The synthesis of chloropropyl-functionalized silica hybrid monolithic column with modification of N,N-dimethyl-N-dodecylamine for capillary electrochromatography separation

Minghuo Wu\textsuperscript{a}, Yingzhuang Chen\textsuperscript{b,c}, Ren'an Wu\textsuperscript{a,\ast}, Ruibing Li\textsuperscript{a}, Hanfa Zou\textsuperscript{a,\ast}, Bo Chen\textsuperscript{b,c}, Shouzhuo Yao\textsuperscript{b,c}

\textsuperscript{a} CAS Key Laboratory of Separation Sciences for Analytical Chemistry, National Chromatographic R & A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, China
\textsuperscript{b} State Key Laboratory of Chemo/Biosensing & Chemometrics, College of Chemistry & Chemical Engineering, Hunan University, Changsha 410082, China
\textsuperscript{c} Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University, Changsha 410081, China

1. Introduction

Monoliths as the chromatographic separation media in liquid chromatography (LC) and capillary electrochromatography (CEC) have attracted great attention in recent years due to its advantages of low backpressure drop, high mass-transfer rate and easy preparation as compared to those of conventional particulate-packed columns [1–5]. The monolithic columns can be mainly classified into organic polymer-based and silica-based monolithic columns. In most cases, the organic monolithic columns could provide the good pH stability in a wide pH range and great flexibility to tune the chemical properties of monoliths by using a variety of functional monomers and crosslinkers [5], and the inorganic silica-based monolithic columns could provide the high mechanical stability, good solvent resistance and high efficiency [6–9]. Nevertheless, the deficiencies of mechanical stability and the desirable porous structure for organic polymer-based monolithic columns, and the lack of the pH stability and the tedious chemical modification for silica-based monolithic columns are the recognizable inherent disadvantages for both monoliths, respectively.

The organic–inorganic hybrid monoliths, incorporating organic moieties into inorganic (usually silica) monolithic matrices via the co-condensation of organofunctional tri-alkoxysilanes and conventional tetra-alkoxysilanes (i.e. tetramethoxysilane, TMOS or tetraethoxysilane, TEOS) by sol–gel process, have been announced the promising separation materials against the polymer-based or silica-based monoliths in chromatography. The organic–inorganic hybrid monoliths may combine the advantages of organic polymer-based and inorganic silica-based monoliths, such as easy fabrication, wide pH range tolerance, good mechanical stability and high permeability. Since Hayes and Malik introduced a C18 functionality into the silica matrix via the sol–gel process to...
obtain an organic-silica hybrid monolithic column in 2000 [10], a variety of hybrid monolithic columns including octyl, phenyl, propyl, etc. have been prepared and directly applied in chromatography and solid-phase extraction (SPE) [11–14]. However, due to the diversity of the organofunctional tri-alkoxysilane, the preparation conditions for the organic–inorganic hybrid monolithic columns vary dramatically, which consequently result in the apparent difficulty in the preparation of novel organic-silica hybrid monolithic columns via the direct synthesis. Therefore, the modification of the synthesized organic–inorganic hybrid monolithic column seems a good choice for obtaining the desired functional hybrid monolithic columns by introducing the reactive groups onto the organic–inorganic hybrid monolithic matrices before the modification. So far, only the vinyl, amino and mercapto groups have been introduced onto the organic–inorganic hybrid monolithic columns by using the corresponding precursors for the purpose of further modifications [15–18]. Such as, Colon et al. introduced an alkyl (C6) group onto an allyl-silica hybrid monolithic column to offer increased retention performance for benzenes by modifying with hexane via free radical polymerization procedure using 2,2′-azobisisobutyronitrile (AIBN) as the catalyst [15]. Tian et al. prepared a calix[4] open-chain crown ether modified organic-silica monolithic column on vinyl-silica hybrid monolith by copolymerization with calix[4] open-chain crown ether using AIBN as initiator [16]. Ma et al. demonstrated the immobilization of an enzyme (trypsin) onto an aminopropyl-silica hybrid monolithic column via the glutaldehyde activation on the aminopropyl-silica hybrid monolithic column, where the glutaraldehyde activation was carried out at room temperature for 6 h and the immobilization of enzyme was undertaken for 24 h at 4 °C by continuously pumping the trypsin solution through the monolithic column [17]. Xu et al. prepared a sulfonic functionalized silica monolithic column from mercaptopropyl-silica hybrid monolith by oxidation with hydrogen peroxide (30%, w/w) and applied in SPE [18]. However, the polymerization reaction via the vinyl hybrid monolithic matrix seemed not satisfactory yet, since the retention factor k′ of ethylbenzene on the hexene modified allyl-silica hybrid monolithic column was only 1.26 with 30% ACN as the running buffer in CEC [15]. Additionally, the gelation of the sol solution in the preparation of amino hybrid monolith occurred very quickly, which required the immediate introduction of the sol solution into the capillary and consequently made the operation and the control of column reproducibility difficult.

In this paper, we introduced a novel approach to modify the organic–inorganic hybrid monolithic column starting from a chloropropyl-functionalized silica hybrid monolith (CP-silica), which was synthesized via the co-condensation of (3-chloropropyl)-trimethoxysilane (CPTMS) and tetramethoxysilane (TMOS). The chloropropyl group on the CP-silica hybrid monolithic matrix could easily react with the tertiary amine of N,N-dimethyl-N-dodecylamine (DMDA) to provide a long carbon chain (C12) onto the CP-silica hybrid monolith with no need of adding any catalyst. In this case, the DMDA not only provided the long carbon chain (C12) onto the CP-silica hybrid monolithic column but also was applied as the strong electroosmotic flow (EOF) generator in a wide pH range for CEC due to the resulting quaternary ammonium groups on the monolith. To the best of our knowledge, this is the first report on using chloropropyl-functionalized organic–inorganic hybrid monolith as the precursory material for the modification of hybrid monolithic column. Since chloropropyl can react with other nucleophiles such as NaOH, NaOR, NaCN or NH3 to form –OH, –OR, –CN or –NH2 functionalities via the nucleophilic substitution reaction, the CP-silica hybrid monolith will be a promising initial material for the preparation of a variety of novel functional monolithic columns.

Fig. 1. Scheme of the CP-silica hybrid monolithic column preparation (A) and subsequent modification with DMDA (B).

2. Experimental details

2.1. Materials

Tetramethoxysilane (TMOS) was purchased from Chemical Factory of Wuhan University (Wuhan, China). (3-Chloropropyl)trimethoxysilane (CPTMS, 97%), N,N-dimethyl-N-dodecylamine (DMDA) and poly(ethylene glycol) (PEG, Mn = 10 000) were purchased from Aldrich (Milwaukee, WI). Fused-silica capillary with 75 μm i.d. and 375 μm o.d. was purchased from the Reafine Chromatography Ltd. (Hebei, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA). HPLC-grade acetonitrile (ACN) was used for the preparation of mobile phases. Other chemical reagents were of analytical grade.

2.2. Preparation of the CP-silica hybrid monolithic column

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 dried by nitrogen gas at room temperature for further use. A mixture of acetic acid (HAC, 0.01 M, 5.0 mL), PEG (10 000 MW, 540 mg), urea (800 mg), TMOS (1.8 mL) and CPTMS (0.6 mL) was stirred at 0 °C for 4 h to form a homogeneous precondensation solution. One millilitre of this precondensation mixture was sonicated for 3 min, and was manually introduced into the pretreated capillary to an appropriate length by a syringe. After both ends of the capillary were sealed with rubbers, the condensation reaction was carried out at 55 °C for 12 h. The obtained chloropropyl-silica (CP-silica) hybrid monolithic columns were then flushed with water and methanol to remove the PEG and other residuals.

2.3. Modification of the CP-silica hybrid monolithic column

To modify the synthesized CP-silica hybrid monolithic column, the column was first flushed by ethanol for 20 min. After that, the DMDA ethanol solutions with different concentrations of 20, 40, 60 and 80% (v/v) were pumped through the CP-silica hybrid monolithic columns for 30 min using a manual syringe pump. And then, the modification of the hybrid monolithic columns was carried out at 70 °C for 12 h in a water bath. The resulted DMDA modified (C12-silica) hybrid monolithic columns were flushed with ethanol extensively to remove the residues. The procedures including the preparation of CP-silica hybrid monolith and the further modification with DMDA were illustrated in Fig. 1.

2.4. Capillary electrochromatography

All CEC experiments were carried out on a CE instrument-ACE™ MDQ System ( Beckman, Fullerton, CA, USA) equipped with a UV detector, while the column temperature was set at 25 °C and...
the detection wavelength was set at 214 nm. A detection window was made by removing the polyimide coating of a fused-silica capillary with a razor blade in the empty section of the capillary at the edge of the organic-silica continuous bed. The total length of the prepared capillary column was 31 cm with effective length of 21 cm. The monolithic column was first preconditioned by running buffer for at least 30 min with a manual syringe pump, and then equilibrated on CE instrument by applying a low separation voltage (10 kV, ramping time for 10 min) until a stable current was obtained. All data obtained were based on three runs. 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 analyte and thiourea in this work, respectively.

3. Results and discussion

3.1. Preparation of CP-silica hybrid monolithic column

The preparation of CP-silica hybrid monolith within the confines of a capillary includes two steps: the hydrolyzation of the silanes of TMOS and CPTMS and the condensation of the hydrolyzed precursors. The hydrolyzation of precursors of TMOS and CPTMS was carried out in a 0.01 M HAC solution with stirring at 0◦C. The amounts of TMOS, CPTMS and PEG of 1.8 mL, 0.6 mL and 540 mg, respectively, were chosen in this work. Since CPTMS is immiscible in aqueous solution, it took ca. 4 h to obtain the homogeneous hydrolyzed mixture of TMOS and CPTMS. To form a homogeneous monolithic matrix within the pretreated capillary, two crucial parameters including the use of urea and the condensation temperature were examined in the preparation of this hybrid monolithic column. The use of urea in the silica monolithic column has been reported, where the urea was used to generate ammonia to promote the uniform mesopore structure in the prepared silica monolithic skeletons due to the thermal decomposition of urea at the elevated temperature while the effect of urea on the condensation procedure was neglected [9,19]. Interestingly, during the preparation of CP-silica hybrid monolithic column, urea played an important role in the formation of the CP-silica hybrid monolithic matrix within the confines of a capillary. It was observed that without the use of urea in the reaction mixture monolith was not filled in the inner space of a capillary. After using urea as an additive in the reaction mixture, the capillary column fully filled with homogenous CP-silica hybrid monolith was thus obtained. The investigation of the use of the urea on the formation of the fully filled monolith within a capillary was carried out by changing the content of urea in precondensation mixture and the morphologies of resulted CP-silica hybrid monoliths under optical microscopy were shown in Table 1. These microscopy images clearly show that the urea as the additive did exert its influence in the formation of monoliths in the sol–gel process. Without the addition of urea in the sol–gel process, there only very limited monolithic matrix formed (Column A, Table 1); as adding 450 mg of urea, the obtained monolithic matrix increased to a partial filling status within the confines of the capillary (Column B, Table 1), which became a fully filling monolith as the addition of urea up to 800 mg (Column C, Table 1). It is difficult to find the exact mechanism to explain this effect of urea on the formation of silica hybrid monolith via the sol–gel process. Artaki et al. [20] have investigated the effects of polar, less polar and nonpolar solvent additives (formamide, dimethyl formamide, acetonitrile and dioxane) on the condensation of sol–gel process by utilizing Raman spectroscopy, molybdenum chemical reaction as well as the electron microscopy methods. They found that the changes in hydrogen bonding and electrostatic interactions caused by solvent additives might explain the sol–gel condensation process. Urea, a polar compound, has also been routinely applied in the biopolymer denaturation of proteins. However, the mechanisms including hydrogen bonding and solvent environment change in protein denaturation by urea remain arguable yet [21]. In our work, the addition of urea has seemed to change the sols condensation environment. The possible interactions including the hydrogen bonding and the electrostatic interactions happening between urea and hydrolyzed silica monomers likely stabilized the precondensed siloxane oligomers to some extent, which in turn facilitated the condensation and gelation of monolithic network. The result of using urea has indicated that the effect of urea in silica hybrid monolithic preparation via the sol–gel process should be included as the better studied control parameter despite the unclear mechanism underneath.

As can be seen from Table 1, the condensation temperature was also crucial for the formation of the CP-silica hybrid monolith. Three different condensation temperatures of 50, 55 and 60 ◦C were adopted to examine the formation of the monolith within the capillary. At lower condensation temperature (50 ◦C), the monolithic matrix was seriously detached from the inner wall of the capillary (Column D). With the increase of the condensation temperature (55 ◦C), the obtained monolithic matrix became homogeneous and was able to fully fill in the inner space of the capillary (Column C). As the condensation temperature further increased to 60 ◦C, the obtained hybrid monolith showed denser matrix structure (Column E) and also the poorer permeability as compared to Column C. The change tendency of the CP-silica hybrid morphology under the microscope depending on the condensation temperature was similar to that of our previous observations that higher condensation temperature would lead a denser monolith with poorer permeability [22].

The SEM images of the obtained CP-silica hybrid monolithic matrix under the optimized reaction conditions were shown in Fig. 2. It can be seen that the capillary is fully filled with the homogenous monolithic matrix. Additionally, the morphology of the CP-silica monolithic matrix strongly resembles to that of a pure silica monolithic matrix with the similar homogenous macropore

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<th>No.</th>
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Other parameters: TMOS, 1.8 mL; CPTMS, 0.6 mL; PEG, 540 mg; condensation time, 12 h.
structure. Such homogenous macropore structure would provide the decreased mass-transfer resistance and the large surface area of the monolithic matrix [23].

3.2. Modification of CP-silica hybrid monolith with DMDA

Due to the incorporation of (3-chloropropyl)-trimethoxysilane in the preparation of this organic–inorganic hybrid monolithic column, the obtained CP-silica hybrid monolith thus possesses the chloropropyl groups on the surface of the formed monolithic matrix, which are the reactive sites for the further functionalization of the monolithic column via the nucleophilic substitution reaction with a variety of nucleophiles. In this work, the DMDA, a tertiary amine with a long carbon chain (C12), would be the good nucleophile to modify the synthesized CP-silica hybrid via the nucleophilic substitution reaction between the chloropropyl and tertiary amine groups. The modification of the CP-silica hybrid monolith with DMDA could introduce a C12 moiety onto the matrix surface, which would afford the hydrophobicity for the modified monolithic column in the separation of analytes in reversed-phase mode in CEC. Fig. 3 displayed the separation electrochromatograms of alkylbenzenes on the intact CP-silica hybrid monolithic column and DMDA (20%, v/v ethanol solution) modified C12-silica hybrid monolithic column. It can be seen that the alkylbenzenes were baseline separated with 40% ACN running buffer on the CP-silica hybrid monolithic column. The elution order of these alkylbenzenes was related with the hydrophobicities of alkylbenzenes, which indicated that the CP-silica hybrid monolithic column showed the sufficient hydrophobicity for the separation of neutral hydrophobic analytes by the RP-CEC. As a comparison, the separation of alkylbenzenes on the DMDA modified C12-silica hybrid monolithic column was also illustrated in Fig. 3 to display the performance difference between both columns. The elution order on the C12-silica hybrid monolithic column was the same as that on the CP-silica hybrid monolithic column. The retention of these alkylbenzenes on C12-silica hybrid monolithic column was enhanced remarkably as compared to that on the CP-silica monolithic column. For instance, the retention factor ($k'$) of butylbenzene on the C12-silica hybrid monolithic column was 5.37, which was only 1.57 on the CP-silica hybrid monolithic column. Fig. 4 shows the relationship of the retention factors ($k'$) of alkylbenzenes on the C12-silica hybrid monolithic column and the ACN content of mobile phase in CEC. As can be seen from Fig. 4, the retention factors ($k'$) of these alkylbenzenes decreased with the increase of the ACN content in mobile phase, which indicated the typical reversed-phase chromatographic retention mechanism.

To investigate the effect of the concentration of DMDA used for the modification of CP-silica hybrid monolithic column, DMDA ethanol solutions with concentrations changed from 20, 40, 60 and 80% (v/v) were utilized for the modification. When using ethanol modification solutions with DMDA concentration greater than 40%,
the resulted monolithic columns were in failure of flushing residuals out of the monolithic column. Thus, only the concentrations of 20% and 40% of DMDA ethanol solution were used for the investigation. Fig. 5 displays the separation of alkylbenzenes on the C12-silica hybrid monolithic columns, which were modified with the 20% and 40% DMDA ethanol solutions, respectively. As shown in Fig. 5, the baseline separation of alkylbenzenes was achieved on both C12-silica hybrid monolithic columns. While, the C12-silica hybrid monolithic column demonstrated the better resolution and higher efficiency on 40% DMDA modified column than that of 20% DMDA modified column. It was considered that the peak tailing was mainly due to the nonspecific interaction between the residual silanols and solutes. The existence of the quaternary ammonium on the surface may shelter the residual silanol groups to some content [24]. Modification with high DMDA concentration 40% would form more quaternary ammonium groups and provided better shelter effect. The stronger EOF and the higher retention factors of the solutes indicated that the modification with high DMDA concentration could provide more quaternary ammonium and more C12 functionalities. A quicker separation was also observed in 40% modified column compared to that of 20% modified column since more quaternary ammonium were formed which lead to a stronger EOF. The column efficiencies of 189 700 to 221 000 N/m were achieved for alkylbenzenes on this 40% DMDA modified monolithic column.

3.3. The EOF of the C12-silica hybrid monolithic column

After the modification of the CP-silica hybrid monolithic column with DMDA, we found that the direction of the EOF on the C12-silica hybrid monolithic column was reversed as compared to that on the CP-silica monolithic column. This is because of the formation of the quaternary ammonium groups on the modified C12-silica hybrid monolithic column from the reaction of tertiary amine groups of DMDA with the chloropropyl groups on the CP-silica monolith. The reversed EOF direction and the increased column hydrophobicity both confirmed the successful modification of the C12-silica hybrid monolithic column by DMDA. Additionally, the EOF generated on the 40% DMDA modified monolithic column was ca. 12.4% higher than that on the 20% DMDA modified monolithic column with the separation of 5 alkylbenzenes achieved in less than 3 min (Fig. 5).

The effect of pH value on the EOF of the prepared C12-silica hybrid monolithic column was tested by changing the pH value of mobile phase. As showed in Fig. 6, the EOF generated on the C12-silica hybrid monolithic column decreased from 15.02 to 2.99 cm² kV⁻¹ min⁻¹ when the pH value increased from 3 to 8. This was because of the ion suppression of the silanol groups of the C12-silica hybrid monolithic column at the lower pH values. As the increase of the pH value of mobile phase, the silanol groups started to dissociate to the negatively charged anions, which resulted in the decrease of the net positive charge on the surface of C12-silica hybrid monolith and the consequent decrease of the EOF. However, due to the high amount of quaternary ammonium groups derived from chloropropyl moieties by DMDA, an EOF of 2.95 cm² kV⁻¹ min⁻¹ was remained at pH up to 8.

3.4. Separation of aromatic compounds

Besides the separation of alkylbenzenes on the C12-silica hybrid monolithic column, the separation of aromatic compounds of hydroquinone, phenethyl alcohol, phenol, p-cresol, 2,3-dimethylphenol, p-nitrotoluene, p-tert-butylphenol and p-xylene were also examined on this C12-silica hybrid monolithic column by CEC. Fig. 7 demonstrated the CEC separation of aromatic analytes on the C12-silica hybrid monolithic using 10 mM citric-Na₂HPO₄ (pH 3) containing 40% ACN as the mobile phase. These compounds were baseline separated within 5 min, which indicated this column was able to separate such solutes in a short time by CEC.

Fig. 5. The separation of alkylbenzenes on C12-silica hybrid monolithic columns prepared with two DMDA concentrations in the modification solution. Conditions: 10 mM Na₂HPO₄-citric acid buffer containing 70% ACN at pH 3; other conditions are the same as shown in Fig. 3.

Fig. 6. The relationship of EOF generated on C12-silica hybrid monolithic column and pH value of mobile phase. Conditions: 10 mM Na₂HPO₄-citric acid buffer containing 40% ACN at different pH values; other conditions are the same as shown in Fig. 3.

Fig. 7. Separation of aromatic compounds on C12-silica hybrid monolithic column. Conditions: mobile phase, 10 mM Na₂HPO₄-citric acid buffer at pH 3 containing 40% ACN; separation voltage, −25 kV; injection, −5 kV for 1 s; other conditions are the same as shown in Fig. 4.

Analytes: (0) thiourea, (1) hydroquinone, (2) phenethyl alcohol, (3) phenol, (4) p-cresol, (5) 2,3-dimethyl phenol, (6) p-nitrotoluene, (7) p-tert-butylphenol, and (8) p-xylene.
The run-to-run reproducibility was evaluated on a single C12-silica hybrid monolithic column. The relative standard deviations (RSDs) for retention factors of analytes (phenol, p-cresol and 2,3-dimethyl phenol) on the C12-silica hybrid capillary monolithic column were less than 0.97% for 5 successive runs. Both column-to-column and batch-to-batch reproducibilities for the preparation on these monolithic columns were also evaluated in terms of the RSDs of retention factors of analytes, which were less than 4.1 (n = 3) and 5.6% (n = 3), respectively. This C12-silica hybrid monolithic column was continuously used for 3 weeks (more than 300 runs) at pH 3 without obvious loss of column efficiency and retention ability which indicated its good stability.

4. Conclusions

A novel chloropropyl-functionalized silica hybrid monolithic column was prepared by the hydrolyzation and co-condensation of CPTMS and TMOS. The obtained CP-silica hybrid monolith showed a homogeneous macroporous morphology and well attached to the inner capillary wall. The synthesized intact CP-silica hybrid monolithic column could be used as the reversed-phase stationary phase for the separation of neutral solutes by CEC. The convenient modification of the chloropropyl groups on monolithic matrix by nucleophiles of tertiary amines is the significant merit of this CP-silica hybrid monolithic column. The quaternary ammonium groups formed after this modification could provide the strong EOF in wide pH range for CEC. DMEDA was used as a model tertiary amine to introduce a long carbon chain (C12) functionality onto this hybrid monolith surface to obtain the C12-silica hybrid monolithic column. After the modification, the retentions of analytes were greatly increased in the RP-CEC mode as compared to CP-silica hybrid monolithic column, and 5 alkylbenzenes were baseline separated within 3 min with high column efficiency in CEC by using a mobile phase containing 70% ACN. The chloropropyl-functionalized hybrid monolith is the promising organic–inorganic hybrid monolith for the further modification with other nucleophiles such as NaOH, NaOR, NaCN or NH3 to obtain –OH, –O–R, –CN, –NH2 functionalities on the surface for other applications.

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