Research Article

Preparation of polyhedral oligomeric silsesquioxane based hybrid monoliths by ring-opening polymerization for capillary LC and CEC

A new organic–inorganic hybrid monolith was prepared by the ring-opening polymerization of octaglycidyldimethylsilyl polyhedral oligomeric silsesquioxane (POSS) with 1,4-butandiamine (BDA) using 1-propanol, 1,4-butanediol, and PEG 10 000 as a porogenic system. Benefiting from the moderate phase separation process, the resulting poly(POSS-co-BDA) hybrid monolith possessed a uniform microstructure and exhibited excellent performance in chromatographic applications. Neutral, acidic, and basic compounds were successfully separated on the hybrid monolith in capillary LC (cLC), and high column efficiencies were achieved in all of the separations. In addition, as the amino groups could generate a strong EOF, the hybrid monolith was also applied in CEC for the separation of neutral and polar compounds, and a satisfactory performance was obtained. These results demonstrate that the poly(POSS-co-BDA) hybrid monolith is a good separation media in chromatographic separations of various types of compounds by both cLC and CEC.

Keywords: Chromatographic separation / Hybrid monoliths / Polyhedral oligomeric silsesquioxane / POSS / Ring-opening polymerization

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1 Introduction

Polyhedral oligomeric silsesquioxanes (POSSs), which exist as cage-type nanostructures with the formula $R_n(SiO_{1.5})_n$ ($n = 6, 8, 10, \ldots$), can be regarded as organic–inorganic hybrid materials at a molecular level [1–3]. Due to their unique inorganic–organic hybrid nanostructures, facile chemical modification, high temperature, and oxidation resistance properties, they have become some of the most intriguing examples of nanostructured organic–inorganic hybrid building blocks, and have been widely used in synthesis and the reinforcement of polymer matrices, heterogeneous catalysts, biomaterials, chemical vapor deposition (CVD) coatings, optical or electrical devices, and lithography [4–8].

Their unique physical and chemical properties make POSSs an ideal alternative to alkoxylsilanes for preparation of silica-based materials, such as organic–silica hybrid monoliths [7, 9–13]. As a typical and novel state-of-the-art format of monolithic materials, hybrid monolithic columns, combining merits of both organic and inorganic monoliths, such as easy preparation, high permeability, pH tolerance, and mechanical strength [14–16], have attracted extensive attention as separation media in all chromatographic methods to separate small molecules and large biomolecules [11, 17–21]. By replacing the alkoxysilanes with a POSS regent, the preparation of POSS-containing hybrid monoliths can be significantly simplified. Besides, ascribed to the unique physical and chemical properties of POSS, the mechanical and pH stabilities of POSS-based hybrid monoliths were remarkably improved [9]. However, the heterogeneous (irregular) microstructure caused by commonly used free-radical polymerization still exists in POSS-based hybrid monoliths, leading to some negative effects, such as low permeability and larger eddy diffusion in flow-through systems [22–26].

Recently, we developed a facile approach for the preparation of POSS-based hybrid monoliths by ring-opening polymerization [11]. A POSS monomer octaglycidyldimethylsilyl POSS (POSS-epoxy) was selected as the precursor to react with diamines or polyamines to form the framework of the
monoliths. Benefiting from the moderate phase separation process of ring-opening polymerization, the hybrid monoliths possess a well-controlled 3D skeletal structure [11, 25, 27, 28] and are promising for chromatographic separations with ultrahigh column efficiency [11, 25]. The unique hybrid monoliths are, of course, of interest to be applied for a myriad of potential applications. However, so far, the application of such monoliths was concentrated in capillary LC (cLC) and rarely involved CEC analysis. Herein, to further confirm the versatility of this novel preparation approach, an organic–inorganic hybrid monolith was prepared by the ring-opening polymerization of POSS-epoxy and 1,4-butanediamine (BDA). The obtained hybrid monolith was characterized by SEM and evaluated systematically in cLC. In addition, the monolith was also investigated in CEC for a comprehensive understanding of its chemistry and efficiency.

2 Materials and methods

2.1 Chemicals and materials

POSS-epoxy, PEG (Mn = 10 000), and (3-aminopropyl)triethoxysilane (APTES) were purchased from Aldrich (Milwaukee, WI, USA). BDA was obtained from J&K Scientific (Beijing, China). 1-Propanol, 1,4-butanediol, thiourea, alkylbenzenes, phenols, and other standard compounds were all purchased from Sigma (St. Louis, MO, USA). The fused-silica capillaries with dimensions of 50 or 75 μm id and 365 μm od were obtained from Refine Chromatography (Yongnian, Hebei, China). HPLC-grade acetonitrile (ACN) was used for the preparation of mobile phases. The water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). Other chemical reagents were all of analytical grade.

2.2 Preparation of the poly(POSS-co-BDA) hybrid monolith

Before the preparation of the monolithic column, the fused-silica capillary was pretreated and rinsed by 1.0 M NaOH for 4 h, water for 30 min, 1.0 M HCl for 14 h, and water for another 30 min, successively, and then dried by a nitrogen stream at room temperature, according to our previous report [17]. A mixture of 3-aminopropyl)triethoxysilane/methanol (50%, v/v) was used to introduce the –NH2 group onto the inner surface of the capillary for anchoring monolith matrices to the capillary inner wall, as described in a previous report [29]. POSS-epoxy, BDA, 1-propanol, 1,4-butanediol, and PEG were mixed under ultrasoundication to form a homogeneous solution. For cLC, the mixture was injected into the modified 40 cm long capillaries with a syringe. For CEC, the mixture was introduced in the capillaries (total length 40 cm) to a length of 28 cm. The filled capillaries were then sealed with rubber stoppers and immersed in a water bath at 50°C for 12 h. After that, the capillaries were flushed with methanol to remove the unreacted residuals. Prior to CEC, a detection window was created by burning out a 2–3 mm segment in the empty section of the capillary at the edge of continuous bed, which was located as close to the monolithic matrix as possible. The monolithic column was then cut to an effective length of 24.5 cm with a total length of 33 cm before use.

2.3 Instrumentation and methods

An Agilent CE system (Hewlett-Packard, Waldbronn, Germany) equipped with a UV detector was used for all electrochromatographic experiments. The data were acquired and processed by Agilent ChemStation software (Hewlett-Packard). The mobile phases with different contents of ACN and varying concentrations of phosphate buffer were filtered prior to use. The standard sample mixtures were prepared with ACN with a concentration of 1.0–10.0 mg/mL. The detection wavelength was set at 214 nm, and both the sample injection and separation were performed at room temperature. Thiourea was used as the EOF marker. The EOF velocity was calculated according the equation, μe = l · I/(V · t0), where μe is the effective mobility, l and I are the effective and total length of the capillary, respectively, V is the applied voltage, and t0 is the migration time of the EOF marker.

The capillary liquid chromatographic evaluation of hybrid monolithic columns was performed on an LC system equipped with an Agilent 1100 (Hewlett-Packard) micropump and a UV detector (K-2501, Knauer, Germany). The detection wavelength was set at 214 or 254 nm. All data were collected and processed by a chromatography workstation (Beijing Cailu Scientific Instrument, Beijing, China). A 7725i injector with a 20 μL sample loop was used for sample loading. For obtaining a flow rate of nanoliters per minute (nL/min), a T-union connector was used to serve as a splitter with one end connected to the capillary monolithic column and the other end to a blank capillary (95 cm long, 50 μm id, and 365 μm od). The split ratio was controlled at about 1/280. The outlet of the hybrid monolithic column was connected with a Teflon tube to an empty fused-silica capillary (75 μm id and 365 μm od), where a detection window was made by removing a 2 mm length of the polyimide coating in a position 5.5 cm from the separation monolithic column outlet.

SEM images were obtained by using a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan).

3 Results and discussion

3.1 Preparation of the poly(POSS-co-BDA) hybrid monolith

The poly(POSS-co-BDA) hybrid monolith was prepared by ring-opening polymerization (Fig. 1) [11]. As the feed recipe and reaction temperature usually play vitally important
roles in the preparation of a monolith, the effect of these parameters was systematically investigated. Considering the results of the preparation of POSS-based hybrid monoliths by ring-opening polymerization in our recent report [11], the amounts of POSS-epoxy and BDA were kept at 50 mg and 10 μL, respectively, and a solution consisting of propanol, 1,4-butanediol, and PEG 10 000 was also chosen as the porogenic solvent mixture. As shown in Table 1, when the content of propanol was low, the monolith was loose and detached from the inner wall of the capillary (column A). As the amount of propanol increased, the monolith became gradually dense (column B). However, further increasing the amount of propanol would result in a slack monolith, which could not be attached to the inner wall of the capillary (column C). Differently, when the amount of 1,4-butanediol increased, the monolith became increasingly dense and transparent under an optical microscope (columns B, D, and E). A similar phenomenon was also observed when the reaction temperature increased, which resulted in a decrease of the permeability (columns B, H, I, and J). In contrast, a transformation of monoliths from dense to slack occurred as the amount of PEG 10 000 increased (columns B, F, and G). After careful evaluation of these results, the conditions used for column B were adopted for the preparation of poly(POSS-co-BDA) hybrid monoliths for further experiments.

### 3.2 Characterization of the poly(POSS-co-BDA) hybrid monolith

Figure 2 presents the cross-section morphology of the hybrid monolith. It was observed that homogenous matrix had uniform macro through-pores and a well-defined 3D skeleton well anchored to the inner wall of capillary without any disconnection. The highly ordered microstructure perhaps benefited from the ring-opening polymerization, which would result in a relatively moderate phase separation process, and could overcome the inherent disadvantages of free-radical polymerization to some extent [25, 28].

The obtained hybrid monolith showed good mechanical stability as the measured back-pressure was linearly increased even over 19.0 MPa when the flow rate increased from 40 to 400 nL/min with a linearity coefficient of $R = 0.9995$ (data not shown). Meanwhile, good permeability

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**Table 1.** Effect of synthesis parameters on the formation of poly(POSS-co-BDA) hybrid monoliths

<table>
<thead>
<tr>
<th>Column</th>
<th>Propanol (μL)</th>
<th>1,4-Butanediol (μL)</th>
<th>PEG (mg)</th>
<th>$T (°C)$</th>
<th>Morphology&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Permeability&lt;sup&gt;b)&lt;/sup&gt; ($\times 10^{-14} \text{ m}^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>150</td>
<td>40</td>
<td>25</td>
<td>50</td>
<td>Detached from the inner wall</td>
<td>Not detected</td>
</tr>
<tr>
<td>B</td>
<td>200</td>
<td>40</td>
<td>25</td>
<td>50</td>
<td>Homogenous</td>
<td>4.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>250</td>
<td>40</td>
<td>25</td>
<td>50</td>
<td>Detached from the inner wall</td>
<td>Not detected</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>0</td>
<td>25</td>
<td>50</td>
<td>Detached from the inner wall</td>
<td>Not detected</td>
</tr>
<tr>
<td>E</td>
<td>200</td>
<td>80</td>
<td>25</td>
<td>50</td>
<td>Transparent</td>
<td>Too hard to pump through</td>
</tr>
<tr>
<td>F</td>
<td>200</td>
<td>40</td>
<td>20</td>
<td>50</td>
<td>Transparent</td>
<td>Too hard to pump through</td>
</tr>
<tr>
<td>G</td>
<td>200</td>
<td>40</td>
<td>30</td>
<td>50</td>
<td>Nonrigid</td>
<td>40.82</td>
</tr>
<tr>
<td>H</td>
<td>200</td>
<td>40</td>
<td>25</td>
<td>60</td>
<td>Homogenous</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>200</td>
<td>40</td>
<td>25</td>
<td>70</td>
<td>Homogenous</td>
<td>2.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>200</td>
<td>40</td>
<td>25</td>
<td>80</td>
<td>Homogenous</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Light brown</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a)</sup> Observed with an optical microscope.

<sup>b)</sup> Permeability, $B_0 = \frac{F_0L}{\eta r^2 \Delta P}$, $F$: linear velocity of mobile phase; $\eta$: dynamic viscosity of mobile phase; $L$: effective column length; $r$: inner radius of column; $\Delta P$: pressure drop across the column [30], measured at room temperature.
at $4.31 \times 10^{-14}$ m$^2$ of the monolith was also measured and calculated according to Darcy’s law [30] of permeability, $B_0 = F \eta L/(\pi r^2 \Delta P)$, where $F$ is the linear velocity of the mobile phase, $\eta$ is the dynamic viscosity of the mobile phase, $L$ and $r$ are the effective length and inner radius of the column, respectively, and $\Delta P$ is the pressure drop across the column.

The hybrid monolith also showed high reproducibility, which was evaluated through the RSD for the retention factors ($k'$) of benzene as the model analyte (thiourea as the void time marker) in cLC. The run-to-run and day-to-day repeatability were 0.17% ($n = 3$) and 0.24% ($n = 3$), respectively. Besides, the column-to-column and batch-to-batch reproducibility were also investigated, which gave RSD values of 0.36% ($n = 3$) and 0.51% ($n = 3$), respectively, indicating satisfactory reproducibility for the preparation of hybrid monoliths. In addition, no significant decrease of column efficiency or obvious column deterioration was observed even after continuous use for hundreds of injections under neutral and acidic mobile phases, which demonstrated the high stability and good pH resistance of the poly(POSS-co-BDA) hybrid monoliths.

### 3.3 Chromatographic evaluation of the poly(POSS-co-BDA) hybrid monolith

#### 3.3.1 cLC evaluation of the poly(POSS-co-BDA) hybrid monolith

Due to the hydrophobicity of POSS-epoxy, the prepared poly(POSS-co-BDA) hybrid monolith was applied for chromatographic separation in RP mode. Five alkylbenzenes were well separated with good peak shapes according to their hydrophobicity in order from low to high (Fig. 3A). The effect of ACN content in the mobile phase on the retention of these alkylbenzenes was investigated. It could be observed that all retention factors varied inversely with the ACN content in the range of 30–70% (Supporting Information Fig. S1), revealing a typical RP retention mechanism. The relationship of the plate heights of these alkylbenzenes versus the flow rate was also investigated. It could be found that a column efficiency of about 100 000 N/m at the optimal flow velocity was achieved on the hybrid monolith (Fig. 3B). The high column efficiency may be attributed to the well-defined 3D skeleton and uniform microstructure formed by the progressive phase separation process in ring-opening polymerization [28], as the hybrid monolith possessed a low surface area of 9.6 m$^2$/g and an average pore diameter of 484.1 nm, demonstrating a lack of mesopores and nanopores (Supporting Information Fig. S2). It has been proved that such a highly ordered microstructure would accelerate the mass transfer during the separation process and be beneficial to improve the column efficiency [31]. In addition, the column showed a relatively high efficiency within a wide range of flow rates from 0.25 to 1.4 mm/s, which means that the hybrid monolith may be promising for rapid separation in cLC applications.

For further validation of the separation ability, polycyclic aromatic hydrocarbons, phenols, benzoic acids, anilines as well as purines and pyrimidines were separated on the hybrid monolith. As can be seen, naphthalene, acenaphthene, 4,4’-dimethylbiphenyl, p-terphenyl, and pyrene were all baseline separated with good peak shapes (Fig. 4A), and high column efficiencies for these compounds ranged from 68 000 to 79 000 N/m. Figure 4B exhibits the chromatogram of separation of three phenols and two benzoic acids derivatives. Five compounds were well separated with ultra-high column efficiencies, especially for hydroxybenzoic acid and benzoic acid, whose column efficiencies were calculated at 215 000 and 226 000 N/m, respectively. Besides, from Fig. 4B, it could be found that hydroquinone and resorcinol were baseline separated, which revealed that the poly(POSS-co-BDA) hybrid may have a special selectivity for phenol isomers. However, only hydroxybenzoic acid and benzoic acid were separated, and other benzoic acid derivatives with lower $pK_a$ values could not be eluted under a mobile phase with 40 mM NH$_4$Ac (pH 2.98). It may be ascribed to the electronic interaction between the amino groups on the surface of matrix and the dissociated carboxyl group on the analytes. Perhaps a mobile phase with a higher salt concentration or lower pH would improve the separation performance of benzoic acid derivatives.

Figure 4C presents the separation of six basic anilines, and satisfactory performance was performed with a column
efficiency varying from 35 800 to 52 700 N/m. Figure 4D shows the chromatographic separation of four purines and pyrimidines on the POSS–BDA hybrid monolith, and high column efficiencies ranging from 73 200 to 82 200 N/m were achieved. However, the two pyrimidines (thymine and uracil) cannot be separated, maybe due to the low selectivity of the poly(POSS–co-BDA) hybrid monolith for pyrimidine.

3.3.2 CEC evaluation of the poly(POSS–co-BDA) hybrid monolith

As a combination of LC and CE, CEC shows unparalleled advantages in low consumption of sample, solvent, and stationary phases [32–34], and has been widely used in the separation of both small molecules and (bio)macromolecules [19, 35, 36]. The POSS-based monoliths prepared by ring-opening polymerization have exhibited excellent performance with respect to cLC applications [11], however, such monoliths have not been applied in CEC. Here, we evaluated the application of the poly(POSS–co-BDA) monolith in CEC for the first time. As a number of secondary amine groups are produced by the ring-opening reaction, the generation of an anodic EOF was anticipated. As we expected, the analytes cannot be detected when a positive voltage was applied. In contrast, the separation could be performed normally when a negative voltage was applied. Since the pH value of the mobile phase would affect the degree of protonation of the amino groups, resulting in a pH-dependent EOF, the influences of the pH value of the mobile phase on EOF was investigated. It was observed that the EOF decreased from $1.62 \times 10^{-8}$ to $6.54 \times 10^{-9}$ m$^{2}$V$^{-1}$s$^{-1}$ with an increase of pH from 3.5 to 8.5.

Baseline separation of five alkylbenzenes was obtained within 11 min by applying a voltage of $-28$ kV (Fig. 5A), and their elution order illustrated a typical RP retention mechanism. The effect of ACN content in the mobile phase on the retention of five alkylbenzenes was studied. A decrease of the retention factor could be observed for these compounds when the ACN content increased from 30 to 70% (data not shown), which further confirmed the RP retention mechanism of
alkylbenzenes on the poly(POSS-co-BDA) hybrid monolith. By applying a voltage ranging from $-3$ to $-28$ kV, the column efficiency of the poly(POSS-co-BDA) hybrid monolith in CEC was also investigated. It should be pointed that the Joule heating is negligible as the current increased linearly as the voltage increased ($-3$ to $-28$ kV; data not shown). Figure 5B shows the Van Deemter curves of the unretained thiourea and retained benzene, exhibiting the minimum plate height of 2.5 and 5.9 $\mu$m at the optimal flow velocity by corresponding to 401 500 and 169 900 N/m, respectively. Besides, no significant decrease of the column efficiencies occurred within a wide range of flow rates from 0.23 to 1.35 mm/s, and showed typical monolithic behavior (efficiency being maintained at higher velocities), promising for rapid separation in CEC applications. In addition, it was found that the column efficiencies in CEC were obviously much higher than those in cLC, which may be due to the effective suppression of eddy diffusion in CEC.

The phenols were also separated on the hybrid monolith in CEC. Six phenols were all well separated with good peak shapes (Fig. 6), the highest column efficiency was calculated at 183 300 N/m for hydroquinone. Particularly, three positional isomers, hydroquinone, pyrocatechol, and resorcin, were almost baseline separated, which demonstrated the high separation ability of the poly(POSS-co-BDA) hybrid monolith. Furthermore, pyrocatechol and phenol were well separated in CEC, in comparison, the baseline separation of pyrocatechol and phenol could not be achieved in cLC even after optimizing the operating conditions (Supporting Information Fig. S3). These results demonstrated the higher separation ability of the poly(POSS-co-BDA) hybrid monolith in CEC.

4 Concluding remarks

A new poly(POSS-co-BDA) organic–inorganic hybrid monolithic column was prepared by the ring-opening polymerization of POSS-epoxy and BDA. The obtained hybrid monolith possessed a well-defined 3D skeleton and high mechanical stability. Benefiting from the uniform microstructure, the hybrid monolith exhibited excellent performance in cLC, in which alkylbenzenes, polycyclic aromatic hydrocarbons, phenols, anilines, and purines and pyrimidines were all successfully separated with high column efficiencies. In addition, the hybrid monolith was applied for the separation of neutral and polar compounds in CEC, and a satisfactory performance was obtained. It was demonstrated that the poly(POSS-co-BDA) hybrid monolith is a good chromatographic media for cLC as well as CEC. In order to further assess the breadth of this hybrid monolith in CEC application, more characterization and separation should be explored in the future.

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5 References


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