



Modification of VTMS hybrid monolith via thiol-ene click chemistry for capillary electrochromatography

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ARTICLE INFO

Article history:

Received 28 September 2011

Received in revised form

30 December 2011

Accepted 6 January 2012

Available online 12 January 2012

Keywords:

Vinyl-based monolithic column

Thiol-ene reaction

Click chemistry

n-Octadecanethiol

3-Mercapto-1-propane-sulfonate

ABSTRACT

An n-octadecanethiol (C₁₈)/3-mercapto-1-propane-sulfonate (MPS) modified organic–inorganic hybrid silica monolithic column possessing vinyl ligands through thiol-ene click chemistry for capillary electrochromatography (CEC) is described. The proposed column is prepared via the sol–gel process by in situ co-condensation using vinyltrimethoxysilane (VTMS) and tetra-methoxysilane (TMOS) as precursors. Examination by SEM shows that the capillary has homogenous macroporous morphology and is well attached to the inner wall of the capillary. The obtained C₁₈-MPS-VTMS silica hybrid monolithic column demonstrated an enhanced hydrophilic property and could be applied as a reversed-phase stationary phase in CEC directly. Compared with unmodified VTMS silica hybrid monolithic column, stronger EOF was observed using this monolithic column. VTMS/TOMS ratios in the reaction mixture were varied and 1:3 was found to be optimum. Good separations of benzenes, aromatic amines, acids and peptides were achieved, the lowest plate height of ~3 μm was obtained, the peak symmetry range from 0.98 to 1.29. The resulting C₁₈-MPS-VTMS silica hybrid monolithic column can be used in different separation modes, including reversed phase mode and ion exchange mode.

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1. Introduction

Monolithic materials have emerged in the late 1980s and early 1990s as stationary phases for HPLC [1,2]. It has attracted much attention for capillary electrochromatography due to its advantages of easy preparation, excellent permeability, and high efficiency compared with traditional packed columns. The monolithic columns can be mainly classified into organic polymer-based [3–5] and inorganic silica-based [6–10] monolithic columns. Generally speaking, the organic monolithic columns can provide good pH stability over a wide pH range and satisfactory flexibility to tune the chemical properties of monoliths by using a variety of functional monomers and crosslinkers [11]. However, due to its deficiencies of mechanical stability and desirable porous structure, the organic monolith still has flaws in some applications. On the contrary, despite the high surface area and low shrinkage of monolithic matrix, the inorganic silica-based monolithic column also has

its deficiency of nonspecific adsorption caused by silanol groups on the monolithic matrix surface.

Thus, it is compulsory to improve the techniques mentioned above. The organic–inorganic hybrid monoliths may combine the advantages of organic polymer-based and inorganic silica-based monoliths [9,12,13]. Wu [14,15] reported a “one-pot” process for fabrication of organic–silica hybrid monolithic capillary columns. However, the reported functional monomer required complicated preparation conditions.

Conventional surface derivatization based on pure silica monolithic column is covalently linked to the silica support through Si–O–Si–C linkages [16]. Recently, surface derivatization based on hybrid organic–inorganic silica monolithic matrix was developed [17–20]. Wu et al. introduced *N,N*-dimethyl-*N*-dodecylamine onto a chloropropyl-functionalized silica hybrid monolithic column through C–N linkages [17]. Cao [18] used gold nanoparticles as intermediate ligands through Au–S linkages for capillary columns with varying surface functionalities. Tian [19] introduced calix [4] open-chain crown ether to modify vinyl-functionalized hybrid silica monolith for capillary electrochromatography. Preinerstorfer developed a thiol-modified monolithic capillary and made in-column surface functionalization by radical addition [20].

Click chemistry was firstly introduced by Sharpless in 2001 [21]. It becomes more and more attractive not only in organic chemistry, but also in biology [22] and material science [23]. Liang's group [24] has used copper-catalyzed azide–alkyne cycloaddition for preparation of stationary phases, especially the hydrophilic one.

Abbreviations: C₁₈, n-octadecanethiol; CEC, capillary electrochromatography; MPS, 3-mercapto-1-propane-sulfonate; VTMS, vinyltrimethoxysilane; TMOS, tetra-methoxysilane; EOF, electroosmotic flow; V50, 2,2'-azobis(2-methylpropionamide) dihydrochloride; PEG, poly (ethylene glycol); CY-6, H-Cys-Asp-Glu-Val-Asp-Tyr-OH; CY-9, H-Cys-Gly-Gly-Asp-Glu-Val-Asp-Tyr-OH; Cy-15, H-Cys-Gly-Asp-Asp-Gly-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Tyr-OH.

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These stationary phases showed good separation performance, however, the synthesis process was complicated, and had to employ copper(I) which can contaminate the silica-matrix. Thiol-ene reaction is a new kind of click chemistry reaction [25]. It is used in polymer chemistry, organic synthesis, biological tag, etc. However, its application in surface-modification for chromatography was seldom reported.

In this work, we introduced thiol-ene click reaction to modify the organic-inorganic hybrid monolithic column starting from a vinyl-functionalized silica hybrid monolith, which was synthesized via the co-condensation of VTMS and TMOS. The vinyl group on the VTMS-silica hybrid monolithic matrix could easily react with *n*-octadecanethiol and 3-mercapto-1-propanesulfonate to provide a long carbon chain (C_{18}) and increase the hydrophobic. It was also applied as the strong electroosmotic flow (EOF) generator over a wide pH range for CEC. To the best of our knowledge, this is the first report on using thiol-ene click chemistry in VTMS silica hybrid monolithic column. Since vinyl ligands can react with other thiol compounds, this hybrid monolithic column will be a promising initial material for the separation of neutral, acidic, or alkaline molecules and proteins. Thus, the technique provides a novel approach for the preparation of a variety of novel function monolithic columns by using different functional monomers.

2. Experimental

2.1. Reagents and chemicals

All water was deionized using a Milli-Q synthesis A10 water purification system (Millipore Inc., Milford, MA), and degassed by ultrasonic for 5 min prior to use. Tetramethoxysilane (TMOS, 99%), 2,2'-azobis (2-methylpropionamide) dihydrochloride (V50, 99%) and vinyltrimethoxysilane (VTMS, 98%) were obtained from Aldrich (Oakville, ON, Canada). Phosphorus tribromide (99%), vinylimidazole (98%) and poly (ethylene glycol) (PEG, MN = 20 000) were purchased from Aladdin (Shanghai, China). Fused-silica capillary with 75 μm i.d. and 375 μm o.d. was purchased from Reafine Chromatography Ltd. (Yongnian, China). All other reagents were of analytical grade and used as received.

2.2. VTMS hybrid monolithic capillary column preparation

Preparation of the monolithic CEC column involved the following procedures. Firstly, the fused-silica capillary was pretreated by rinsing with 1.0 M HCl for 12 h, water for 30 min, 1.0 M NaOH for 12 h, and water for another 30 min, respectively, and then dried by nitrogen gas at room temperature for further use. Secondly, the sol-gel solution was prepared by mixing acetic acid (0.01 M, 1.25 mL), PEG (20 000 MW, 0.135 mg), urea (0.175 mg),

TMOS (0.45 mL) and VTMS (0.15 mL) in a 10 mL flask. Then the sol-gel solution was stirred at 0 °C for 4 h to form a homogeneous mixture solution. The mixture solution (1 mL) was sonicated at 0 °C for 5 min, and then introduced into the pretreated capillary with 35 cm length by a syringe. Thirdly, both ends of the capillary were sealed with rubbers, and the condensation reaction was carried out at 47 °C for 12 h. Finally, the obtained VTMS silica hybrid monolithic column was then flushed with water and ethanol to remove PEG and other residuals. With both ends sealed by silicon rubbers, the capillary monolithic column was stored at 4 °C in darkness before use.

2.3. Modification of VTMS silica hybrid monolithic capillary column

To modify the synthesized VTMS silica hybrid monolithic column, the column was first flushed by water and ethanol for 30 min, respectively, to remove the progen. After that, ethanol solution of the functional monomer was pumped through the monolithic column for 5 min using a manual syringe pump. Then, the modification of the hybrid monolithic column was carried out at 60 °C for 5 h in water bath. The synthesis of the VTMS silica hybrid monolith is schematically illustrated in Fig. 1. The resulting modified hybrid monolithic column was flushed with ethanol extensively to remove the residues.

2.4. Morphology characterization of the biphasic hybrid monolithic capillary column

Microscopy and scanning electron microscopy (SEM) were used for the characterization of monolithic matrixes in hybrid monolithic columns. Microscopic pictures were taken with an inverted microscope Leica DMI4000B equipped with a Leica DFC480 camera. Scanning electron micrographs were obtained using a JEOL JSM-840 scanning electron microscope, operated at 15 kV and a filament current of 60 mA. The samples were acquired from sections of biphasic hybrid monolithic column initially cut into equal lengths (0.5 cm) and positioned longitudinally within a retractable aluminum stage. These samples were used to depict the surface view of the sol-gel coating from a longitudinal section of the open tubular column. This stage, with the mounted capillary segments, was then placed into a Balzers SCD 050 sputter coating chamber and coated with a gold/palladium alloy at 40 mA for 60 s to avert subsequent charging.

2.5. Tryptic digestion of BSA

BSA (5 mg) was dissolved in 5 mL of water, and then the solution was incubated at 90 °C for 1 h to inactivate protein; subsequently, the mixture was incubated at room temperature in dark for 30 min;

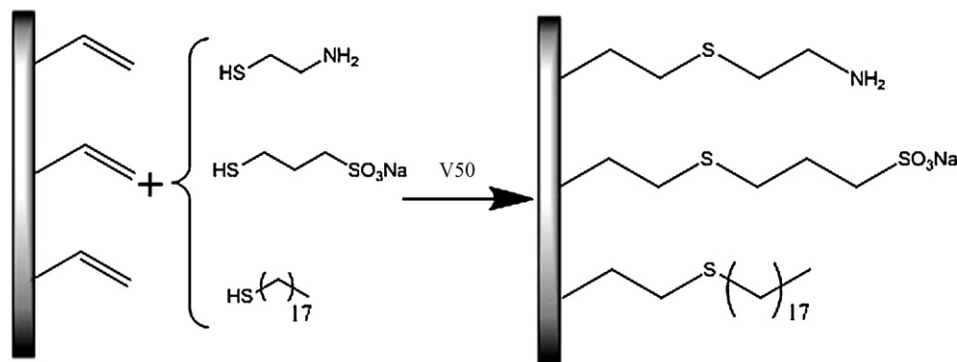

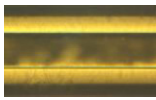


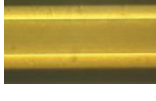



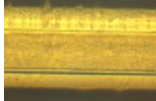


Fig. 1. Scheme for the modification of the VTMS hybrid monolith with functional monomer compounds.

Table 1
Effect of urea content, ratio of VTMS to TMOS (v/v, mL) and temperature on structure of hybrid monolithic (other conditions: 0.135 g of PEG 20 000, 1.25 mL of 0.01 M acetic acid).

	T (°C)	Urea content (g)	Ratio (VTMS/TMOS, v/v)	Permeability	Morphology	Microscope Image
1	45	0.1	0.2/0.4	Poor	Opaque detached	
2	45	0.175	0.12/0.48	Poor	Opaque detached	
3	45	0.25	0.15/0.45	Good	Detached	
4	47	0.1	0.12/0.48	Poor	Detached	
5	47	0.175	0.15/0.45	Good	Homogeneous transparent	
6	47	0.25	0.2/0.4	Poor	Detached	
7	49	0.1	0.15/0.45	Poor	Homogeneous	
8	49	0.175	0.2/0.4	Poor	Detached	
9	49	0.25	0.12/0.48	Poor	Seriously detached	

and then 5 mg trypsin was added; finally, the mixture was digested at 37 °C for 12 h.

2.6. Capillary electrophoresis

A VTMS silica hybrid monolithic capillary column capillary was installed in a P/ACE™ MDQ System (Beckman, Fullerton, CA, USA) equipped with a PDA detector. The total length of the capillary column was 35 cm with the length from the detection window to the outlet being 8.5 cm. The effective length of the column was 27 cm. Prior to operation, the vinyl-silica hybrid monolithic column was conditioned by rinsing with running buffer followed by electrokinetic conditioning at 5 kV for 20 min.

3. Results and discussion

3.1. Preparation of VTMS silica hybrid monolithic column

The formation of VTMS silica hybrid monolithic column involves two major reactions: the poly-condensation of hydrolyzed precursors of TMOS and VTMS, and the copolymerization of the precondensated siloxanes and vinyl organic monomers. The physical and chromatographic properties of monolithic matrixes are

controlled by changing various factors during the preparation procedure. In our work, the influences of urea content, TMOS/VTMS ratio, and poly-condensation temperature on the column morphology were studied in a multivariate approach for the optimization.

To investigate the effect of the amount of VTMS in the reaction mixture on the formation of the hybrid monolith, the ratio of VTMS to TMOS (v/v) from 1:4 to 1:2 were evaluated by referring to values from the literature [14]. The obtained columns are displayed in Table 1, with their synthesis parameters and resulting optical microscope images. As seen from these images, column 5 is the best choice. The lower content of VTMS (as for columns 2, 4 and 9) in the reaction mixture would result in slack monolith with opaque aggregation inside the capillary; while the higher content of VTMS (as for columns 1, 6 and 8) could result in the semi-transparent monolith matrixes, however, the permeability is poor. So, the careful adjustment of silane monomer in the reaction mixture will be necessary for obtaining the desirable monolith column.

The importance of urea in the monolithic column preparation has been reported [26]. Due to the thermal decomposition of urea at elevated temperature, urea was used to generate ammonia to promote the uniform mesopore structure in the prepared silica monolithic skeletons. However, the effect of urea on the condensation procedure was neglected. Wu et al. [17] discussed

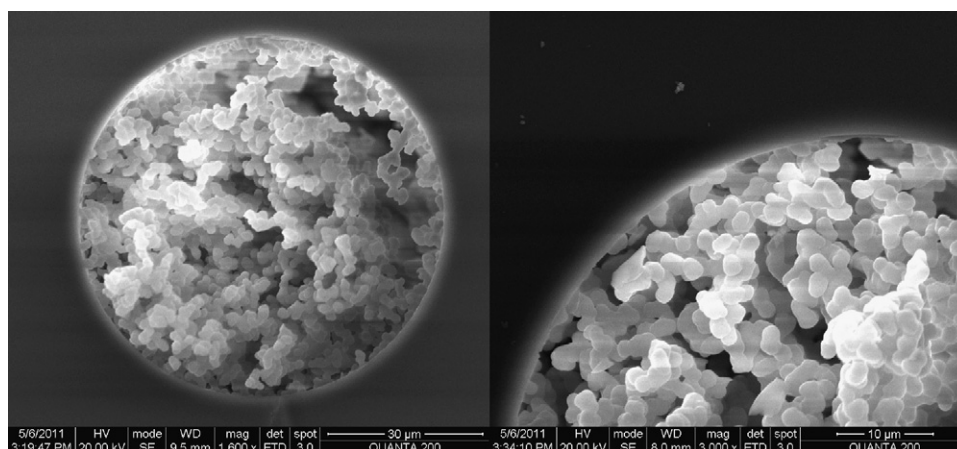


Fig. 2. SEM photographs of silica hybrid monolith: (A) 1500 \times and (B) 3000 \times .

the infection of urea in the formation of trimethoxysilane hybrid monolithic column. In this work we found that, without urea in the reaction mixture, the monolith was not filled in the inner space of capillary. Adding of urea in the reaction mixture, the mesopore structure of capillary column was obtained. The influence of urea dosage on the infection of shaping the fully filled capillary was studied by changing the urea content in pre-condensation mixture and the morphologies of resulted VTMS silica hybrid monolith under inverted fluorescence microscope are shown in Table 1. As seen from the microscopy images, the lower content of urea with 0.1 g in the reaction mixture would result in the slack monolith with opaque aggregation inside the capillary; the higher content of urea could result in the homogeneous and semitransparent monolithic matrix within the confines of the capillary.

Because the polycondensation is temperature sensitive, the effect of temperature on the morphology and permeability of obtained monolithic matrixes was studied in detail. The copolymerization was carried out under 45, 47 and 49 $^{\circ}$ C respectively. It was clearly seen that at lower condensation temperature (45 $^{\circ}$ C), the monolithic matrix formed a loose porous structure, which rigidity was not strong enough for monolithic matrixes we need. While at 47 $^{\circ}$ C, the obtained monolithic matrixes became homogeneous and fully filled in the capillary. As the temperature further increased to 49 $^{\circ}$ C, the monolithic matrixes were seriously detached from the inner capillary wall due to the incomplete co-condensation of silane monomers. Hence, 47 $^{\circ}$ C was used for subsequent experiments. Based on the investigations, the VTMS to TMOS ratio of 1:3, the urea content of 0.175 g, and the condensation temperature of 47 $^{\circ}$ C were used in the preparation of the VTMS hybrid silica monolithic column.

Fig. 2 illustrates the VTMS silica hybrid monolithic structure prepared inside a 75- μ m internal diameter matrix under the optimized reaction conditions. It can be seen that the capillary is fully filled with the homogenous monolithic matrix. An interconnected, globular skeleton structure can be seen with flow-through channels of about 2 μ m diameter. Additionally, the morphology of the VTMS silica monolithic matrix strongly resembles to that of pure silica monolithic matrix with the similar homogenous macropore structure. Such homogenous macropore structure would provide decreased mass-transfer resistance and large surface area of the monolithic matrix [27].

3.2. Thiol-ene reaction on surface of VTMS hybrid monolith

Characterization of effect of thiol-ene reaction on the surface of VTMS hybrid monolithic column was taken by a

probe of fluorescein isothiocyanate. Due to the incorporation of vinyltrimethoxysilane in the preparation of this organic–inorganic hybrid monolithic column, the obtained VTMS silica hybrid monolithic column thus possesses the ethylene linkage on the surface of the formed monolithic matrix. Then, mercaptoethylamine was introduced in prepared TMOS and VTMS silica hybrid monolithic columns, respectively. After that, the probe of fluorescein isothiocyanate was introduced in both silica hybrid monolithic columns. Finally, both columns were flushed with ethanol to remove spare fluorescein. Results display that TMOS silica hybrid monolithic column has no fluorescence reaction; on the contrary, VTMS silica hybrid monolithic column shows a tenacious fluorescence reaction, as it is shown in Fig. S-1. That means mercaptoethylamine was connected to the surface of the monolithic column, and the thiol-ene reaction was substantial.

3.3. Column efficiency of the VTMS hybrid monolithic column

The column efficiency of the VTMS hybrid monolithic column was evaluated in CEC by changing the applied voltage from 3 to 28 kV. Linear flow velocity, namely EOF velocity (v_{EOF}) is calculated by $v_{\text{EOF}} = L_d/t_m$, where L_d is the length of the column from the inlet to the detection window, t_m is the migration time of EOF marker. Plate height (H) is calculated by $H = L_d/N$, where N is the theoretical plate number obtained from the electrochromatograms by the 32 Karat software. The relationship between the flow velocity and the plate height of thiourea and phenylmethanol are demonstrated in Fig. 3. The lowest plate height of $\sim 3 \mu\text{m}$ was obtained, which corresponded to column efficiency (theoretical plates, N) of $\sim 320\,000$ plates/m. Also, it can be seen that the column remained at high efficiency in CEC with the linear velocity ranging from 0.5 to 2.5 mm/s.

3.4. Generation of EOF on the VTMS silica hybrid monolithic column

In an attempt to evaluate EOF on CEC column, thiourea was used as model compound. Generally, CEC with monolithic column bearing positively charged groups or negatively charged groups generate the anodic EOF or cathodic EOF, respectively. EOF is calculated by $\mu_{\text{EOF}} = L_d L_t / t_m V$, where L_d is the length of the column from the inlet to the detection window, L_t is the total length, t_m is the migration time of EOF marker, V is separation voltage. The VTMS silica hybrid monolithic column with octadecatrienyl groups and sulfonic groups resulted in a strong anion exchanger and, therefore, the generation of cathodic EOF, which can easily be controlled via the pH value of running buffer. Fig. 4 depicts the effect of running buffer pH on EOF on the VTMS modified with

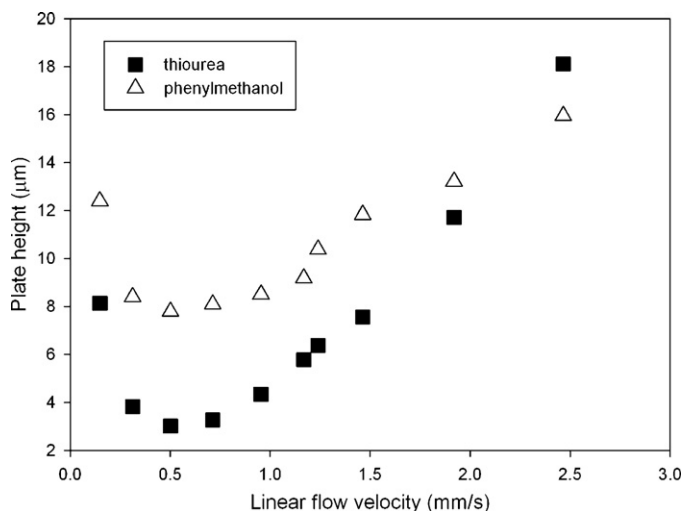


Fig. 3. van Deemter plots for thiourea and phenylmethanol on the C_{18} -MPS-VTMS silica hybrid monolithic column. Experimental conditions: 20 mM borate buffer at pH 9.0 containing 20% v/v ACN; separation voltage, from 3 to 28 kV; injection, 10 kV for 3 s; total length, 35 cm; effective length, 27 cm; detection wavelength, 203 nm.

3-mercaptopropylsulfonate (MPS) hybrid silica monolithic column. Normal EOF of $0.135 \text{ cm}^2 \text{ kV}^{-1} \text{ s}^{-1}$ was obtained by using the running buffer at pH 2.96. With the increase in pH value of running buffer, the normal EOF increased as the ionization of sulfonic groups became stronger. The normal EOF reached to the maximum of $0.244 \text{ cm}^2 \text{ kV}^{-1} \text{ s}^{-1}$ with the pH of the running buffer at 10.00. Whereas, the effect of running buffer pH on EOF on the VTMS without MPS is different from that on the VTMS modified with MPS. At the low pH of 2.96, 3.30 and 4.46, respectively, no EOF was generated. That means when the pH is below 5, the hydroxyl group on the surface of monolithic matrix cannot be hydrolyzed. Therefore, there is no free charge distribution on surface of monolithic column. With the increase in the pH value of running buffer, due to the increased amount of ionized hydroxyl groups, the EOF is generated, and reached to the maximum of $0.1998 \text{ cm}^2 \text{ kV}^{-1} \text{ s}^{-1}$ with the pH of the running buffer at 10.00. The above results indicate that the modification of ethylene bond was successful and notable. And the EOF on the prepared MPS-VTMS silica hybrid monolithic capillary column was pH dependent and can be controlled easily by altering the pH value of the running buffer. As shown in Fig. 4, the normal

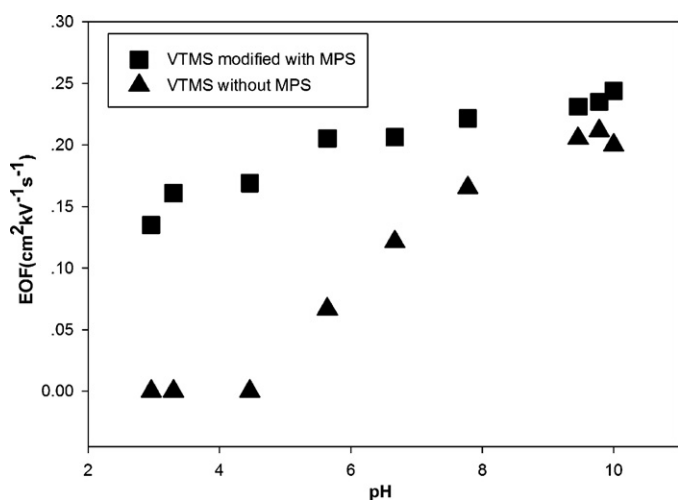


Fig. 4. The relationship of EOF generated on silica hybrid monolithic column and pH value of mobile phase. Conditions: 4 mM $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ containing 30% ACN at different pH value; separation voltage, 20 kV; injection, 10 kV for 3 s; total length, 35 cm; effective length, 27 cm; detection wavelength, 203 nm.

EOF increased slightly when the pH of the running buffer increase above 5. And with a series of pH optimizing experiments, pH 5 was found to be optimal for small molecule weight compounds. In spite of that, as the separation of biomolecular compounds need neutral buffer, so that pH 7 was chosen for analysis of BSA tryptic digests as shown in Fig. 7.

3.5. Retention mechanism of hybrid monolithic stationary phase

The effect of ACN concentration in the mobile phase on the retention of weak acidic solutes in CEC with hybrid column was studied. As shown in Fig. S-2, its hydrophilic effect on hybrid silica monolithic column is in evidence. Here, the effect of organic modifier content in the mobile phase was studied with thiourea, phenylmethanol and naphthalene as exemplar compounds by varying the volume percentage of acetonitrile (ACN) from 10% to 90% in the mobile phase.

The influence of ACN content in the mobile phase on the retention time of these three tested compounds is shown in Fig. 5A. Thiourea is used as a normal dead volume marker. With the ACN content in the mobile phase increasing from 10 to 50%, the retention time of thiourea slightly decreased. Then, it changed a little as the ACN content increased sequentially. Phenylmethanol behaved similar to thiourea. As a nonpolar compound, naphthalene's retention time was much longer than the other two compounds at low ACN concentration. The decrease tendency of its retention time was drastic when the ACN content in the mobile phase increased from 10 to 50%. And then, the retention time decreased mildly when ACN content further increased. Notably, when ACN content increased to about 65%, a crossing point was observed. This interesting phenomenon indicated their reverse chromatography behavior, their peaks overlapped when the ACN content achieved about 60% and the peak position changes when the ACN content increased to above 70%. The nonpolar compound eluted faster in high ACN content mobile phase than the polar compound. On the contrary, in low ACN content mobile phase, the polar molecule was eluted faster than nonpolar molecule. As for thiourea, its retention time leveled off with the variation of ACN content. And for the tag of dead time, the retention time of thiourea was stationary and advisable. In summary, when the ACN content raised to a certain degree, their peaks overlapped and the separation performance was affected. Therefore, the ACN content in buffer must be controlled at a low level to provide a sufficient peak capacity for separating homologous compounds. We finally chose 10% ACN for separation of small molecular weight compounds, and 15% ACN for separation of biomolecular compounds, respectively, as the optimum option.

As we can see from Fig. 5B–D, respectively, there is another vital factor which influences the retention time of the tested compounds. The different retention time of analytes in the same mobile phase is probably caused by the modification of the surface on the C_{18} -MPS-VTMS silica hybrid monolithic column. We decorated the monolithic matrix with different mole ratio of MPS/ C_{18} of 1:0, 3:1, 1:1, 1:3, respectively. Fig. 7 shows the difference between the four kinds of modified silica hybrid monolithic column. It is obvious that the retention time of nonpolar compound naphthalene becomes longer with the increase of hydrophobic C_{18} group. However, as a tag of dead time, the retention time of thiourea remained stable. Hence, we chose ratio mole ratio of MPS/ C_{18} of 1:3 for following separation capability experiments. In that case, it can integrate both remarkable reserve capillary of C_{18} and free charge for EOF of MPS.

3.6. Applications

3.6.1. Analysis of small molecule weight compounds

In our experiments, C_{18} and MPS were used as post-modification reagents. The sulfo-acid group of MPS is negatively charged in

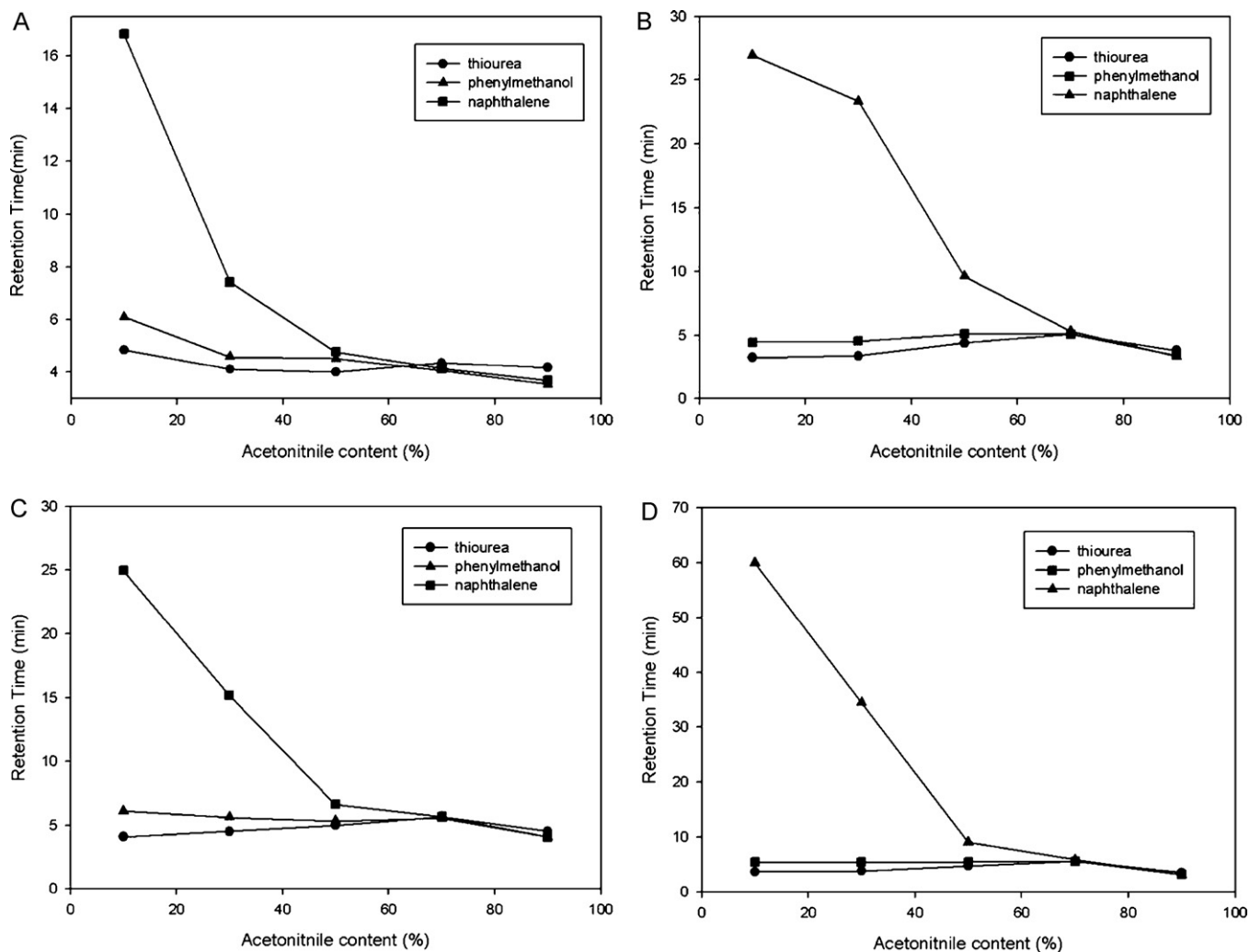


Fig. 5. The relationship between the retention time of thiourea, phenylmethanol, naphthalene on the hybrid silica monolithic column of different mole fractions of C_{18} /MPS and the ACN concentration of the mobile phase. (A) 100% MPS, (B) 75% MPS: 25% C_{18} , (C) 50% MPS: 50% C_{18} and (D) 25% MPS: 75% C_{18} . Conditions: the same as shown in Fig. 5.

buffers, so it can function as cation-exchanger and interact with positively charged compounds through electrostatic interaction. Another decoration reagent, C_{18} , provides the monolithic column with the character of hydrophobicity. A mixture of closely related neutral compounds of benzene series including benzene, toluene, ethylbenzene, isopropyl benzene, p-xylene and butyl benzene was tested, to see if the proposed technique could be used to separate them that are difficult to retain and separate by conventional CEC. It can be seen from Fig. 6, that good separation was obtained.

3.6.2. CEC analysis of bovine serum albumin (BSA) tryptic digests with C_{18} -MPS-VTMS silica hybrid monolithic column

Silica-based monolithic column has hierarchical meso- and macroporous structures, which lead to the fast mass transfer kinetics and low backpressure during separation [28]. On account of such unparalleled properties, the separation of peptide mixtures derived from the tryptic digestion of BSA was attempted using the VTMS silica hybrid monolithic column by a CEC system. The base peak chromatogram of the tryptic digestion is illustrated in Fig. 7. It can be seen that this hybrid monolithic column did exhibit sufficient hydrophobicity due to the MPS moiety in the silica monolithic matrix. On the basis of the database search of

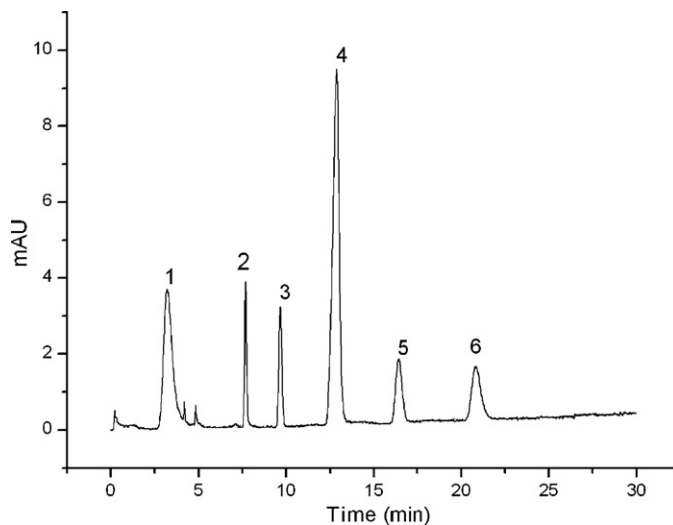


Fig. 6. Separation of alkylbenzenes on the monolithic column. Conditions for column: 4 mM Na_2HPO_4 - KH_2PO_4 at pH 5.0 containing 10% ACN; separation voltage, 20 kV; injection, 10 kV for 3 s; total length, 35 cm; effective length, 25 cm; detection wavelength, 203 nm. Analytes: 1 - benzene; 2 - toluene; 3 - ethylbenzene; 4 - isopropyl benzene; 5 - p-xylene; 6 - butyl benzene.

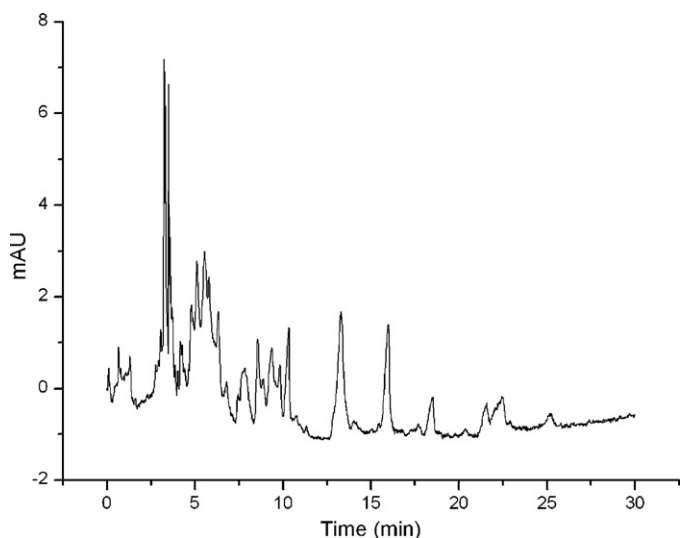


Fig. 7. Separation of tryptic digests of BSA on column, conditions for column: $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ at pH 7.0 containing 15% ACN. Samples: tryptic digests of BSA.

the obtained chromatogram of BSA tryptic digest, 24 unique peptides were found. The results demonstrated that it is an excellent means to introduce the thiol-functional groups into the silica-monolithic matrix via this thiol-ene click chemistry process to provide the desirable functionalities for the separation of complex samples.

3.6.3. Advantages of separation capability

To study the separation capability compared with conventional capillary zone electrophoresis (CZE), a series of small charged molecules were separated on a C_{18} -MPS-VTMS silica hybrid monolithic column and a bare capillary column. The separation mechanism of charged compounds on the monolithic column is the combination of electrophoretic mobility and ion exchange interaction. A mixture of aromatic amines including *p*-phenylenediamine, aniline, *p*-toluidine, *N*-methyl aniline, *N,N*-dimethylaniline and diphenylamine were almost coeluted on the bare capillary column as shown in Fig. 8-A1. In contrast, C_{18} -MPS-VTMS silica hybrid

monolithic column gave a baseline separation of all the compounds with much longer retention times (Fig. 8-A2).

On the other hand, a mixture of six organic acids was used to further investigate the separation capability of the column. Fig. 8B shows that better separation of these acids was obtained on the prepared monolithic column (B1) compared with bare capillary column (B2). The elution order on the column was salicylic acid, mandelic acid, terephthalic acid, *o*-chlorobenzoic acid, benzoic acid and hippuric acid. The analysis of acidic compounds is relatively difficult on bare capillary column. However, the charged solutes were all eluted before void time on C_{18} -MPS-VTMS silica hybrid monolithic column, which showed the good stability of the monolithic column.

Moreover, we chose three peptides to investigate the separation effect of prepared monolithic column and micellar electrokinetic capillary chromatography (MEKC). Fig. 8-C2 demonstrates that the prepared monolithic column can give effective and fast separation of peptides, the elution order on the column was H-Cys-Asp-Glu-Val-Asp-Tyr-OH (CY-6), H-Cys-Gly-Gly-Asp-Glu-Val-Asp-Tyr-OH (CY-9) and H-Cys-Gly-Asp-Asp-Gly-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Tyr-OH (CY-15) which possibly caused by the sulfoacid groups that contribute to generating a strong electroosmotic flow, thus enhancing the native electrophoretic migration of peptides. Nevertheless, the open tube column with SDS coating can also separate the peptides, the speed is lower and UV absorption is much weaker (Fig. 8C2). Chromatogram map shows that this monolithic column is advantageous over traditional CEC approaches including CZE and MEKC.

3.7. Reproducibility and detection limit

The reproducibility and detection limit of the C_{18} -MPS-VTMS silica hybrid monolith was evaluated using CY-9 as the test solute. The intraday relative standard deviation (RSD) for the retention times was 1.97% ($n=3$). The inter day RSD for the retention times was 4.23% ($n=3$); the detection limit was 50 μM . A C_{18} -MPS-VTMS silica hybrid monolithic column showed stable chromatographic performance for more than three months. These results demonstrated that the prepared VTMS silica hybrid monolithic column has good stability and reproducibility.

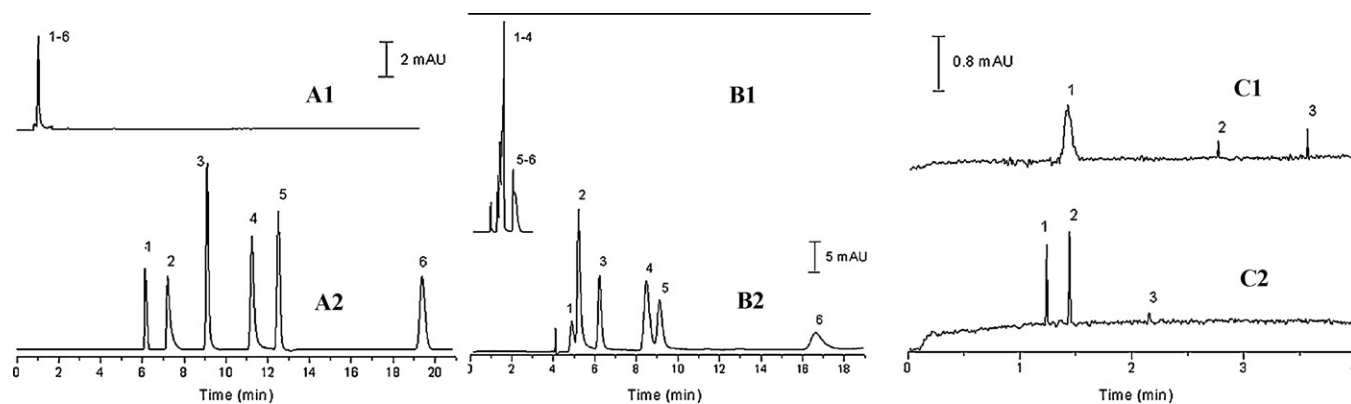


Fig. 8. Separation of charged molecules using conventional CZE and MEKC and C_{18} -MPS-VTMS silica hybrid monolithic column. (A) Separation of aromatic amines on monolith column and bare capillary. 1 – *p*-Phenylenediamine; 2 – aniline; 3 – *p*-toluidine; 4 – *N*-methyl aniline; 5 – *N,N*-dimethylaniline; 6 – diphenylamine. Conditions: 20 mM borate buffer at pH 9.0 containing 20% ACN; (A1) C_{18} -MPS-VTMS silica hybrid monolithic column; (A2) bare column. (B) Separation of organic acids on monolith column and bare capillary. 1 – Salicylic acid; 2 – mandelic acid; 3 – terephthalic acid; 4 – *o*-chlorobenzoic acid; 5 – benzoic acid; 6 – hippuric acid. Conditions: 20 mM borate buffer at pH 7.4 containing 20% ACN; (B1) C_{18} -MPS-VTMS silica hybrid monolithic column; (B2) bare column. (C) Separation of peptides on monolith column and bare capillary. 1 – CY-6; 2 – CY-9; 3 – CY-15; conditions: (C1) C_{18} -MPS-VTMS silica hybrid monolithic column; 20 mM borate buffer at pH 9.0 containing 20% ACN; (C2) bare column; 20 mM borate buffer and 10 mM SDS at pH 9.0 containing 20% ACN; Other conditions are the same as shown in Fig. 3.

4. Conclusions

A novel kind of vinyl-functionalized hybrid silica monolithic column was prepared via catalytic sol–gel process. The use of thiol-ene click chemistry in hybrid monolithic matrix surface modification enables the induction of anticipated functional groups through surface modification. The reaction parameters including monomer ratio, pH and the water content are tailored for controlling the porosity, mechanical property and chemical structure of the resulting gel. The optimized monolithic column showed a homogeneous macroporous morphology without detachment, and satisfactory separation of small-molecule compounds or biomolecular compounds was achieved. The thiol-ene click chemistry provides a new way for preparing organic–inorganic monolithic matrix of surface modification with good functionalities.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (Nos. 20975032, 21075031) and the National Basic Research Program of China (973 Program, No. 2009CB421601).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.01.009.

References

- [1] F. Svec, J.M.J. Fréchet, *Anal. Chem.* 64 (1992) 820–822.
- [2] Q.C. Wang, F. Svec, J.M.J. Fréchet, *Anal. Chem.* 65 (1993) 2243–2249.
- [3] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, *Anal. Chem.* 70 (1998) 2288–2295.
- [4] I. Gusev, X. Huang, C. Horváth, *J. Chromatogr. A* 855 (1999) 273–290.
- [5] L.J. Sondergeld, M.E. Bush, A. Bellinger, M.M. Bushey, *J. Chromatogr. A* 1004 (2003) 155–165.
- [6] M.T. Dulay, R.P. Kulkarni, R.N. Zare, *Anal. Chem.* 70 (1998) 5103–5107.
- [7] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, N. Tanaka, *Anal. Chem.* 72 (2000) 1275–1280.
- [8] D. Allen, Z. El Rassi, *Electrophoresis* 24 (2003) 3962–3976.
- [9] J.D. Hayes, A. Malik, *Anal. Chem.* 72 (2000) 4090–4099.
- [10] E. Haghedooren, L. Peeters, S. Dragovic, J. Hoogmartens, E. Adams, *Talanta* 78 (2009) 665–671.
- [11] G. Guiochon, *J. Chromatogr. A* 1168 (2007) 101–168.
- [12] H. Colon, X. Zhang, J.K. Murphy, J.G. Rivera, L.A. Colon, *Chem. Commun.* (2005) 2826–2828.
- [13] L. Yan, Q. Zhang, *J. Chromatogr. A* 1121 (2006) 92–98.
- [14] M. Wu, R. Wu, F. Wang, L. Ren, J. Dong, Z. Liu, H. Zou, *Anal. Chem.* 81 (2009) 3529–3536.
- [15] Z. Zhang, M. Wu, R.a. Wu, J. Dong, J. Ou, H. Zou, *Anal. Chem.* 83 (2011) 3616–3622.
- [16] A. Sayari, S. Hamoudi, *Chem. Mater.* 13 (2001) 3151–3168.
- [17] M.H. Wu, Y.Z. Chen, R.A. Wu, R.B. Li, H.F. Zou, B. Chen, S.Z. Yao, *J. Chromatogr. A* 1217 (2010) 4389–4394.
- [18] Q. Cao, Y. Xu, F. Liu, F. Svec, J.M.J. Fréchet, *Anal. Chem.* 82 (2010) 7416–7421.
- [19] Y. Tian, L. Zhang, Z. Zeng, H. Li, *Electrophoresis* 29 (2008) 960–970.
- [20] B. Preinerstorfer, W. Bicker, W. Lindner, M. Lämmerhofer, *J. Chromatogr. A* 1044 (2004) 187–199.
- [21] H.C. Kolb, M.G. Finn, K.B. Sharpless, *Angew. Chem. Int. Ed.* 40 (2001) 2004–2021.
- [22] C.W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* 67 (2002) 3057–3064.
- [23] J.E. Moses, A.D. Moorhouse, *Chem. Soc. Rev.* 36 (2007) 1249–1262.
- [24] Y. Hu, X. Liang, J. Wang, Z. Zheng, X. Hu, *J. Org. Chem.* 68 (2003) 4542–4545.
- [25] A.K. Tucker-Schwartz, R.A. Farrell, R.L. Garrell, *J. Am. Chem. Soc.* 133 (2011) 11026–11029.
- [26] C. Xie, M. Ye, X. Jiang, W. Jin, H. Zou, *Mol. Cell. Proteomics* 5 (2006) 454–460.
- [27] J. Ou, X. Li, S. Feng, J. Dong, X. Dong, L. Kong, M. Ye, H. Zou, *Anal. Chem.* 79 (2006) 639–646.
- [28] N. Ishizuka, H. Kobayashi, H. Minakuchi, K. Nakanishi, K. Hirao, K. Hosoya, T. Ikegami, N. Tanaka, *J. Chromatogr. A* 960 (2002) 85–96.