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Preparation of phenyl-silica hybrid monolithic column with "one-pot" process for capillary liquid chromatography

Zhenbin Zhang[a,b], Hui Lin[a,b], Junjie Ou[a,**], Hongqiang Qin[a,b], Ren'an Wu[a], Jing Dong[a], Hanfa Zou[a,*]

[a] CAS Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R & A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

A phenyl-silica hybrid monolithic column for capillary liquid chromatography (cLC) was prepared through "one-pot" process by con-currently using benzyl methacrylate and alkoxysilanes. The effects of the molar ratio of tetramethoxysilane/vinyltrimethoxysilane (TMOS/VTMS), polycondensation temperature, content of supramolecule template (cetyltrimethylammonium bromide, CTAB), ratio of N,N′-dimethylformamide/methanol (v/v), the volume of benzyl methacrylate on the morphologies of the prepared phenyl-silica hybrid monolithic columns were investigated in detail. The permeability of the hybrid monolithic column was calculated as 3.23×10^{-13} m^2, and the minimum plate height was determined as 8.38 μm which corresponding to 119,000 theoretical plates per meter. Separation of various neutral, polar and basic analytes as well as small peptides on the hybrid monolithic column was achieved by cLC and showed high efficiency and satisfactory reproducibility. Moreover, the prepared hybrid monolithic column was also applied for the analysis of tryptic digests of bovine serum albumin (BSA), ovalbumin, α-casein, cytochrome C and myoglobin by cLC tandem mass spectrometry (cLC–MS/MS), and the results showed that the separation performance was close to that of the octadecylsilane (C18) packed capillary column which demonstrating its potential in proteome analysis. Moreover, since the prepolymerization system was mainly consisted of organic solvents (methanol and N,N′-dimethylformamide), various hydrophobic monomers could be potentially used to prepare organic-silica hybrid monolithic columns through "one-pot" approach.

1. Introduction

Capillary liquid chromatography (cLC) has been gaining the rising attention in separation field due to its distinct merits [1–6], such as high column efficiency, good reproducibility, low consumption of sample and solvent, strongly reduced chromatographic dilution during separation process, great compatibility with mass spectrometry (MS), etc. Taking advantage of the great sensitivity by coupling capillary columns to MS, the cLC–MS system has been widely applied in the fields of proteomics, genomics and metabolomics [7–9].

In the past few decades, a comprehensive attention in the field of cLC has been paid to the novel state-of-the-art stationary phases, the porous monoliths, which were synthesized by *in situ* preparation of polymer- or silica-based monoliths with various specific functionalities arising from the versatile chemical reactions and/or post-modifications [10,11]. Compared to conventional particulate packed columns, the *in situ* prepared monoliths are chemically attached onto the inner wall of capillaries and no retaining frits are required to support the monolithic matrixes. Based on the nature of the matrix, monolithic columns can be mainly categorized into three types: organic polymer-based, silica-based and organic-silica hybrid monolithic columns. Organic polymer-based monolithic columns, such as polystyrenes [12–14], polymethacrylates [15,16], and polyacrylamides [17,18] have good stability towards pH changes and are easy to tune the surface chemistry by tailoring both porogenic solvents and monomers in the prepolymerization solutions. Unfortunately, the swelling in organic solvents would lead to the short lifetime and unsatisfactory retention reproducibility of organic polymer-based monoliths in some cases. Alternatively, the silica-based monolithic column demonstrates better solvent resistance and higher mechanical stability [19,20]. But the surface functionalization of silica-based monolithic column is still time-consuming and laborious. In recent few years, the organic-silica hybrid monolithic column has attracted great attentions since it combines the merits of both organic polymer-based and silica-based monoliths, such as ease of preparation, pH stability and good mechanical stability [21,22].

* Corresponding author. Tel.: +86 411 84379610; fax: +86 411 84379620.
** Corresponding author. Tel.: +86 411 84379576; fax: +86 411 84379620.
E-mail addresses: junjieou@dicp.ac.cn (J. Ou), hanfazou@dicp.ac.cn (H. Zou).

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We have recently developed a simple "one-pot" approach for preparing the organic-silica hybrid monolithic capillary columns, which represented a new way to prepare organic-silica hybrid monoliths with variety of organic monomers [23]. However, there was a great limitation that the organic monomers must be water-soluble in order to form a homogeneous prepolymerization mixture. More recently, the reaction system composed of methanol (MeOH), N,N′-dimethylformamide (DMF) and water has been developed to overcome the limitation of using water-soluble organic monomers in previously developed "one-pot" method [24].

It is well known that octadecylsilane (C18) stationary phase is one of the most widely used stationary phases for reversed phase (RP) liquid chromatography due to its great resolving ability for a wide range of analytes. Meanwhile, phenyl-type stationary phases are also proven the resolving ability for the separation of species in which π–π interactions may be exploited during the retention process [25]. However, current standard methods for the separation and analysis of polycyclic aromatic hydrocarbons (PAHs) with C18 stationary phase involve longer analysis time and using higher percentage of organic solvent in mobile phase, usually acetonitrile (ACN). The preparation of organic–inorganic hybrid phenyl monolithic column based on the co-condensation of tetraethoxysilane and phenyltriethoxysilane by a two-step catalytic sol–gel process has been reported [26], however, the process would be suffered from the limitation and difficulty of using or synthesizing the functional organic-trialkoxysilanes. Alternatively, the phenyl-silica hybrid monolithic column for cLC was prepared by con-currently using benzyl methacrylate through "one-pot" approach in this work. The effects of various preparation conditions on the morphologies of the prepared phenyl-silica hybrid capillary monolithic columns were investigated in detail. The morphology of the column was characterized by scanning electron microscopy (SEM). Various neutral, polar and basic analytes as well as small peptides were separated on the prepared phenyl-silica hybrid monolithic column. The resulting column was also applied in the analysis of tryptic digests of mixed proteins consisting of bovine serum albumin (BSA), ovalbumin, α-casein, cytochrome C and myoglobin by cLC–MS/MS for demonstrating its potential in proteome analysis.

2. Experimental

2.1. Chemicals and materials

Benzyl methacrylate was purchased from Sigma Chemical Co. (St Louis, MO, USA). Vinyltrimethoxysilane (VTMS) and cetyltrimethylammonium bromide (CTAB) were purchased from Aldrich (Milwaukee, WI, USA). Tetramethoxysilane (TMOS) was obtained from Chemical Factory of Wuhan University (Wuhan, China). 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Plant (Shanghai, China) and re-crystallized in ethanol prior to use. Fused-silica capillary with 75 μm i.d. and 375 μm o.d. was purchased from the Reafine Chromatography LTD. (Hebei, China). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Shanghai Chemical Plant (Shanghai, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA). Other chemical reagents were of analytical grade.

2.2. Preparation of the phenyl-silica hybrid capillary monolithic column

The schematic preparation of the phenyl-silica hybrid monolithic capillary column was illustrated in Fig. 1. Before the preparation of the hybrid monolithic column, the fused-silica capillary was pretreated by rinsing by 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 the capillary was dried by nitrogen stream at room temperature. For preparation of the phenyl-silica hybrid monolithic column, a prepolymerization mixture was prepared by mixing MeOH (190 μL), DMF (60 μL), H_2O (62 μL), TMOS (125 μL), VTMS (150 μL), CTAB (5 mg), benzyl methacrylate (30 μL), 10 μM ammonia solution (50 μL), and AIBN (1 mg) at room temperature and sonicating for 10 min to obtain a homogeneous solution. Using a syringe and a short Teflon sleeve, the mixture was manually introduced into a 40-cm pretreated capillary. By sealing both ends of the capillary with two pieces of rubbers, the capillary was incubated at 40 °C and 60 °C for 12 h, respectively, for condensation and polymerization. The obtained phenyl-silica hybrid monolithic capillary column was then flushed with MeOH to remove CTAB and other residuals.

2.3. Instruments and methods

cLC experiments were performed on an Agilent 1100 LC system (Hewlett–Packard) equipped with a micropump. The flow rate of pump was set at 40–200 μL/min. For obtaining a flow rate of nanoliters per minute, a T-union connector was used to serve as a splitter, with one end connected to the monolithic column and another end connected to a blank capillary (50 μm i.d.). The actual flow rate in the monolithic column was 80–400 nL/min, resulting in split ratio about 500:1. A 7725i injector equipped with a 20 μL sample loop was connected between the micropump and T-union to load the samples with split mode. The other end of monolithic column was connected to a 15-cm long blank capillary (75 μm i.d.) by polymer tubing, where a detection window was made by removing the polyimide coating of a fused-silica capillary with a razor blade. A UV detector K-2501 from Knauer (Berlin, Germany) was used, and the detection wavelength was set at 214 nm. All chromatographic data were collected and evaluated using HW-2000 software from Qianpu Software LTD. (Shanghai, China). The retention factor (k') was defined as $(t_r - t_0)/t_0$, where t_r and t_0 represent the retention times of an analytes and an unretained compound, respectively.

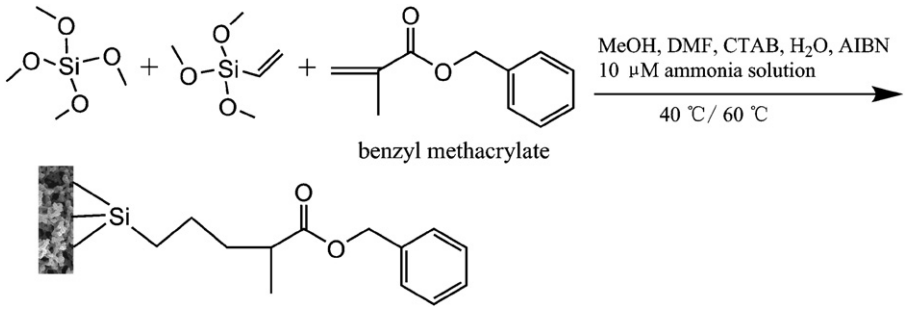


Fig. 1. Schematic preparation of phenyl-silica hybrid monolith.

SEM images were obtained by using a JEOL JSM-5600 scanning electron microscope.

2.4. Tryptic digestion of proteins

The proteins including BSA, ovalbumin, α-casein, cytochrome C and myoglobin were mixed with equal molar ratio and dissolved in 1 mL of denaturing buffer containing 8 M urea and 50 mM ammonium bicarbonate. After added 20 μL dithiothreitol (50 mM), the mixture was incubated at 60 °C for 1 h to reduce the disulfide bonds of proteins. Subsequently, 40 μL iodoacetamide (50 mM) was added, and then, the mixture was incubated in dark at room temperature for 45 min. Finally, the mixture was diluted 10 fold with 50 mM ammonium bicarbonate buffer (pH 8.2) and digested with trypsin at the ratio of enzyme-to-substrate of 1:40 (w/w) at 37 °C for 16 h. After digestion, the pH value of the obtained solution was adjusted to 2.7 by 10% trifluoroacetic acid. Followed by solid-phase extraction with a homemade C18 cartridge, the tryptic digests were dried in vacuum concentrator and dispersed in 0.1% formic acid (FA) with the concentration of 1 pmol/μL before cLC–MS/MS analysis.

2.5. Mass spectrometry detection

The cLC–MS/MS experiments were performed by interfacing a surveyor MS pump to a Finnigan LTQ ion trap mass spectrometer (Finnigan MAT, ThermoFinnigan, San Jose, CA). Buffer A was water (0.1% FA), and buffer B was 100% ACN (0.1% FA). Tryptic digests were automatically injected onto the column with buffer A for 3 min at the flow rate of 5 μL/min. After then, the trapped peptides were separated at a flow rate of ca. 150 nL/min on a phenyl-silica hybrid monolithic column (12 cm × 75 μm i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of the monolithic capillary column [27]. The LTQ linear ion trap mass spectrometer equipped with a nanospray ion source. The temperature of the ion transfer capillary was set at 200 °C. The spray voltage was set at 1.8 kV, and the normalized collision energy was set at 35.0%. One microscan was set for each MS and MS/MS scan. All MS and MS/MS spectra were acquired in the data dependent mode. The mass spectrometer was set that one full MS scan was followed by six MS/MS scans on the six most intense ions. The dynamic exclusion function was set as follows: repeat count 2, repeat duration 30 s, and exclusion duration 90 s. System control and data collection were done by Xcalibur software version 1.4 (Thermo). The scan range was set from m/z 400 to m/z 1600.

2.6. Data analysis

The acquired MS/MS spectra were searched on a database using the MASCOT (version 2.2.04) protein identification platform (Matrix Science, London, UK), and the MS/MS spectra of pull-down were searched against IPI_bovine BOVIN_3.32 (32,946 sequences; 16,109,453 residues). Cysteine residues were searched as fixed modification of 57.0215 Da, and methionine residues as variable modification of 15.9949 Da. Peptides were searched using fully tryptic cleavage constraints and up to two internal cleavages sites were allowed for tryptic digestion. The mass tolerances were 2 Da for parent masses and 1 Da for fragment masses.

3. Results and discussion

3.1. Preparation of phenyl-silica hybrid monolithic column

Our works aimed at preparation of the phenyl-silica hybrid monolithic column by directly incorporating the phenyl functionalities into the porous monolithic matrixes. Due to the hydrophobic property of the TMOS, VTMS and benzyl methacrylate, these reactants could not be mixed homogeneously when water was utilized as the sole solvent in the reaction. On the other hand, when water was replaced by organic solvents (DMF and MeOH), or a little water was used in the prepolymerization mixture, the hydrolysis of both TMOS and VTMS could not be proceeded completely, therefore, the subsequent condensation and polycondensation were inhibited. To address the aforementioned problems, a mixture of water, MeOH and DMF was used. Detailed studies demonstrated that the most suitable volume of water was 62 μL in total 637 μL polymerization mixtures. The physical and chromatographic properties of the monolithic matrixes are easily controlled by changing various factors during the preparation process. In this work, the effect of the molar ratio of TMOS/VTMS, polycondensation temperature, content of supramolecule template (CTAB), ratio of DMF/MeOH and volume of benzyl methacrylate on the column morphology and chromatographic properties were systematically studied.

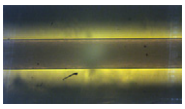
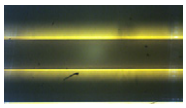
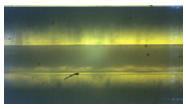
The formation of the phenyl-silica hybrid monolithic capillary column involves three major reactions: hydrolysis, polycondensation and copolymerization of the precondensated siloxanes and benzyl methacrylate. The effect of the molar ratio of VTMS/TMOS in the reaction mixture on the formation of hybrid monolithic column was investigated. The lower content of VTMS (the molar ratio of VTMS/TMOS was less than 0.542:1) in the reaction mixture would result in a transparent monolith inside the capillary, while the higher content of VTMS (the molar ratio of VTMS/TMOS was more than 0.95:1) would result in the slack monolith. Only the molar ratio of VTMS/TMOS at 0.82:1 could result in homogeneous and semi-transparent monolithic matrixes within the confine of a capillary. It was worth noting that the molar ratio of VTMS/TMOS in the prepolymerization mixture was higher than the previously reported one of 0.25:1 [28], which means there were more than 3 times higher vinyl groups in the monolith available for copolymerization of benzyl methacrylate.

As we know, the morphology and permeability of the resultant monolithic matrix was greatly affected by the polycondensation temperature. The columns were prepared at the polycondensation temperature of 35 °C, 40 °C and 45 °C, respectively. The results indicated that the monolithic matrix was seriously detached from the inner capillary wall when the polycondensation temperature was 35 °C. Further increasing the condensation temperature to 40 °C, the obtained monolithic matrix was homogeneous and fully filled in the capillary. However, when the condensation temperature was 45 °C, it would result in the transparent monolith with low permeability forming inside the capillary.

The surfactant CTAB acts as supramolecular template in the formation of the sol–gel monolith. The formation of phenyl-silica hybrid mesostructure is the result of the delicate balance of two competitive processes, organization of the template and polymerization. Therefore, the content of CTAB has dramatic effect on the column morphology and chromatographic properties. According to the experimental results, it was found that the hybrid monolithic column could not be obtained if no CTAB was added in the prepolymerization mixture. The permeability of the monolithic column was improved with an increase of the content of CTAB in the prepolymerization mixture. However, if the content of CTAB was higher than 14.8 mg CTAB in total 637 μL polymerization mixture, it would lead to a substantial deterioration of column efficiency, even the detachment of the monolithic matrix from the inner wall of the capillary (as column A3 in Table 1). The optical microscopy images of the columns prepared with 2.5 mg, 4.9 mg, 14.8 mg CTAB in total 637 μL polymerization mixtures, respectively, responding to A1, A2 and A3, are shown in Table 1.

The initial purpose of adding DMF or MeOH in the prepolymerization mixture was to dissolve the hydrophobic organic monomer. Therefore, more benzyl methacrylate would be dissolved by

Table 1
The effect of CTAB content on the formation of phenyl-silica hybrid monolith.[a]

Column	A1	A2	A3
CTAB/(mg)	2.5	4.9	14.8
Permeability	1.3×10^{-13} m^2	3.23×10^{-13} m^2	3.23×10^{-10} m^2
Morphology			

[a] Other preparation conditions: TMOS, 125 μL; VTMS, 150 μL; MeOH, 190 μL; H_2O, 62 μL; DMF, 60 μL; 10 μM ammonia solution, 50 μL; benzyl methacrylate, 30 μL; condensation temperature, 40 °C; AIBN, 1 mg.

increasing the volume of DMF or MeOH in the prepolymerization mixture. Additionally, the presence of DMF in the prepolymerization mixture would act as drying control chemical additive, which may avoid the shrinkage of the resulting monolithic columns [29]. As a result, the ratio of DMF/MeOH has great effect on the morphology and permeability of the prepared capillary monolithic column. When the ratio of DMF/MeOH (v/v) changed from 20:230 to 60:190, more benzyl methacrylate could be dissolved in the prepolymerization mixture, and no significantly changes in the morphology and permeability of the resulting capillary monolithic column were observed. However, when the ratio of DMF/MeOH (v/v) is further increased to 100:150, the siloxanes could not be dissolved in the prepolymerization mixture to form a homogeneous solution, and the prepared monolithic matrix in the capillary is transparent and inhomogeneous. As a result, the ratio of DMF/MeOH (v/v) at 60:190 was selected in the following experiments.

Additionally, the effect of the content of benzyl methacrylate on the morphology of the hybrid monolithic column was also investigated. Based on the results of experiments, it was found that the morphology of the obtained monolithic column was almost unaffected by the content of benzyl methacrylate when its volume was less than 50 μL in the total 637 μL reaction solution. However, further increasing the content of benzyl methacrylate would lead to formation of transparent matrix in the capillary, and difficultly allowing the mobile phase flowing through.

3.2. Characterization of the phenyl-silica hybrid monolithic column

The SEM images of the phenyl-silica hybrid monolithic column are presented in Fig. 2. As shown in Fig. 2a, a uniform monolithic matrix with large through-pores was observed. It can be seen from Fig. 2b that the phenyl-silica monolithic matrix was well attached to the inner wall of a capillary. It may be attributed to the silanol groups at the inner wall of the capillary taking part in the polycondensation during the monolithic column preparation. Additionally, the mechanical stability of the obtained hybrid monolithic column was examined by connecting the column to a cLC pump with the flow rate ranging from 40 to 120 μL/min using ACN/H_2O = 40/60 (v/v) as mobile phase. The back pressure increased linearly with an increase of flow rate, which indicated that the hybrid monolith possessed good mechanical stability under the pressure of 20 MPa. Using the Darcy's Law of permeability $B_0 = F\eta L/(\pi r^2 \Delta P)$ [30], where F is the flow rate of mobile phase, η is the viscosity of mobile phase and the value of 0.801 cP was from Ref. [31], L is the effective length of column, r is the inner radius of the column and ΔP is the pressure drop of the column, the permeability of the monolithic column was calculated as 3.23×10^{-13} m^2, which indicated a good permeability of the prepared monolithic column.

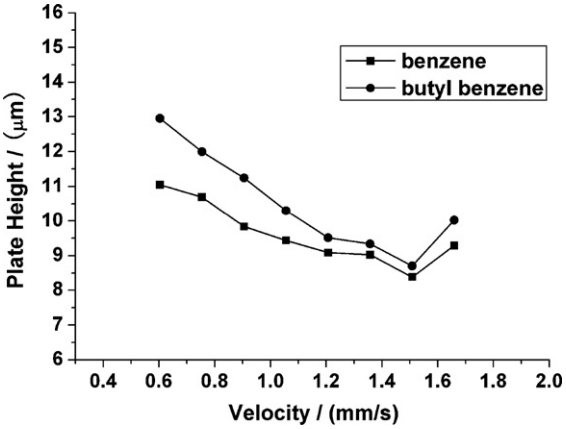


Fig. 3. Dependence of the plate height of analytes on the linear velocity of mobile phase by the phenyl-silica hybrid monolithic capillary column. Experimental conditions: effective length of 20 cm × 75 μm i.d.; mobile phase, TEAA buffer at pH 4.2 containing 50% ACN; test compounds, benzene and butyl benzene; detection wavelength, 214 nm.

The column efficiency of the phenyl-silica hybrid monolithic column was evaluated on cLC by changing the flow rate from 40 to 130 μL/min (before split). The relationship between the flow velocity and the plate height of alkylbenzenes was shown in Fig. 3. The lowest plate height of ca. 8.4 μm was obtained, corresponding

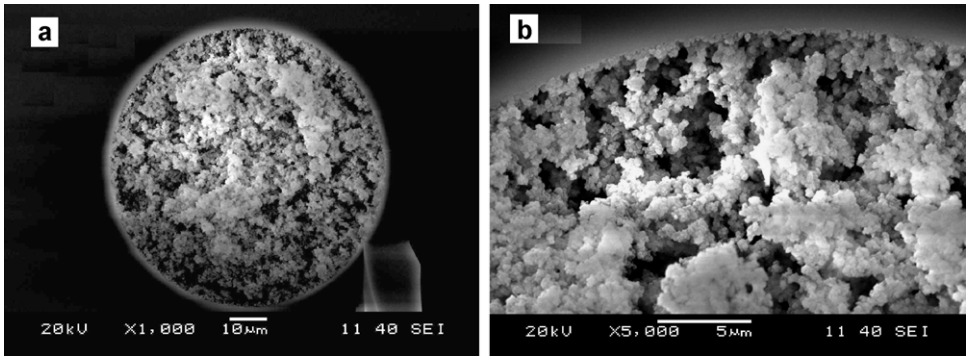


Fig. 2. SEM images of phenyl-silica hybrid monolith: (a) 1000× and (b) 5000×.

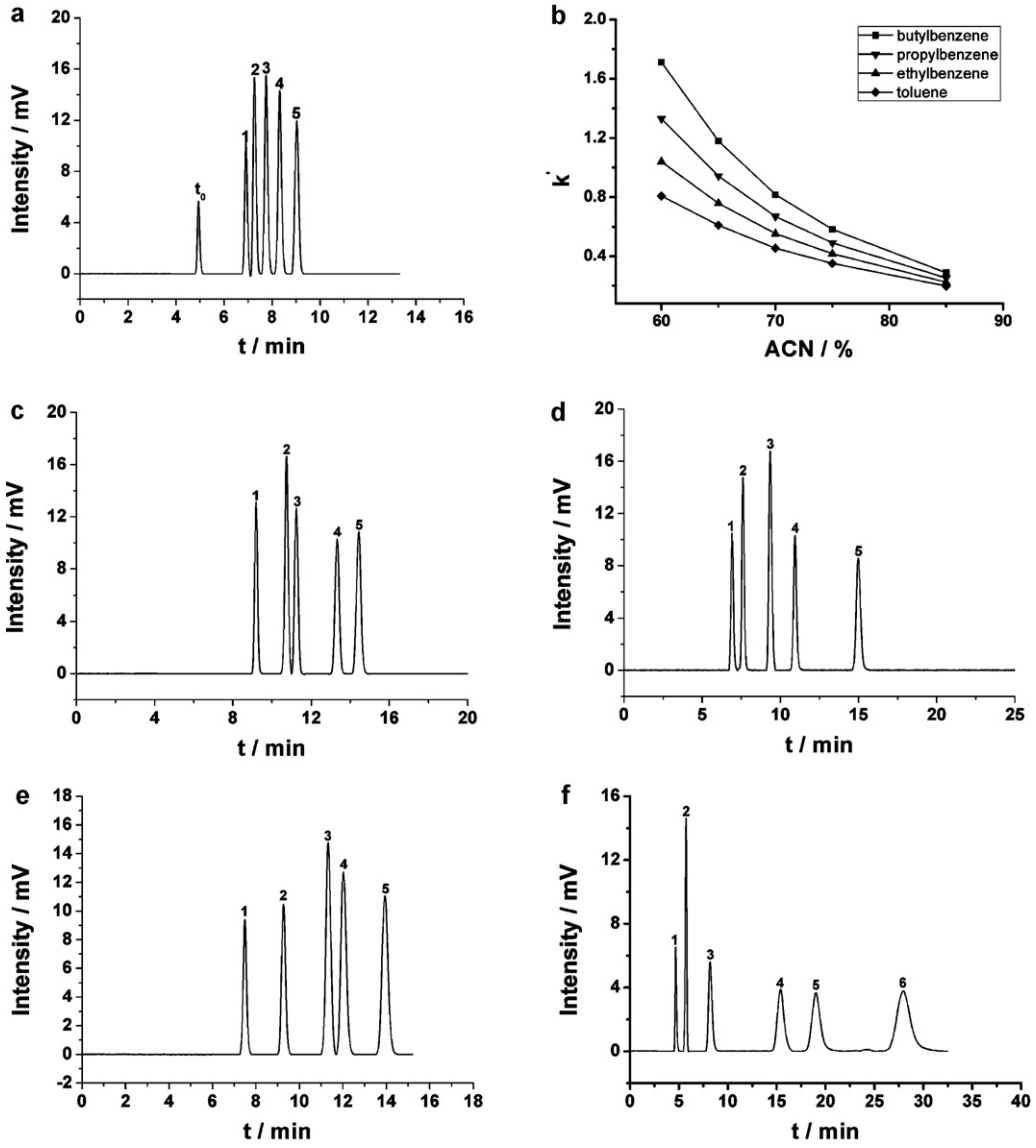


Fig. 4. Separation of various compounds on phenyl-silica hybrid monolithic column by cLC. (a) separation of alkylbenzene, solutes: t_0, thiourea, (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene; (b) the effect of ACN content in the mobile phase on the retention factor of alkylbenzene. (c) separation of PAHs, solutes: (1) naphthalene, (2) acenaphthene, (3) 4, 4′-dimethylbiphenyl, (4) pyrene, (5) p-terphenyl. (d) separation of basic compounds, solutes: (1) gramine, (2) benzidine, (3) caffeine, (4) 1,2-diphenyl hydrazine, (5) 2-nitroaniline; (e) separation of polar compounds, solutes: (1) phloroglucinol, (2) 4-cresol, (3) 2,4-dichlorophenol, (4) 4-tert-butylphenol, (5) 2,4,5-trichloro phenol; (f) separation of small peptides, solutes: (1) Arg-Gly, (2) Trp-Ala, (3) Trp-Tyr, (4) Phe-Trp, (5) Trp-Phe, (6) Trp-Trp. Experimental conditions: phenyl-silica hybrid monolithic capillary column with effective length of 20 cm × 75 μm i.d.; mobile phase for (a and c), ACN/TEAA buffer at pH 4.2 = 60/40 (v/v); mobile phase for (d and e), ACN/TEAA buffer at pH 4.2 = 40/60 (v/v); mobile phase for (f), ACN/TEAA buffer at pH 4.2 = 8/92 (v/v); flow rate, 120 μl/min (before split); detection wavelength, 214 nm.

to ca. 119,000 theoretical plates per meter. Moreover, the relative standard deviations (RSDs) of both column-to-column and batch-to-batch reproducibilities for the preparation of monolithic columns were also evaluated in term of the RSDs of retention factors of analytes, and the RSDs were less than 3.25% (n = 3) and 4.87% (n = 3), respectively. These results indicated that the prepared phenyl-silica hybrid monolithic columns have good stability and reproducibility.

3.3. Application of phenyl-silica hybrid monolithic column

The phenyl-silica hybrid monolithic column was first applied to the separation of alkylbenzenes. Fig. 4a represents the cLC separation of five alkylbenzenes on the monolithic column. The analytes were eluted in the order of thiourea < benzene<toluene < ethylbenzene < propylbenzene < butylbenzene according to their increased hydrophobicities. The effect of the amount of ACN in the mobile phases on the retention factors of alkylbenzenes was investigated, and the result was exhibited in Fig. 4b. It can be seen that the retention factor of these solutes decreases with the increasing amount of ACN in the mobile phase. These results confirmed that the separation of alkylbenzenes on the phenyl monolithic column is mainly based on typical RP chromatographic retention mechanism.

In addition, PAHs, basic compounds, phenolic compounds and small peptides were also separated on the phenyl-silica hybrid monolithic column with good peak shape, and chromatograms were shown in Fig. 4c–f, respectively. The separation of basic compounds is always suffered from peak tailing in previous reports due to non-specific adsorption on the stationary phases. However, this phenomenon was not observed on the phenyl-silica hybrid monolithic column.

Table 2
The sequence coverages of proteins identified by cLC–MS/MS analysis with the C18 particle packed column and phenyl-silica hybrid monolithic column.

Column	BSA (%)	Ovalbumin (%)	α-Casein-S1 (%)	α-Casein-S2 (%)	Cytochrome C (%)	Myoglobin (%)
C18 packed	60.63	39.64	33.80	9.91	32.69	73.20
Phenyl monolith	59.97	39.64	38.97	16.22	38.46	58.82

Besides the separation of aromatic compounds and peptide mixture, the separation of tryptic digests of mixed proteins was also attempted on this hybrid monolithic column by cLC–MS/MS. The tryptic digests was loaded onto the phenyl-silica hybrid monolithic column (12 cm × 75 μm i.d.) with a capillary, and cLC–MS/MS analysis with RP mode was performed, the chromatogram was shown in Fig. 5. The elution profile of the moderate intensity peptide of K.AEFVEVTK.L extracted from the separation chromatogram is shown in Fig. 6a. The peak capacity of 144 can be theoretically measured for a 12 cm monolithic capillary column. The performance of a C18-particulate packed capillary column (12 cm × 75 μm i.d.) with an integrated ESI tip was also evaluated with the same digests. Based on the elution profile of the same peptide shown in Fig. 6b, a peak capacity of 143 was obtained. Thus the separation performance of the phenyl-silica monolithic capillary column is close to that of the C18-particulate packed capillary column. Moreover, the enhanced permeability of monolithic capillary column results in lower backpressure than that of the packed capillary column, enabling the choice of longer columns to achieve higher separation performance. Based on the database search of the obtained chromatogram of tryptic digest, the protein coverage obtained from

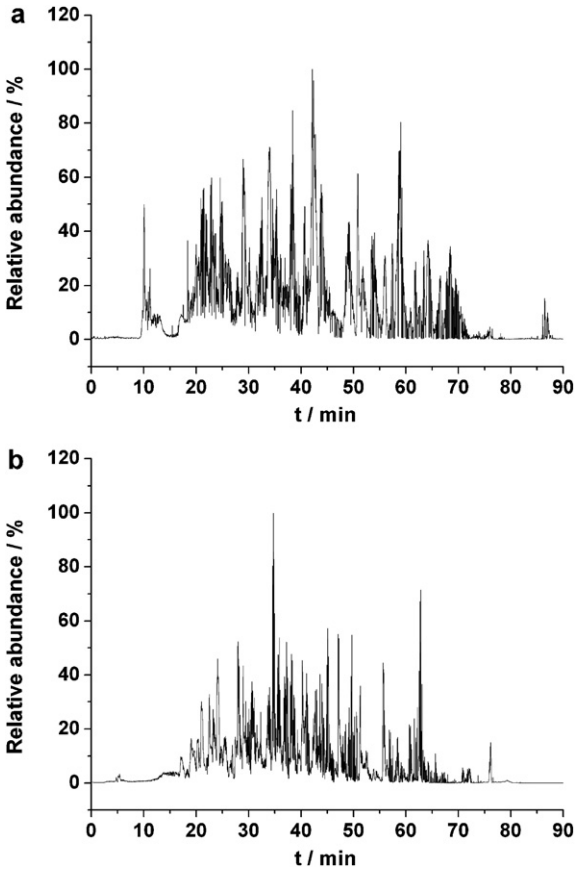


Fig. 5. Base peak chromatograms of cLC–MS/MS analysis of tryptic digests on (a) phenyl-silica hybrid monolithic column and (b) C18-particulate packed column. Experimental conditions: effective length, 12 cm for both phenyl-silica monolithic column and C18-particulate packed column; detection wavelength, 214 nm. The separation was performed by a 2 min gradient elution from 0 to 10% buffer B and a 90 min gradient elution from 10 to 35% buffer B.

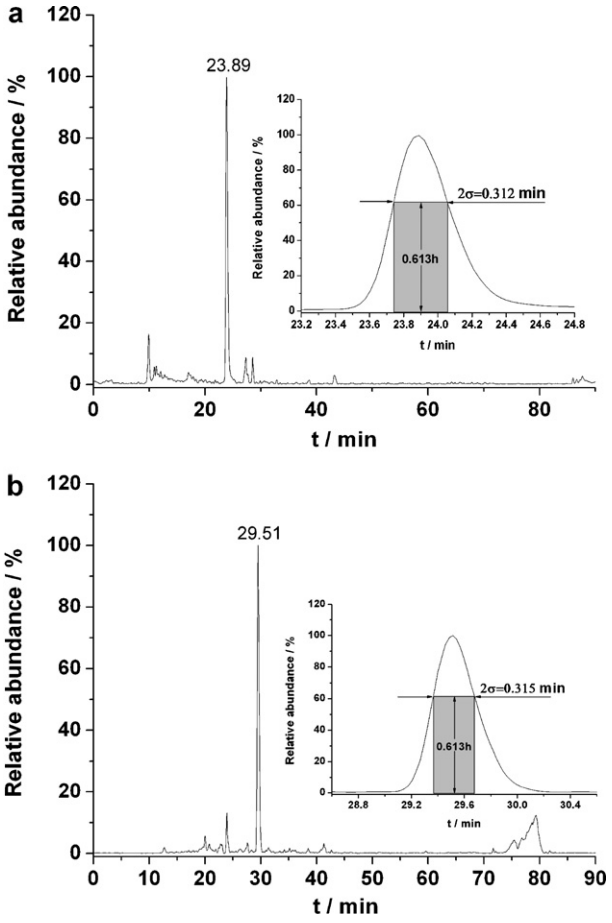


Fig. 6. Peak profiles using extracted ion chromatogram corresponding to individual peptide (K.AEFVEVTK.L) for calculating peak capacity of the column. (a) Phenyl-silica hybrid monolithic column and (b) C18-particulate packed column.

the phenyl-silica hybrid monolithic column and a C18-particulate packed commercial column was shown in Table 2. These results also indicated that the phenyl-silica monolithic capillary column is a potential tool for efficient proteome analysis.

4. Conclusion

In this study, a phenyl-silica hybrid monolithic column for cLC was successfully prepared through "one-pot" process by concurrently using benzyl methacrylate and alkoxysilanes. The SEM images of the phenyl-silica hybrid monolithic column showing a uniform monolithic matrix and tightly bonded onto the capillary wall. Separation of various neutral, polar, basic analytes as well as small peptides on the phenyl-silica hybrid monolithic column was achieved by cLC. Moreover, the prepared phenyl-silica hybrid capillary monolithic column was also applied in the analysis of tryptic digests of BSA, ovalbumin, α-casein, cytochrome C and myoglobin by cLC–MS/MS and the results showed that the separation performance was close to that of the C18-particulate packed capillary column which demonstrating its potential in proteomics research. Moreover, the limitation of using hydrophobic organic monomer in a water-soluble system was circumvented, and more hydrophobic

organic functional groups may be incorporated into organic-silica hybrid monolith in this manner.

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References

[1] K.E. Karlsson, M. Novotny, Anal. Chem. 60 (1988) 1662.
[2] R.T. Kennedy, J.W. Jorgenson, Anal. Chem. 61 (1989) 1128.
[3] K. Miyamoto, T. Hara, H. Kobayashi, H. Morisaka, D. Tokuda, K. Horie, K. Koduki, S. Makino, O. Nunez, C. Yang, T. Kawabe, T. Ikegami, H. Takubo, Y. Ishihama, N. Tanaka, Anal. Chem. 80 (2008) 8741.
[4] Y. Hirata, M. Novotny, J. Chromatogr. 186 (1979) 521.
[5] B. Chankvetadze, I. Kartozia, J. Breitkreutz, M. Girod, M. Knobloch, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 251.
[6] K.B. Tomer, Chem. Rev. 101 (2001) 297.
[7] F.J. Wang, J. Dong, M.L. Ye, R.A. Wu, H.F. Zou, J. Chromatogr. A 1216 (2009) 3887.
[8] F.J. Wang, J. Dong, M.L. Ye, R.A. Wu, H.F. Zou, Anal. Chim. Acta 652 (2009).
[9] F.J. Wang, G.H. Han, Z.Y. Yu, X.N. Jiang, S.T. Sun, R. Chen, M.L. Ye, H.F. Zou, J. Sep. Sci. 33 (2010) 1879.
[10] R.A. Wu, L.H. Hu, F.J. Wang, M.L. Ye, H.F. Zou, J. Chromatogr. A 1184 (2008) 369.
[11] H.F. Zou, X.D. Huang, M.L. Ye, Q.Z. Luo, J. Chromatogr. A 954 (2002) 5.
[12] A. Premstaller, H. Oberacher, C.G. Huber, Anal. Chem. 72 (2000) 4386.
[13] A. Premstaller, H. Oberacher, W. Walcher, A.M. Timperio, L. Zolla, J.-P. Chervet, N. Cavusoglu, A. van Dorsselaer, C.G. Huber, Anal. Chem. 73 (2001) 2390.
[14] W.H. Jin, H.J. Fu, X.D. Huang, H. Xiao, H.F. Zou, Electrophoresis 24 (2003) 3172.
[15] S. Eeltink, L. Geiser, F. Svec, J.M.J. Frechet, J. Sep. Sci. 30 (2007) 2814.
[16] Y.Y. Li, H.D. Tolley, M.L. Lee, J. Chromatogr. A 1218 (2011) 1399.
[17] F.M. Plieva, J. Andersson, I.Y. Galaev, B. Mattiasson, J. Sep. Sci. 27 (2004) 828.
[18] M.Q. Liu, H.Y. Liu, Y.K. Liu, L.G. Bai, G.L. Yang, C.L. Yang, J. Cheng, J. Chromatogr. A 1218 (2011) 286.
[19] N. Ishizuka, H. Kobayashi, H. Minakuchi, K. Nakanishi, K. Hirao, K. Hosoya, T. Ikegami, N. Tanaka, J. Chromatogr. A 960 (2002) 85.
[20] V.V. Tolstikov, A. Lommen, K. Nakanishi, N. Tanaka, O. Fiehn, Anal. Chem. 75 (2003) 6737.
[21] M.H. Wu, R.A. Wu, Z.B. Zhang, H.F. Zou, Electrophoresis 32 (2011) 105.
[22] L.G. Bai, H.Y. Liu, Y.K. Liu, X.H. Zhang, G.L. Yang, Z.Y. Ma, J. Chromatogr. A 1218 (2011) 100.
[23] M.H. Wu, R.A. Wu, F.J. Wang, L.B. Ren, J. Dong, Z. Liu, H.F. Zou, Anal. Chem. 81 (2009) 3529.
[24] Z.B. Zhang, M.H. Wu, R.A. Wu, J. Dong, J.J. Ou, H.F. Zou, Anal. Chem. 83 (2011) 3616.
[25] S. Kayillo, G.R. Dennis, R.A. Shalliker, J. Chromatogr. A 1126 (2006) 283.
[26] L.J. Yan, Q.H. Zhang, W.B. Zhang, Y.Q. Feng, L.H. Zhang, T. Li, Y.K. Zhang, Electrophoresis 26 (2005) 2935.
[27] C.H. Xie, M.L. Ye, X.G. Jiang, W.H. Jin, H.F. Zou, Mol. Cell. Proteomics 5 (2006) 454.
[28] H. Colon, X. Zhang, J.K. Murphy, J.G. Rivera, L.A. Colon, Chem. Commun. (2005) 2826.
[29] L.L. Hench, J.K. West, Chem. Rev. 90 (1996) 33.
[30] R.D. Stanelle, L.C. Sander, R.K. Marcus, J. Chromatogr. A 1100 (2005) 68.
[31] S. van der Wal, Chromatographia 2 (1985) 274.