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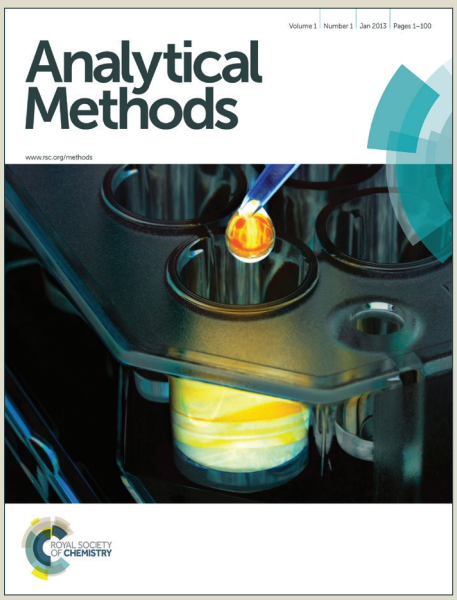

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Layer-by-layer self-assembly of polydopamine/gold nanoparticles/ thiols coating as the stationary phase for open tubular capillary electrochromatography

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Abstract

Much attention has been paid to utilizing polydopamine (PDA) as stationary phases in open-tubular capillary electrochromatography (OT-CEC) owing to its diverse properties, such as strong adhesive to various surface, latent reactivity toward amine and thiol groups and metal ions chelating/redox activities. In this study, a novel open-tubular capillary column coated with polydopamine/gold nanoparticles/thiols (PDA/Au NPs/thiols) was fabricated based on multiple properties of PDA for the first time. The capillary inner surface was firstly functionalized with a layer of PDA/Au NPs using the strong adhesive and metal ions redox properties of PDA. Thiols was then introduced and covalently react with the hybrid coating based on the Michael addition reaction of PDA and thiols and also Au-S bonds. Moreover, benefited from the porosity of PDA, layer-by-layer (LBL) self-assembly was further applied to increase the amounts of stationary phase (Au NPs and thiols), which can significantly enhance the separation effectiveness and stability of the coated column. The formation of PDA/Au NPs/thiols coating in the capillary was confirmed and characterized by scanning electron microscopy (SEM), Energy Dispersive Spectrometer (EDS) and AFM (Atomic Force Microscope). Then the separation effectiveness of the PDA/Au NPs/thiols@capillary was verified by the separation of alkylbenzenes, which can achieve baseline separation easily with high column efficiency. In addition, the column showed long lifetime and good stability. The relative standard deviations (RSDs) for intra-day and inter-day repeatability of the PDA/Au NPs/thiols @capillary were lower than 5%. Therefore, the layer-by-layer

self-assembly of PDA/Au NPs/thiols on capillary inner-surface could be an effective capillary modification strategy.

1. Introduction

Capillary electrochromatography (CEC) is a powerful analytical technique which combines high selectivity of HPLC and high efficiency of capillary electrophoresis (CE).[1-3] The capillary columns are critical to the development of CEC[4-7] and the commonly used columns in CEC include packed column, monolithic column and open-tubular column. Among these CEC columns, OT-CEC has some advantages such as the ease of preparation and operation, the low back-pressure and the absence of bubble formation.[8-10] Therefore, OT-CEC have been increasingly used in a variety of fields such as chiral separation,[1, 4] natural products analysis[9, 11] and protein analysis.[5, 10]

The stationary phases in OT-CEC mainly consist of chemically bonded phases and physically adsorbed phases[7]. To obtain chemically bonded phases with good stability and long lifetime, the tedious and time-consuming process are required in general[7, 12]. Deposition of physical coating on the capillary inner surfaces is an easier approach to fabricate OT-CEC column with less cost,[7, 13] although the coating is less stable and has a shorter lifetime than those of covalent coating in some instances. On the other hand, the low separation capability of OT-CEC caused by the low phase ratio of stationary phase, hinders its applications. To increase the phase ratio of OT-CEC, some capillary modification strategies have been presented, such as etching,[14] polyelectrolyte multilayer coating (PEM),[15, 16] porous polymer[17, 18] and nanoparticle (NP) modification.[19-21] Although the inner surface area of etched capillary is dramatically increased, the high density of silanol groups of the etched

capillary may cause high density stationary phase, which seriously blocks mass transfer.[7] PEM coatings have good reproducibility and stability, which are based on the layer-by-layer (LBL) self-assembly of cationic and anionic polyelectrolytes. Nevertheless, the polymers with precise mole fractions of different monomers are needed and a limited variety of polyelectrolytes can be used.[15,16] In addition, the modification strategies using porous polymers or nanoparticles also can greatly increase the effective surface area of capillary.[17-21] But the synthesis procedure of porous layers or nanoparticles is complex and costly. Hence, it is essential to further develop various, novel and permanent coating columns with good applicability and high phase ratio of stationary phase.

Mussel-inspired surface chemistry on the polydopamine (PDA) has attracted extensive interest in different research fields,[22, 23] owing to its intriguing properties, including strong adhesive property,[22-24] metal ions chelating[23, 25] and redox activities,[23 , 25] and latent reactivity with many functional molecules[23, 26, 27], etc. PDA-based coating columns possessed a simple preparation process and had a good stability similar to chemical bonded coating.[28] Therefore, PDA-based coating is a promising and effective capillary modification strategy for OT-CEC.[28-35] Based on the strong adhesion of PDA, Yin and co-workers first reported the use of PDA as a stationary phase in OT-CEC for the determination of auxins.[28] Wang's group anchored antifouling amine-functionalized PEG on the capillary inner surface for the protein separation, which was based on the latent reactivity of PDA.[29] Furthermore, on the basis of metal ions redox property of PDA, Liang and co-workers coated

polydopamine and gold nanoparticles on PDMS microchip for separating amino acids in OT-CEC.[30] However, the existing PDA-based OT-CEC were commonly fabricated solely based on one or two properties of PDA. Consequently, the application potential of PDA in the fabrication of OT-CEC columns could not completely show and the amounts of PDA-based materials coated in the capillary inner surface were limited.

Recently, a new methodology of LBL self-assembly related to the sequential assembly of PDA and some other functional materials has been proposed,[36-38] which can render the pristine surfaces with desired properties such as super-hydrophobicity and anti-adhesion property. For example, Cai and coworkers utilized LBL assembly of thiols/Ag nanoparticles/PDA on PET bottles for the enrichment of organic pollutants from water samples.[36] However, to our knowledge, the potential use of PDA-based LBL self-assembly in OT-CEC has not been studied.

Herein, a novel method for the preparation of permanent coating columns with high phase ratio based on LBL self-assembly of polydopamine/gold nanoparticles/ thiols (PDA/Au NPs/thiols) was developed for the first time, which was based on multiple properties of PDA, including adhesive property to deposit on the capillary surface, metal ions redox property to reduce $HAuCl_4$ to Au NPs, latent reactivity toward amine and thiol groups and property of porosity. The PDA/Au NPs/thiols@capillary combined the advantages of LBL self-assembly and nanoparticles on increasing the phase ratio of OT-CEC. Moreover, the resultant coating possessed abundant interaction sites, including the hydrophobic interaction

provided by thiols and the π-π stack interaction introduced by PDA. Besides, related references have reported the porosity of PDA.[39-42] Hian Kee Lee and co-workers characterized the morphology of PDA and confirmed its porosity by SEM.[39] Benefited from the porosity, the mass transfer and permeability of analytes in the LBL self-assembly coatings would not be interfered severely by the increased sequential assembly steps. Hence, it can be expected that the layer-by-layer self-assembly of PDA/Au NPs/thiols on capillary inner-surface could be an excellent capillary modification strategy and the PDA/Au NPs/thiols@capillary may possess good separation efficiency on some hydrophobic analytes. The formation of PDA/Au NPs/thiols coating in the capillary was confirmed and characterized by scanning electron microscopy (SEM), energy dispersive spectrometer (EDS) and AFM (Atomic Force Microscope). The separation ability of the PDA/Au NPs/thiols @capillary was systematically evaluated by the separation of alkylbenzenes.

2. Experimental

2.1. Chemicals and materials

Dopamine hydrochloride, hydrogen tetrachloroaurate ($HAuCl_4 \cdot 4H_2O$), 1,10-decane-dithiol, 1-octadecanethiol, benzene, methylbenzene, ethylbenzene, propylbenzene and n-butylbenzene were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Methanol and acetonitrile (ACN) of HPLC grade were from Adamas Reagent Co. (Shanghai, China). Hydrochloric aid (HCl), sodium hydroxide (NaOH), acetic acid (CH_3COOH), and sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) were all analytical grade and from KeLong Chemical Reagent Co., Ltd. (Chengdu, China). The ultrapure

water was prepared by AK's laboratory water purification system (Tang's Kangning Science and Technology Development Co., Chengdu, China). Fused silica capillaries of 75 μm i.d. × 375 μm o.d. were obtained from Yongnian Optical Fiber Factory (Hebei, China).

2.2. Apparatus

All the CE experiments were performed with an Agilent 7100 3D CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector and an Agilent ChemStation software. The experiments were performed at 20 kV and 25 ℃. The samples were detected at 200 nm and injected for 5 s using a pressure of 35 mbar. The morphology observation and elemental analysis of the PDA/Au NPs/thiols @capillary column were performed by SEM and EDS (Tescan VEGA3 LMH, Czech), and AFM (MFP-3D, Bruker) respectively.

2.3. Sample solutions and buffer preparation

The standard stock solutions of all the alkylbenzenes with the concentration of 1.0 mg/mL were prepared by dissolving analytes in acetonitrile individually and were stored at 4 ^{o}C refrigerator. $HAuCl_4 \cdot 4H_2O$ aqueous solution was prepared by dissolving the analyte in ultrapure water and its concentration was 10.0 mg/mL. Acetic buffer with different concentrations was prepared by dissolving CH_3COONa in ultrapure water and the pH value was adjusted to 4.5-7.0 using acetic acid. The running buffer was prepared by mixing the acetic buffer with an appropriate amount of acetonitrile. All the solutions were filtered through a 0.45 μm membrane filter (Auto science instrument Co., Ltd., Tianjin, China) and degassed by sonication prior to experiments.

2.4. Preparation of PDA/Au NPs/thiols@capillary

The capillary inner surface was firstly filled with a mixed aqueous solution of dopamine (DA) and $HAuCl_4$ for a period of time. As a reductant, DA in situ chemically reduced $HAuCl_4$ to gold nanoparticles (Au NPs), meanwhile $HAuCl_4$ as an oxidant triggered the self-polymerization of DA to PDA, which were simultaneously deposited on the capillary inner surface based on the adhesive property of PDA, generating in situ a well-distributed and robust PDA/Au NPs layer.[30] Then, dithiol was used as the LBL partner of PDA/Au NPs layer because it contained abundant thiol groups which could react with PDA and Au NPs through Michael addition reaction[23, 26] and Au-S bonds.[8, 36] The introduction of dithiol can provide plenty of hydrophobic interaction sites and enhance the coating stability because of the formation of intermolecular cross-linking networks.[8] After the desired numbers of sequential assembly steps were achieved, the PDA/Au NPs layer exposed on the capillary surface was further self-assembly with the alkanethiol. The prepared capillary was named as PDA/Au NPs/thiols@capillary.

The detailed procedure for preparing the PDA/Au NPs/thiols@capillary is schematically shown in Fig. 1. Firstly, the bare fused-silica capillary was preconditioned by flushing with methanol, 1 M HCl, 1 M NaOH for 30 min in sequence, ultrapure water for 10 min, and dried by purging nitrogen gas for 5 min. 30 mg dopamine hydrochloride was dissolved in 5 mL of 10 mM Tris-HCl buffer (pH 8.5) containing $HAuCl_4$ (0.01%, w/w). Then the solution was injected into the pretreated capillary with a syringe and the capillary was kept at 25 ^{o}C for 12 h with both ends

sealed with rubber stoppers. Subsequently, the PDA/Au NPs@capillary was rinsed with water and dried with nitrogen flow for five minutes. For the fabrication of capillary column coated with a monolayer of alkanethiol: firstly, 15 mg/mL 1-octadecanethiol was dissolved in ethanol which was pre-equilibrated by bubbling with nitrogen flow and the solution was injected into the PDA/Au NPs@capillary. The obtained capillary was placed in an oven for 24 h under 50 ^{o}C with both ends sealed. Finally, the excess 1-octadecanethiol solution was removed from the capillary column by rinsing with ethanol and the column was dried with nitrogen flow for five minutes, respectively.

For the PDA/Au NPs/thiols@capillary fabricated by LBL self-assembly: firstly, the PDA/Au NPs@capillary was filled with the ethanol solution of 1,10-decanedithiol (15 mg/mL). The capillary was then placed in an oven for 2 h under 50 ^{o}C with both ends sealed. The excess 1,10-decanedithiol solution was removed from the capillary column by rinsing with ethanol and dried with nitrogen flow for five minutes. Then, 6 mg/mL dopamine hydrochloride dissolved in 5 mL of Tris-HCl solution (10 mM, pH 8.5) containing $HAuCl_4$ (0.01%, w/w) was injected into the resultant capillary and it was kept at room temperature for 2 h. Then the capillary was rinsed with ultrapure water and dried with nitrogen stream for five minutes. The two sequential assembly steps were repeated until the desired numbers of deposition steps were achieved. Finally, the PDA/Au NPs layer exposed on the capillary surface were subjected to self-assembly with 1-octadecanethiol solution as the same as above monolayer procedure.

As to the preparation of PDA/thiols@capillary, it was prepared with minor modification similar as PDA/Au NPs/thiols@capillary only in the absence of $HAuCl_4$. Its mechanism mainly relied on the Michael addition reaction of PDA and thiols. The preparation procedure of the PDA@capillary was similar to that in PDA/thiols@capillary except the assembly steps of thiols.

3. Results and discussion

3.1. Characterization of PDA/Au NPs/thiols@capillary

The morphology of these columns was characterized by SEM, EDS and AFM. As demonstrated in Fig. 2A, the bare fused-silica capillary had a smooth inner surface. After coated by PDA and thiols, the inner surface of the PDA/thiols@capillary became rough with some visible aggregates, which indicated successful fabrication of the coated capillary column (Fig. 2B). After further introduction of $HAuCl_4$ in PDA, the coating of the PDA/Au NPs/thiols@capillary became thicker and rougher. It can be attributed to the formation of Au NPs embedded in the coating (Fig. 2C), because $HAuCl_4$ can be utilized as an oxidizing reagent to trigger DA polymerization and the source of metallic nanoparticles.[43] In order to further demonstrate the formation of Au NPs, EDS and AFM were further applied to characterize the surface properties, respectively shown in Fig. 3 and Fig. S1 (supporting information). The EDS results of PDA/Au NPs/thiols@capillary displayed that the emission lines of Au appeared (Fig. 3b), which further confirmed the successful introduction of Au NPs. Additionally, the particle size of Au NPs was obtained from AFM. Calculation of the average particle size gave a size of 100 nm. The particle size of Au NPs was almost in the range of

40-120 nm, indicating a wide range of size distribution of Au NPs.

3.2. Effect of pH on the EOF mobility

Electroosmotic flow (EOF) drives the running buffer in OT-CEC. The EOF mobility was investigated with dimethyl sulphoxide (DMSO) as the natural marker and calculated as follows:

$$\mu_{eof} = L_d L_t / V t$$

where L_t and L_d are the total length of the capillary (48.5 cm) and effective length (40 cm), V is the voltage applied across the capillary (20 kV), and t is the migration time of DMSO. As shown in Fig. 4, the effect of pH on the EOF mobility of those capillaries was studied. CH_3COONa/CH_3COOH was chosen as the buffer at pH 4.5-7.0. The concentration of CH_3COONa was 20 mM. As can be seen from Fig. 4, the EOF of all the evaluated columns increased with pH in the range of pH 4.5-7.0. The PDA@capillary possessed lower EOF mobility than the bare capillary. It can be attributed to the stronger effect of silanol masking and the existence of catechol and amine groups in the PDA layer. After the PDA@capillary was modified with thiols, the EOF mobilities of the PDA/Au NPs/thiols@capillary and PDA/thiols @capillary are similar and further decreased. The decreased EOF can be interpreted as the decreased amounts of catechol and amine groups exposed on the capillary surface, which was masked by the thiols. The similar values of the EOF mobility suggested that the Au NPs embedded in the PDA layers had little impact on the charge property of the PDA layer exposed on the capillary surface.

3.3. Separation ability of capillaries with different coatings

The separation ability of the PDA@capillary, PDA/thiols@capillary and PDA/Au NPs/thiols@capillary was compared. As exhibited in Fig. 5, when using the PDA@capillary, the hydrophobic compounds were eluted rapidly and the peaks were severely overlapped, which might result from the hydrophillic hydroxyl and amino groups existed in PDA layers. The slight separation trend might be related to the π-π interactions and hydrogen bonding interactions provided by the catechol and quinine functional groups in PDA layers. When dithiol was introduced as a cross-linker and alkanethiol was modified on the surface, the separation selectivity was greatly improved by PDA/thiols@capillary. It is suggested that these alkyl chains, which provided hydrophobic interaction sites, played an essential role in the separation. However, the resolution was only 0.69 for benzene *vs* toluene, and 0.73 for methylbenzene *vs* ethylbenzene. When PDA/Au NPs/thiols@capillary was used, the separation selectivity can be further improved and baseline separation was achieved. The resolution of benzene *vs* toluene was 1.79 and the theoretical plates of benzene was 28,106, which was much better than that of 11,133 on PDA/thiols@capillary. It can be ascribed to that the introduction of Au NPs increased the surface area and the hydrophobic interaction sites. Besides, the additional Au-S bonds made it more stable except the original Michael addition reaction between PDA and thiols, thus resulting in better separation. The comparison of resolution of peaks on PDA/Au NPs/thiols@capillary and PDA/thiols@capillary was summarized in Table 1. Therefore, as expected, the PDA/Au NPs/thiols@capillary displays the best separation ability among capillaries with different kinds of modified coatings.

In addition, a method based on the modification of PDA in a LBL fabricated graphene stationary phase has been used in OT-CEC for the enhancement of CE separation which was put forward by Chen's group.[34] Five alkylbenzenes were also used as the model substances. The resolution of five hydrophobic compounds in this work was as follows: Ben/Methylbenzene and Methyl/Ethylbenzene 1.5; Ethyl/ Propylbenzene and Propyl/n-butylbenzene 2.0 while in our work the resolution was: 1.79, 1.91, 3.12 and 4.43 which was shown in table 1. Obviously, the resolution was improved to some degree. However, the RSDs of the migration time of PDA/Au NPs/thiols@capillary was a little higher than that of their work. But the difference was quite modest. Compared with this literature, our method still possessed its own advantages.

3.4 Influence factors on separation ability

3.4.1. Effect of the number of LBL self-assembly PDA/Au NPs/thiols layers

The monolayer and two-layer of PDA/Au NPs/thiols coating failed to separate five alkylbenzenes (Fig. 6A and Fig. 6B). The reason for this poor separation behavior is perhaps due to the insufficient hydrophobic stationary phase on the surface of capillary. But the separation trend of two-layer PDA/Au NPs/thiols was a little more obvious than that of one-layer. Hence, we put forward with one hypothesis that the separation behavior could be further improved with the increase of the number of LBL self-assembly PDA/Au NPs/thiols layers. To verify this hypothesis, four-layer, and six-layer PDA/Au NPs/thiols coating were fabricated. The thickness of different layers was measured by SEM which was shown in Fig. S2. The monolayer of

PDA/Au NPs/ thiols@capillary was around 110 nm, two-layer was nearly 430 nm, four-layer coating was almost 790 nm and six-layer was about 1370 nm. When the number of self-assembly layers increased to seventh, the prepared PDA/Au NPs/thiols@capillary column was easy to be blocked.

As can be seen from Fig. 6, the separation ability was enhanced as well as the longer retention time of analytes with the increased number of self-assembly PDA/Au NPs/thiols layers. It may be ascribed to the following possible effects: (i) the more layers of PDA/Au NPs/thiols coating there are, the more nanoparticles and alkyl chains are; (ii) Au NPs existed on the surface could increase the surface area and immobilize more thiols by Au-S bonds, thus further increasing column capacity; (iii) the porosity of PDA layers[39-42] which has been demonstrated can ensure that the mass transfer and permeability of analytes in the LBL self-assembly coatings would not be interfered severely by the increased sequential assembly steps. In summary, there were more hydrophobic interaction sites with the increased number of self-assembly layers, which might have stronger interaction with alkylbenzenes, thus resulting in good separation efficiency.

3.4.2. Influence of acetonitrile concentration

The effect of acetonitrile concentration on separation performance of PDA/Au NPs/ thiols@capillary is shown in Fig. 7. It can be found that the baseline separation of five alkylbenzenes can be achieved with 10% acetonitrile in 20 mM CH_3COONa at pH 6.0 and 12% ACN provided the optimal separation. The migration time slightly increased but the resolution among five compounds decreased with the increase of ACN

concentration from 12 to 20% (v/v). Furthermore, at 20% acetonitrile, peaks of benzenze, methylbenzene and ethylbenzene overlapped. These results can be attributed to the following two possible effects: (i) the decrease of the EOF with enhanced contents of the organic modifier, resulting in migration time prolonging; (ii) the eluting power of the mobile phase was enhanced with the increase of acetonitrile concentration, thus weakening the hydrophobic interaction between analytes and stationary phase and leading to the decrease of resolution of peaks. Because of different hydrophobic constants of the five substituted benzenes (benzene 2.13, methylbenzene 2.60, ethylbenzene 3.16, propylbenzene 3.69, n-butylbenzene 4.13), the hydrophobic interaction between the analytes and the stationary phase is n-butylbenzene > propylbenzene > ethylbenzene > methylbenzene > benzene. The peak sequence was in accordance with the order of hydrophobic constant value of alkylbenzenes.

Besides, the effect of pH on separation was also investigated (Fig. S3) and the greatest improvement in resolution of benzene and toluene (1.79) obtained at pH 6.0. Moreover, the effect of acetate buffer concentration ranging from 5 mM to 30 mM on the separation was also investigated (Fig. S4). According to peak shape and resolution, the acetate buffer concentration of 20 mM was selected.

3.5. Stability and column repeatability

The stability of the PDA/Au NPs/thiols@capillary is an important precondition for the practicability of the column. The repeatability was evaluated based on the relative standard deviations (RSDs) of migration time and peak area of alkylbenzenes. As

shown in Table 2, the intra-day and inter-day RSDs were all below 5%, indicating that the PDA/Au NPs/thiols@capillary possessed a good repeatability. The PDA/Au NPs/thiols@capillary could be used for more than 80 runs without obvious changes in separation efficiency. All the results demonstrated that the PDA/Au NPs/thiols @capillary had good stability and practicability.

4. Conclusion

A novel open-tubular capillary column coated with PDA/Au NPs/thiols was fabricated using multiple properties of PDA and LBL self-assembly technique for the first time. The PDA/Au NPs/thiols@capillary combined the advantage of LBL self-assembly and nanoparticles on increasing the phase ratio of OT-CEC. Accordingly, the column capacity, the hydrophobic interaction sites and the stability could all be improved significantly. Compared with PDA/thiols@capillary, the separation capability of PDA/Au NPs/thiols@capillary was greatly enhanced. Higher resolution and number of theoretical plates could be achieved by the introduction of Au NPs. The PDA/Au NPs/thiols@capillary displayed good separation ability towards neutral compounds under organic solvents, continuous pressure of mobile phase and high voltage. Additionally, the columns also had good stability. Therefore, the layer-by-layer self-assembly of PDA/Au NPs/thiols on capillary surface may be an effective capillary modification strategy.

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Figure captions:

Fig. 1. Schematic procedure for the preparation of the PDA/Au NPs/thiols @capillary.

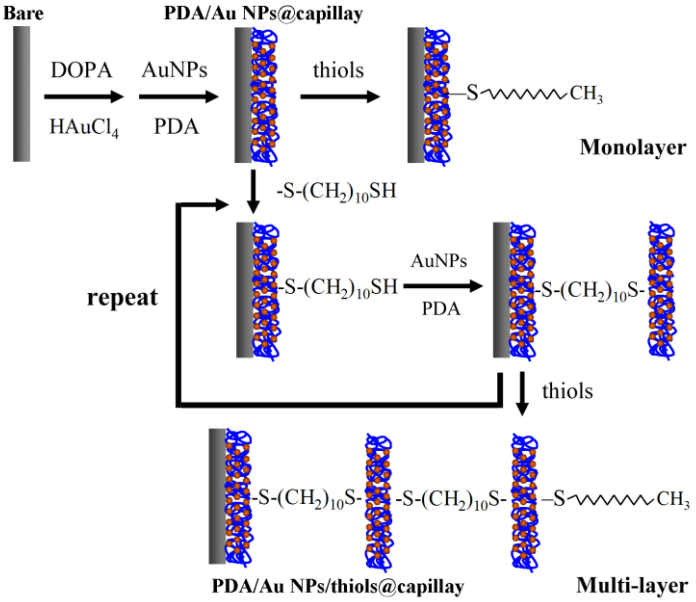


Fig. 2. SEM images of bare silica capillary (A), PDA/thiols@capillary (B), PDA/Au NPs/thiols@capillary (C). The white spots in (C) are the Au NPs. The number of coating layers in (B) and (C) was six.

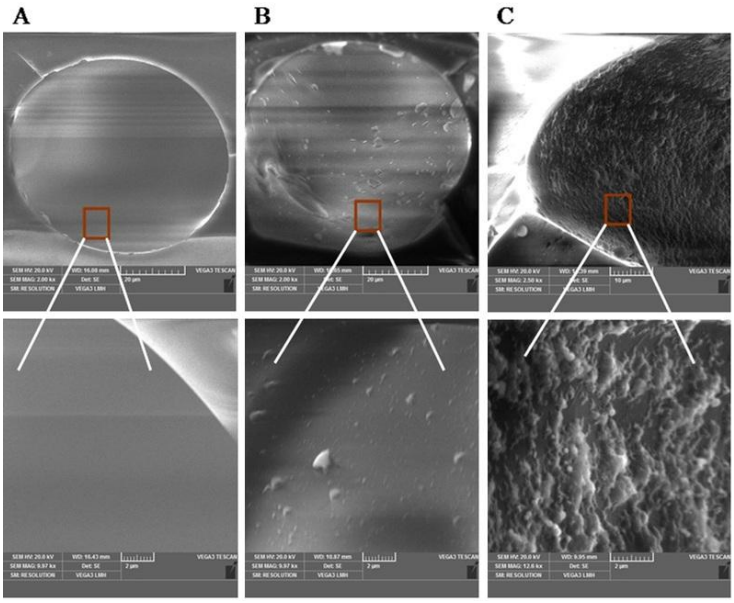


Fig. 3. EDS spectra of the bare silica capillary (a) and PDA/Au NPs/thiols@capillary (b)

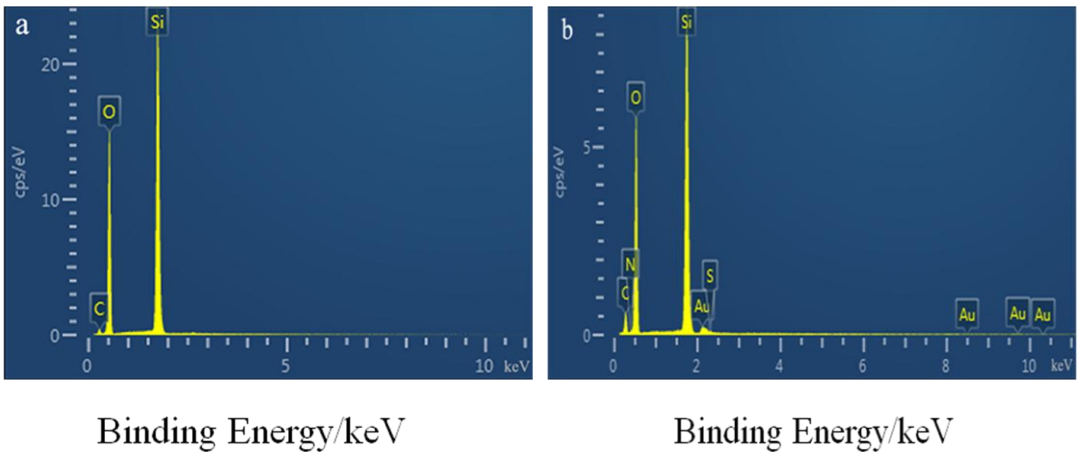


Fig. 4. Effect of buffer pH on the EOF mobility for the capillaries. Experimental condition: 20 mM CH_3COONa (pH 4.5-7.0); separation voltage, 20 kV; injection, 30 mbar × 5 s; detection wavelength, 200 nm; capillary column, 48.5 cm (40 cm effective length) × 75 μm i.d. DMSO was used as EOF marker. The number of LBL self-assembly coating layers of capillaries was six.

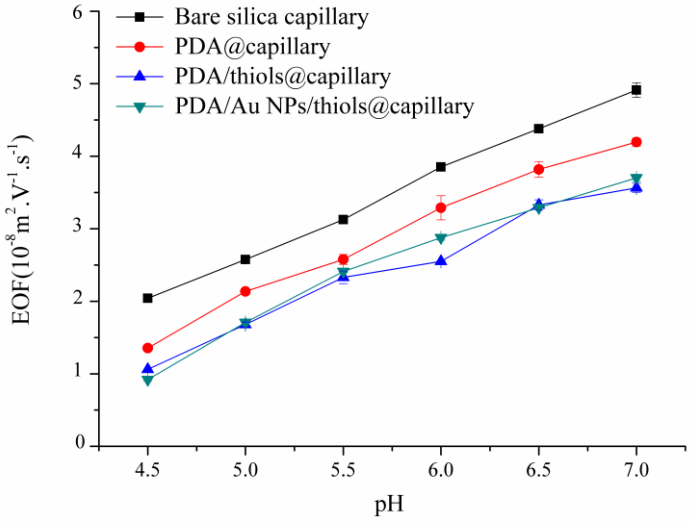


Fig. 5. Separation ability on the PDA@capillary (A), PDA/thiols@capillary (B) and PDA/Au NPs/thiols@capillary (C). Experimental conditions: acetonitrile–20 mM CH_3COONa (12/88, v/v), pH 6.0. The number of coating layers was six. All other separation conditions are the same as mentioned in Fig. 4. Peak identification: 1, benzene (100 μg/mL); 2, methylbenzene (100 μg/mL); 3, ethylbenzene (100 μg/mL); 4, propylbenzene (200 μg/mL); 5, n-butylbenzene (200 μg/mL).

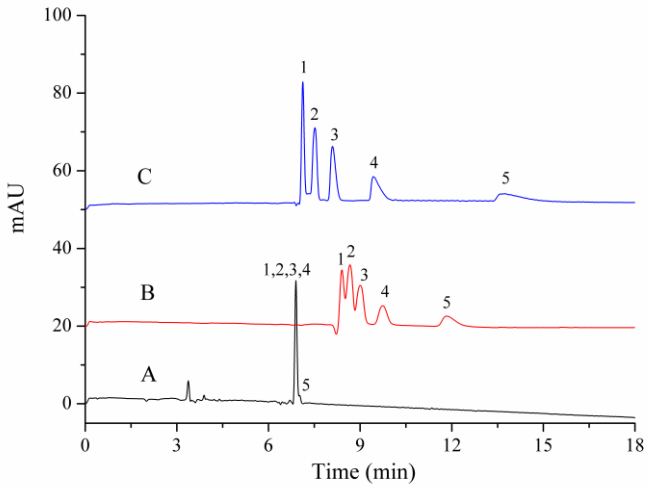


Fig. 6. The effect of the number of LBL self-assembly PDA/Au NPs/thiols layers on separation ability. (A) One layer; (B) two layers; (C) four layers and (D) six layers.

Experimental conditions: acetonitrile–20 mM CH_3COONa (12/80, v/v), pH 6.0. All other separation conditions are the same as mentioned in Fig. 4. Peak identifications are identical to Fig. 5.

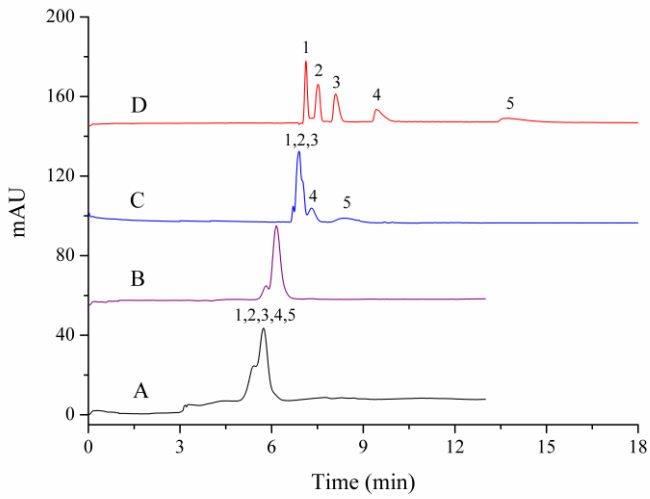


Fig. 7. The effect of acetonitrile concentration on the separation of five alkylbenzenes on the PDA/Au NPs/thiols@capillary. (A) 10% ACN; (B) 12% ACN; (C) 15% ACN and (D) 20% ACN. Experimental conditions: acetonitrile–20 mM CH_3COONa (pH 6.0). All other separation conditions are the same as mentioned in Fig. 4. Peak identifications are identical to Fig. 5.

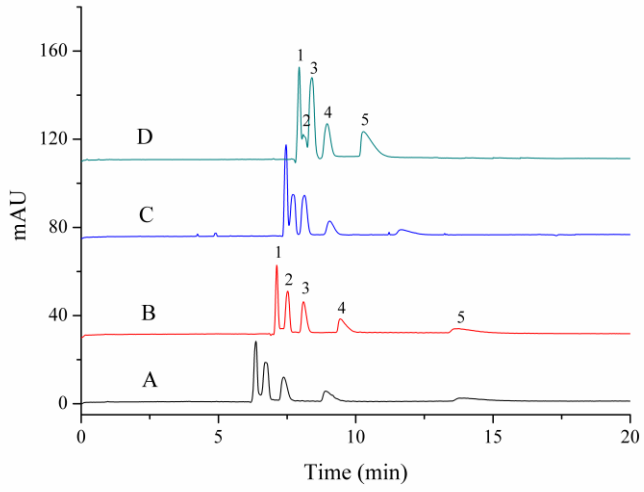
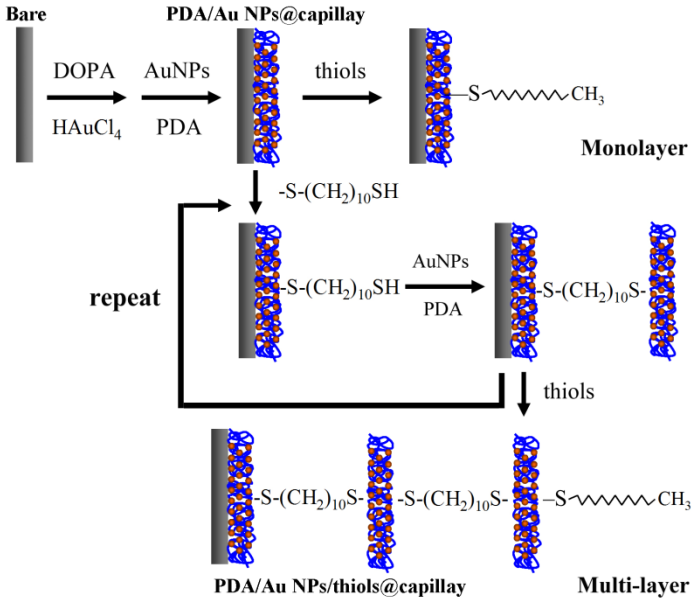


Table 1. Comparison of resolution on PDA/Au NPs/thiols@capillary and PDA/thiols @capillary.

	Column	PDA/Au NPs/thiols @capillary	PDA/thiols @capillary
Rs	Ben/Methylbenzene	1.79	0.69
	Methyl/Ethylbenzene	1.91	0.73
	Ethyl/Propylbenzene	3.12	1.41
	Propyl/n-butylbenzene	4.43	3.11

Table 2. Repeatability of PDA/Au NPs/thiols@capillary.

Compounds	Time (RSD%)		Peak area (RSD%)	
	Intra-day (*n*=3)	Inter-day (*n*=3)	Intra-day (*n*=3)	Inter-day (*n*=3)
Benzene	1.51	3.32	1.56	2.60
Methylbenzene	1.14	3.15	3.15	4.99
Ethylbenzene	1.05	3.11	2.31	3.26
Propylbenzene	1.91	2.95	3.58	3.81
n-butylbenzene	3.10	2.40	3.74	4.58



A novel method for the preparation of permanent coating columns with high phase ratio based on multiple properties of PDA and LBL self-assembly of polydopamine/gold nanoparticles/ thiols was developed for the first time.