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To be cited as: *ChemPlusChem* 10.1002/cplu.201700223

Link to VoR: http://dx.doi.org/10.1002/cplu.201700223

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In Situ Growth of Covalent Organic Framework Shells on Silica Microspheres for Liquid Chromatography

Lu-Liang Wang,[a] Cheng-Xiong Yang,*[a] and Xiu-Ping Yan*[a,b]

Abstract: Exploring of covalent organic frameworks (COFs) as novel stationary phases is intriguing and significant for the current revolution in chromatography. However, the irregular morphology of COFs is the main obstacle for the practical application of COFs in high-performance liquid chromatography (HPLC). Here we report a facile in situ growth strategy to synthesize monodispersed COF@SiO_2 microspheres with uniform and controllable COF shells as the stationary phase for HPLC. TpBD COF constructed from 1,3,5-triformylphloroglucinol (Tp) and benzidine (BD) is taken as the COF shell, while aminosilica (SiO_2-NH_2) is employed as the core to support the TpBD shell. The TpBD shell thickness is adjusted by controlling the concentration of Tp and BD monomers. The TpBD@SiO_2 packed columns show excellent hydrophobic selectivity, good reproducibility for the separation of neutral (toluene and ethylbenzene, polycyclic aromatic hydrocarbons), acidic (hydroquinone, p-cresol and p-chlorophenol) and basic molecules (nucleobases, nucleosides and deoxynucleosides). This work demonstrates the great potential of COFs in separation sciences.

Introduction

Covalent organic frameworks (COFs) with strong covalent bonds formed by organic ligands condensation are an intriguing type of porous crystalline polymers.[1-3] Owing to their periodic molecular arrangement and inherent porosity, COFs have many excellent properties including high surface area, good chemical and thermal stability, and show great potential in diverse areas including gas capture and separation,[4] catalysis[5] and sensing.[6,7]

Chromatography is of prime importance in chemistry, environment, food and life science. The chromatographic column is the core component in chromatography. Exploring of porous materials such as metal organic frameworks (MOFs) [8-26] and COFs [2,27-29] as novel stationary phases is intriguing and significant for the current revolution in chromatography. COFs have been successfully applied as advanced stationary phases in gas chromatography and capillary electrochromatography for the separation of alkanes, aromatic and chiral compounds.[30-33] However, the application of COFs in high-performance liquid chromatography (HPLC) has been lagged behind although their excellent properties in separation science. COFs obtained from the traditional synthesized methods often show irregular shape, sub-micrometer size or broad size distribution, which may cause low column efficiency and high column backpressure of the COFs packed columns, thus being the greatest challenge of COFs in HPLC.[34] Increase of the permeability of the COFs-based columns is an effective way to avoid the above mentioned problems. Recently, *Liu et al*. reported the preparation of methacrylate-bonded COF monolithic columns with good permeability and resolution for enhanced HPLC separation of small molecules.[34] Synthesis of uniform and applicable sized COFs composite microspheres to pack COFs-based columns is another feasible way to overcome the aforementioned challenges.

Horváth and Kirkland put forward the concept of “core-shell silica structure” (solid silica particles coated with a porous shell) in HPLC in the 1960's.[35] The core-shell spherical particles consist of an inner solid silica core and a controlled porosity shell. The HPLC columns packed with core-shell spherical composite can increase column permeability, improve heat dissipation, avoid the band broadening and enhance column efficiency.[36-38] Therefore, controllable synthesis of COF@ silica (COF@SiO_2) core-shell microspheres for HPLC is a pregnant and feasible strategy to overcome the limitations of pure COFs and to expand the application of COFs in HPLC.

Herein, we report an in situ growth strategy to synthesize uniform core-shell COF@SiO_2 microspheres as novel stationary phase for HPLC (Figure 1). In this work, the TpBD (Tp and BD are 1,3,5-triformylphloroglucinol and benzidine, respectively) is selected as the model COF, while the aminosilica (SiO_2-NH_2) is employed as the core to support the TpBD shell. The TpBD shell thickness is easily adjusted by controlling the concentration of Tp and BD monomers. The TpBD@SiO_2 packed columns are evaluated by HPLC for model probe molecules. Nucleobases, nucleosides and deoxynucleosides are the basic and significant components for gene expression and disease diagnose,[39,40] but their base-separation is difficult because of their similar polarity and structures.[41] We demonstrate the good performance of TpBD@SiO_2 packed columns for good separation of nucleobases, nucleosides and deoxynucleosides in the isocratic elution mode. The present in situ growth strategy to the fabrication of uniform COF@SiO_2 core-shell microspheres will trigger a new field for the exploration of COFs in HPLC.

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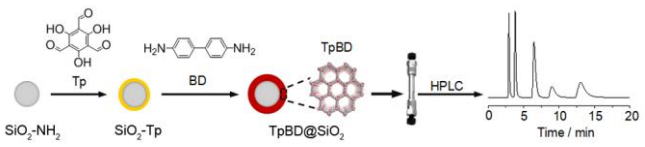

Figure 1. Scheme for the synthesis of the core-shell TpBD@SiO_2 microspheres for HPLC.

Results and Discussion

Fabrication and characterization of TpBD and TpBD@SiO_2 microspheres

A rational selection of solvent and reaction time is vital to the synthesis of highly crystalline COFs.[1,31] The effect of solvent composition on TpBD crystallinity was investigated and monitored by powder X-ray diffraction spectrometry (PXRD). Solvents such as toluene, ethanol and mesitylene were tested to synthesize TpBD under refluxing. However, the intensity of characteristic PXRD peaks at the 3.4° and 5.8° of TpBD were quite low (Figure S1A in the Supporting Information).[30,43] So, a binary solvent of mesitylene and 1,4-dioxane was then considered. The increase of 1,4-dioxane content in the binary solvent resulted in a significant enhancement of the diffraction peaks at 3.4° and 5.8°, indicating the 1,4-dioxane was beneficial to the formation of highly crystalline TpBD (Figure S1A). Finally, pure 1,4-dioxane was applied to prepare highly crystalline TpBD. The effect of reaction time on TpBD crystallinity was also investigated (Figure S1B). The peak intensity of 3.4° and 5.8° was gradually enhanced from 10 min to 30 min, while no obvious increase of the PXRD peak intensity 3.4° and 5.8° was observed from 30 min to 120 min (Figure S1B), indicating the Schiff base reaction of TpBD can be accomplished within 30 min. Therefore, the solvent of 1,4-dioxane and reaction time of 30 min were employed to synthesize TpBD and core-shell TpBD@SiO_2 microspheres.

Core-shell TpBD@SiO_2 microspheres were fabricated by an in situ controllable growth strategy (Figure 1). Spherical SiO_2-NH_2 was selected as both the core and the source of amino group to react with the aldehyde group on Tp to induce the controllable growth of TpBD shell on the surface of SiO_2-NH_2. The obtained SiO_2-Tp was then reacted with BD to form the TpBD shell on the silica core (Figure 1). Such strategy can significantly reduce the formation of irregular and self-aggregated TpBD on TpBD@SiO_2 microspheres (Figure 2, Figure S2). In addition, the thickness of the TpBD shell on TpBD@SiO_2 can be easily controlled by adjusting the concentration of Tp and BD (Table 1).

The obtained TpBD@SiO_2 core-shell microspheres were characterized via PXRD, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), Fourier Transform infrared spectroscopy (FTIR) and N_2 adsorption experiments (Figure 2-3, Figure S2-S5). Only very few TpBD crystals were formed on TpBD@SiO_2-0.10 due to the low concentration of Tp monomer on SiO_2-Tp. Increase of the Tp monomer from 0.10 mmol to 0.30 mmol greatly increased the thickness of TpBD shell on TpBD@SiO_2 microspheres from 50 nm to 150 nm (Figure 2, Figure S4). However, further increase of the Tp monomer to 0.50 mmol led to no obvious improvement of TpBD thickness on TpBD@SiO_2 microspheres, indicating the full occupation of the amino groups on SiO_2-NH_2 by the aldehyde groups on 0.3 mmol Tp. The EDS element mapping images also reveal the uniform distribution of TpBD shell on the surface of SiO_2@TpBD microspheres (Fig. 3C,D).

Table 1. Table Caption. The amounts of SiO_2-NH_2, Tp and BD used for the synthesis of different TpBD@SiO_2 microspheres.

	SiO_2-NH_2	Tp / mmol	BD / mmol
TpBD@SiO_2-0.10	200 mg	0.10	0.15
TpBD@SiO_2-0.30	200 mg	0.30	0.45
TpBD@SiO_2-0.50	200 mg	0.50	0.75

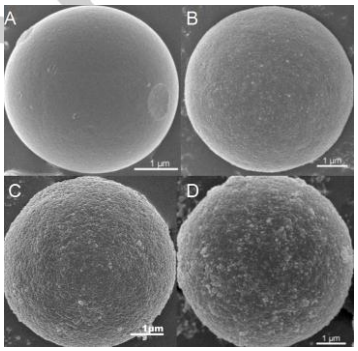

Figure 2. SEM images: (A) SiO_2-NH_2; (B) TpBD@SiO_2-0.10; (C) TpBD@SiO_2-0.30; (D) TpBD@SiO_2-0.50.

The appearance of the peaks at 1653 cm^{-1} (C=N), 1438 cm^{-1} (Ar(C=C)), 1100 cm^{-1} (Si-O-Si), 806 cm^{-1} (SiO-H) and 468 cm^{-1} (Si-O) for Tp and SiO_2-NH_2 in the FTIR spectrum of SiO_2-Tp suggests the successful modification of Tp on SiO_2-NH_2 (Figure S3). The peaks of TpBD at around 1657 cm^{-1} (C=N) and 1458 cm^{-1} (Ar(C=C)) and the peaks of SiO_2-NH_2 at around 1092 cm^{-1} (Si-O-Si), 813 cm^{-1} (SiO-H) and 467 cm^{-1} (Si-O) on TpBD@SiO_2 microspheres confirm the successful formation of TpBD onto the SiO_2-NH_2.[24, 30,] In addition, the increase of the signal intensity at 1657 cm^{-1} (C=N) and 1458 cm^{-1} (Ar(C=C)) from TpBD@SiO_2-0.10 to TpBD@SiO_2-0.50 further demonstrates the controllable growth of TpBD shell onto the silica sphere surface (Figure 3A). The existence of the characteristic peaks at 20-25° for SiO_2-NH_2 and the characteristic peaks 3.4° and 5.8° for TpBD in the PXRD patterns (Figure 3B) confirm the successful synthesis of TpBD@SiO_2 microspheres. The increase of the PXRD peak intensity of TpBD@SiO_2-0.10, TpBD@SiO_2-0.30 and TpBD@SiO_2-0.50 at 3.4° and 5.8° for TpBD also reveals the controllable synthesis of TpBD on SiO_2-NH_2 microspheres.

TpBD@SiO_2-0.30 gave much higher BET surface area (385 m^2 g^{-1}) than SiO_2-NH_2 (224 m^2 g^{-1}) due to the large BET surface area of coated TpBD (596 m^2 g^{-1}) (Figure S5A). Furthermore, TpBD@SiO_2-0.30 showed a large pore (12.3 nm) and a small pore (1.4 nm) (Figure S5B). The small pore came from the shell TpBD (1.4 nm). The large pore was smaller than that of the core

SiO_2-NH_2 (14 nm), indicating the TpBD was also formed on the inner surface of the pore of SiO_2-NH_2.

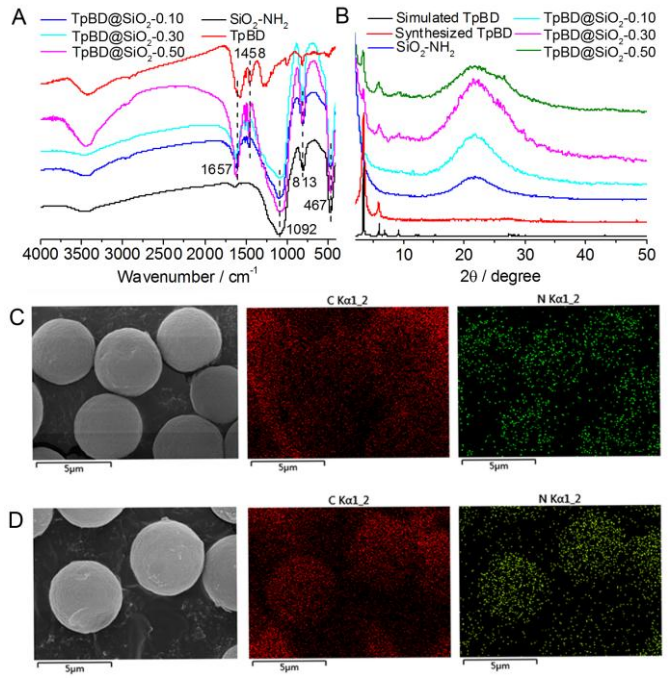

Figure 3. (A) FT-IR spectra and (B) PXRD patterns of SiO_2-NH_2, TpBD@SiO_2-0.10, TpBD@SiO_2-0.30, TpBD@SiO_2-0.50 and TpBD. EDS element mapping: (C) SiO_2-NH_2; D) TpBD@SiO_2-0.30

Evaluation of SiO_2@TpBD packed columns

The prepared TpBD@SiO_2 columns were evaluated with diverse probe molecules.[42] Toluene and ethylbenzene were firstly selected as probe molecules to demonstrate the hydrophobic property of the TpBD@SiO_2 columns. All the TpBD@SiO_2 packed columns using CH_3CN/H_2O (50:50) as the mobile phase gave good separation of toluene and ethylbenzene (Figure 4A). The same elution order of toluene and ethylbenzene on TpBD@SiO_2 packed columns and commercial C18 column reveals the reversed-phase separation behavior of TpBD@SiO_2 columns. The retention factors of toluene ($k'_{toluene}$) and ethylbenzene ($k'_{ethylbenzene}$) on TpBD@SiO_2 columns gradually increased with the TpBD shell thickness (Table S1 in the Supporting Information), indicating the significant role and hydrophobic property of TpBD. Although the retention factors $k'_{toluene}$ and $k'_{ethylbenzene}$ on TpBD@SiO_2 packed columns were less than those on C18 column, the TpBD@SiO_2 packed columns still showed good hydrophobic property.

Neutral polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, 1,2-dihydroacenaphthylene, fluorene, phenanthrene, anthracene and pyrene were tested as nonpolar compounds to further evaluate the hydrophobic property of the TpBD@SiO_2 packed columns (Figure S6, S7). The elution of these PAHs follows an increasing order of naphthalene, 1,2-dihydroacenaphthylene, fluorene, phenanthrene, anthracene and pyrene on TpBD@SiO_2 packed columns, revealing the retention mechanism was based on their increasing hydrophobicity (Table S2).

The separation performance of TpBD@SiO_2 packed columns for acidic and basic compounds was then evaluated (Figure 4B, 4C and Figure S8, S9). The acidic and basic analytes were well resolved on the TpBD@SiO_2 packed columns. The elution of the acidic compounds followed the order of hydroquinone, p-cresol and p-chlorophenol with their increasing acidity. In addition, the basic compounds followed the elution order of n-phenylacetamide, 4-methylaniline, p-nitroaniline with their increasing alkalinity. The different retentions of acidic and basic compounds on the TpBD@SiO_2 packed columns resulted from their different hydrogen-bond and π-π interaction between the TpBD on TpBD@SiO_2spheres and the analytes. Furthermore, the TpBD@SiO_2 packed column gave good separation of basic drug molecules including xanthine, theophylline and caffeine (Figure S10).

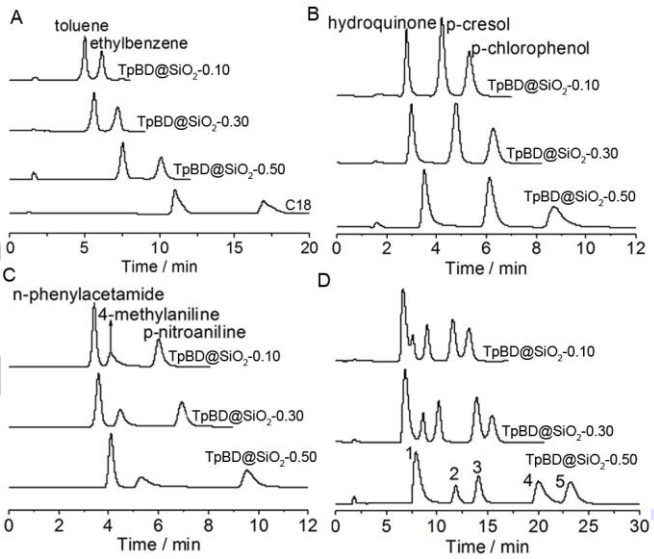

Figure 4. HPLC separation on the TpBD@SiO_2-0.10 packed column, TpBD@SiO_2-0.30 packed column, TpBD@SiO_2-0.50 packed column: (A) toluene and ethylbenzene; (B) acidic compounds; (C) basic compounds; (D) RP5 (1: theophyline; 2: anisole; 3: methyl benzoate; 4: p-nitroaniline; and 5: o-xylene). Mobile phase (1.0 mL min^{-1}): ACN-H_2O (50:50) (A), ACN-H_2O (40:60) (B and C) and ACN-H_2O (30:70); UV detection: 210 nm (A), 254 nm (B, C and D).

We also used theophyline, anisole, methyl benzoate, p-nitroaniline and o-xylene as the five probe molecules (denoted as RP5) to demonstrate the reversed phase separation ability of TpBD@SiO_2 packed columns (Figure 4D). The elution of RP5 followed the order of theophyline, anisole, methyl benzoate, p-nitroaniline and o-xyleneon TpBD@SiO_2 packed columns, which was in accordance with their reversed phase separation mode. The theophylline was usually used to label the dead time of reversed phase columns. However, the theophylline was strongly retained on the TpBD@SiO_2 packed columns, indicating the hydrophobic mechanism was not the only force to retain the theophylline, and the hydrogen-bond interaction between theophylline and TpBD should also play significant roles in the separation. The basic polar molecule (p-nitroaniline) and neutral polar molecules (anisole, o-xylene and methyl benzoate) was well resolved on the TpBD@SiO_2 packed columns. These results reveal the TpBD@SiO_2 packed columns not only show

high resolution for neutral polar molecules, but also give good performance separation for the acidic and basic compounds.

The retention and resolution of all these analytes on TpBD@SiO_2 columns gradually increased with the TpBD shell thickness (Figure 4, Figure S7, S10), suggesting the significant role of TpBD. In addition, the TpBD@SiO_2-0.30 packed columns gave uniform core-shell morphology, high surface area, good reversed phase separation ability and fast separation (Figure 3, 4). The TpBD@SiO_2-0.30 column offered high column efficiency for toluene (22381 plates m^{-1}).

HPLC separation of nucleobases, nucleosides and deoxynucleosides

Nucleobases, nucleosides and deoxynucleosides are the basic and significant components in the living cells.[39] They can not only make different nucleic acids with genetic information, but also be the potential biomarkers of diseases. For example, the detection of 5mdC in the DNA methylation process can be conducive to explore the pathogenesis of cancer.[40] HPLC is the most effective technique to separate and monitor these important componentins. However, the complex buffer system in the mobile phase and the gradient elution mode was usuallyapplied to optimize the separation of these analytes.[39-41] In this work, baseline separation of five nucleobases (Cyt, Ura, Thy, Gua and Ade), five nucleosides (C, U, T, G and A), five deoxynucleosides (dC, dU, dT, dG and dA), 5mdC and dC on the TpBD@SiO_2-0.30 packed column was achieved in the isocratic elution mode without any addition of the buffer in the mobile phase (Figure 5, Figure S11).

The thermodynamics of TpBD@SiO_2-0.30 packed column for the separation of nucleobases, nucleosides, deoxynucleosides, 5mdC and dC was investigated from 25 ^oC to 55 ^oC (Figure S12, S13).[34, 44] Increase of temperature reduced the selectivity and retention of the above analytes on the TpBD@SiO_2-0.30 packed column, indicating that the separation processes of these analytes on TpBD@SiO_2-0.30 packed column were exothermic. The good linearity of the van't Hoff plots shows that the separation mechanism of the above analytes on TpBD@SiO_2-0.30 was unchanged in temperature range of 25-55 ^oC (Figure S13). The negative Gibbs free energy change (ΔG) of the above analytes indicates that the transfer of these analytes from the mobile phase to the TpBD@SiO_2-0.30 stationary phase was a thermodynamically spontaneous process (Table S3). In addition, the analytes with more negative ΔG was apt to have stronger retention on TpBD@SiO_2-0.30 packed column.

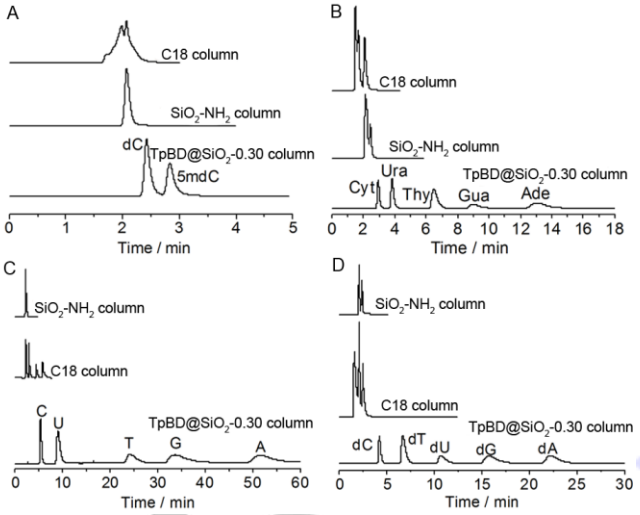

Figure 5. HPLC separation on the SiO_2-NH_2, C18 and TpBD@SiO_2 -0.30 packed columns: (A) dC and 5mdC; (B) nucleobases; (C) nucleosides; (D) deoxynucleosides. Mobile phase (1.0 mL min^{-1}): ACN-H_2O (20:80) (A), ACN-H_2O (10:90) (B and D) and ACN-H_2O (5:95) (C); UV detection: 254 nm

The TpBD@SiO_2-0.30 packed column gave much better resolution than the SiO_2-NH_2 column and C18 column for the separation of these analytes (Figure 5). The maximum kinetic diameters of the above analytes (Figurre. S15) are smaller than the pore size of the TpBD (1.4 nm), we assume that the separation mainly occurred inside the pore of the COF shell.[31,34] For the separation of nucleobases, the elution oder of Cyt, Ura, Thy and Ade is in agreement with their increasing order hydrophobic interaction with the TpBD shell (Figure 5B, Table S4). In addition, Gua and Ade have additional strong π-π interaction with the framework of TpBD (Figurre. S15), so the elution of nucleobases on the TpBD@SiO_2-0.30 packed column followed the order of Cyt, Ura, Thy, Gua and Ade (Figure 5B). The separation of nucleosides, deoxynucleosides was also ascribed to the hydrophobic and π-π interactions between analytes and the TpBD shell of TpBD@SiO_2-0.30 packed column. The TpBD@SiO_2-0.30 packed column gave good reproducibility for seven replicate separations of nucleobases, nucleosides, deoxynucleosides with relative standard deviations (RSD) of 0.4-1.9%, 0.4-3.0% and 0.5-3.0% for peak area, peak height and retention time, respectively (Figure. S14, Table S5). The TpBD@SiO_2 packed column had good stablility and reproducibility to separate nucleobases at least for three months (Figure. S16). These results demonstrate the great potential of TpBD@SiO_2 column as novel stationary phase for the HPLC separation of nucleobases, nucleosides and deoxynucleosides.

Conclusions

In summary, we have shown an in situ growth method for the preparation of monodispersed COF@SiO_2 microspheres with tailored COF shells. The prepared COF@SiO_2 microspheres overcome the main limitations for the application of COFs for HPLC. The COF@SiO_2 columns give good column efficiency, high resolution and good reproducibility for the separation of neutral (toluene and ethylbenzene, polycyclic aromatic hydrocarbons), acidic (hydroquinone, p-cresol and p-

chlorophenol) and basic molecules (nucleobases, nucleosides and deoxynucleosides). This work will trigger a new field for COFs as the stationary phases in liquid chromatography.

Experimental Section

Reagents and chemicals

Tp and BD were purchased from Tongchuangyuan Pharmaceutical Technology Co. (Chengdu, China). Cytidine (C) and Uridine (U) were from Dingguo Changsheng Biotechnology (Beijing, China). Adenosine (A) and guanosine (G) were obtained from Yuanye Biotechnology Co. (Shanghai, China). 5-methyl-2'-deoxycytidine (5mdC) and 2'-deoxycystidine (dC) were from Heowns Biochem Technologies LLC (Tianjin, China). Thymidine (T), cytosine (Cyt), thymine (Thy) and guanine (Gua) were provided by Sigma-Aldrich (St Louis, MO, U.S.A). 5-Methyldeoxyuridine (dT) and 2'-deoxyuridine (dU) were obtained from InnoChem Science & Technology Co. (Beijing, China). Adenine (Ade), uracil (Ura), caffeine, 4-methylaniline, hydroquinone, *n*-phenylacetamid, *p*-chlorophenol, *p*-nitroaniline, theophyline, *p*-cresol and xanthine were bought from Aladdin Co. (Shanghai, China). 2'-Deoxyadenosine (dA) and 2'-deoxyguanosine (dG) was provided by J&K Scientific Co. (Beijing, China). Toluene, 1,2-dihydroacenaphthylene, methyl benzoate, pyrene, fluorene, phenetole, anthracene, ethylbenzene, *o*-xylene, and phenanthrene were provided by Guangfu Fine Chemical Industry Research Institute (Tianjin, China). 1,4-Dioxane, ethanol, mesitylene, acetonitrile (ACN), *N,N*-dimethylformamide (DMF), methanol and methylbenzene were bought from Concord Fine Chemical Institute (Tianjin, China). Aminosilica (5 μm) and C18 silica gel (5 μm) was provided by Borui Jianhe Chromatographic Technology Co. (Tianjin, China). Ultrapure water was given by Wahaha Foods Co. (Tianjin, China). The chemicals used were of analytical grade at least.

Instrumentation

PXRD data were obtained on a D/max-2500 diffractometer with CuKα radiation (Rigaku, Japan). The FT-IR spectra were acquired on a Magna-560 spectrometer (Nicolet, Madison, WI). SEM images were gained on a Shimadzu SS-550 scanning electron microscope. BET surface areas data were performed on a NOVA 2000e analyzer (Quantachrome, USA) using N_2 adsorption at 77 K. Transmission electron microscopy (TEM) was performed on Tecnai G2 F20 (Philips, Holland).

Waters 510 HPLC pump with a 486 tunable UV detector was used for HPLC experiments. The chromatographic data and were acquired on the N2000 system. The column temperature was controlled by CO-5060 column heater (Ameritech, USA).

Synthesis of TpBD

Tp (0.30 mmol) was dissolved with 1,4-dioxane (40 mL) in a 100 mL flask. The BD (0.45 mmol) was quickly added under vigorous stirring, and the mixture were refluxed for 30 min. The light brown TpBD was collected by 5-min centrifugation at 8000 rpm, then washed with ethanol. The synthesized TpBD was refluxed with DMF and ethanol sequentially, and finally dried under vacuum overnight.

Synthesis of TpBD@SiO_2 core-shell microspheres

TpBD@SiO_2 shell-core microspheres were synthesized with different concentrations of precursor solution (Table 1). Tp (0.10, 0.30 or 0.50 mmol) was dissolved with 1,4-dioxane (40 mL) in a 100 mL round-bottom flask. SiO_2-NH_2 (200 mg) was added and refluxed for 30 min. The SiO_2-Tp was then obtained by 3-min centrifugation at 3000 rpm. The BD (0.15, 0.45 or 0.75 mmol) was dissolved with 1,4-dioxane (40 mL) in a 100 mL flask, and mixed with the obtained SiO_2-Tp. The mixture was refluxed for 30 min. The resulting TpBD@SiO_2 were collected by 3-min centrifugation at 1000 rpm, refluxed with DMF and ethanol sequentially. Finally, the TpBD@SiO_2 microspheres were dried under vacuum overnight.

Preparation of TpBD@SiO_2 packed columns

The TpBD@SiO_2 columns were prepared by packing the suspension of TpBD@SiO_2 (3.0 g) in ethanol (20 mL) downward into a stainless steel column (150 mm × long 4.6 mm i.d.) under 6000 psi.[26] The C18 and SiO_2-NH_2 columns were obtained in the same way as for TpBD@SiO_2 columns. Pre-conditioning of the TpBD@SiO_2, C18 and SiO_2-NH_2 packed columns were performed with methanol for 2 h.

Acknowledgements

This work was supported by the National Basic Research Program of China (Grant 2015CB932001), National Natural Science Foundation of China (Grant 21305071), Tianjin Natural Science Foundation (Grants 14JCZDJC37600 and 14JCQNJC06600)

Keywords: covalent-organic framework • stationary phase • liquid chromatography • core-shell composite

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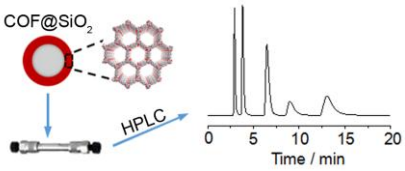
A facile in situ growth strategy was developed to prepare monodispersed covalent organic framework@silica ($COF@SiO_2$) microspheres with controllable COF shells. The synthesized $COF@SiO_2$ microspheres overcome the main obstacles for the application of COFs for liquid chromatography and demonstrate the great potential of COFs in separation sciences.



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