ value for 15 successive separations of benzophenone and biphenyl on Au-MPTMS, Au-APTMS and Au-loosely coated capillaries

| Compound / Column | Mean run-to-run $k'$ (RSD, %) | | |
|---|---|---|---|
| | Au-MPTMS | Au-MPTMS | Au-loosely coated |
| Benzophenone | 0.61 (1.0) | 0.09 (3.4) | 0.045 (342) |
| Biphenyl | 2.6 (0.5) | 2.14 (0.2) | 0.377 (357) |

RSD is in parenthesis.

rapid deterioration due to probable washing-off of the gold particles from the column in the absence of a coupling agent. This results in irreproducible retention values, with RSD values of 342 and 357% for benzophenone and biphenyl respectively. Fig. 10 displays the three chromatograms obtained after 1, 9 and 15 injections on the loosely coated capillary. The retention time for biphenyl rapidly decreases from 3.9 min for $n = 1$, to 2.9 min for $n = 9$, to 2.3 min for $n = 15$ (where $n$ = the number of injections). Thus, reproducible chromatography with capillaries coated with gold nanoparticles requires the use of coupling agents to form stable phase coatings with long column life.

### 3.4. Application to pyrethroid pesticides analysis

Three pyrethroid pesticides were injected in an attempt to separate them on a 20-μm I.D. Au-APTMS capillary. The pesticides, fenpropathrin, fenvalerate and fluvalinate, were analysed with organic modifier varied from 60 to 75% methanol. Fig. 11 shows a chromatogram of the separation of fenpropathrin and fenvalerate at 75% methanol with

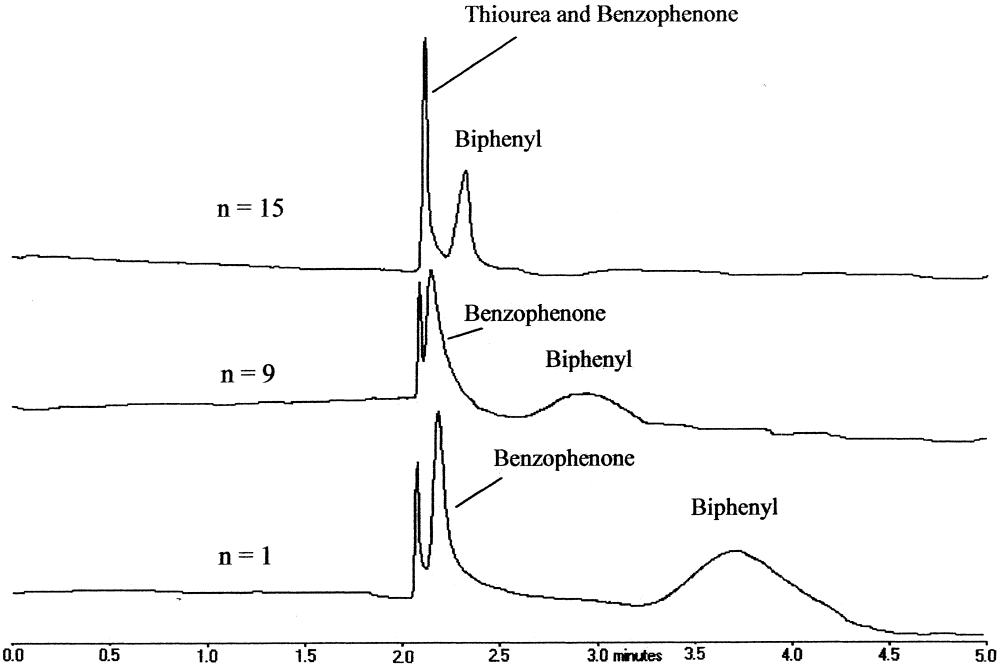


Fig. 10. Comparison of chromatograms obtained for Au-loosely coated capillary after 1, 9 and 15 injections. Conditions: Au-loosely coated 30 cm × 30 μm I.D., mobile phase: MeOH–5 m*M* $Na_2HPO_4$ pH 7.0 (45:55), voltage: 30 kV. Sample: thiourea, benzophenone and biphenyl in methanol–water (70:30). Injection: 0.5 p.s.i. for 4 s.

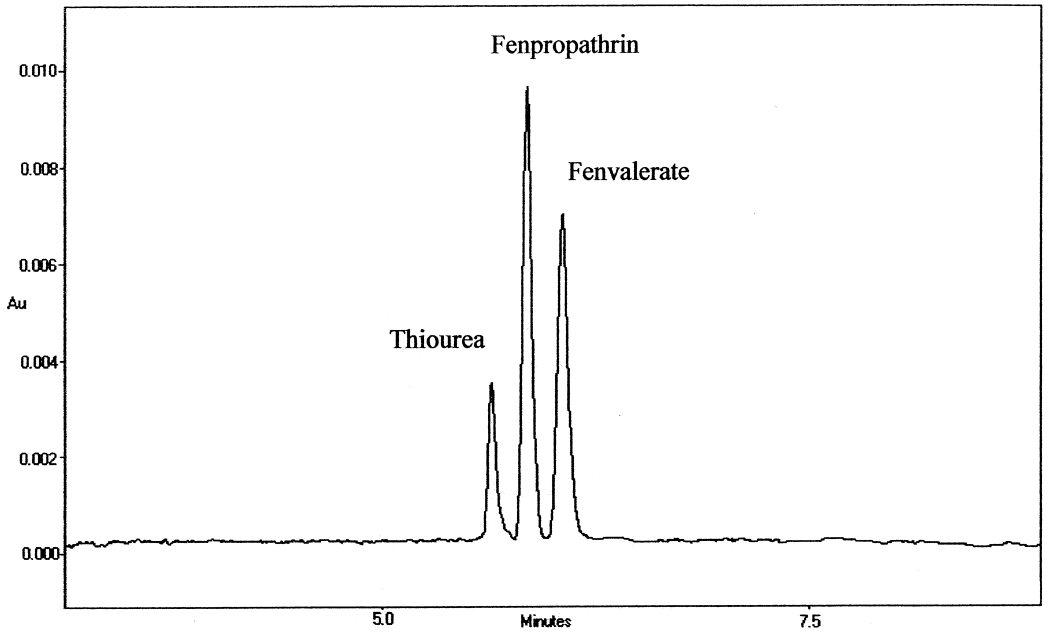


Fig. 11. Electrochromatogram of the separation of fenpropathrin and fenvalerate. Conditions: Au-APTMS coated capillary 30 cm×20 μm I.D.; mobile phase: methanol–25 m*M* $NaH_2PO_4$ (75:25) pH 3.0; voltage: −25 kV; sample: thiourea, fenpropathrin and fenvalerate in 100% methanol. Injection: −5 kV for 3 s, UV detection: 200 nm.

plates/m of 208 914 and 120 656, respectively. Fluvalinate was run separately due to impurities in the sample. The regression coefficients, $r^2$, are 0.9965, 0.9996 and 0.9990 for fenpropathrin, fenvalerate and fluvalinate in log $k'$ versus % MeOH plots.

## 4. Conclusions

Nanomaterials at present have numerous commercial and technological applications, e.g., in electronic, optical and mechanical devices. As the interest in nanoparticles increases due to their unique physical and chemical properties, relative to the bulk material, so too are the applications of these materials in analytical chemistry.

Novel, open-tubular columns for CEC can be formed by immobilising the alkylthiolgold nanoparticles on prederivatised APTMS or MPTMS capillaries. SEM analysis can be used to view the surface topography. The electroosmotic flow characteristics of Au-APTMS and Au-MPTMS capillary columns when studied at varying pH and voltages are altered by the derivatisation and nanoparticle coating steps.

A range of test solutes were analysed on the capillary columns to determine their discriminating ability and efficient separations were achieved for benzophenone and biphenyl solutes on Au-MPTMS and Au-APTMS capillary columns. A study of the reproducibility of retention for these solutes on Au-APTMS, Au-MPTMS and a loosely coated capillary demonstrated the necessity of a coupling agent to prevent the gold nanoparticles from washing-off.

This work confirms the use of alkylthiol gold nanoparticles as a novel phase for open-tubular CEC, demonstrating reproducible retention and characteristic reversed-phase behaviour. The dodecanethiol gold capillary columns are easier to produce and operate than packed capillary columns. The eventual use of such phases in chromatography is expected to be in the biomolecular analysis area, with research still required to determine how the favourable properties of gold nanoparticulate phases will be optimised for such analysis [16].

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