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Preparation and application for separation of small molecules of a new poly(OVS-co-EDMA) hybrid monolithic column

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Abstract

A hybrid monolithic column was prepared using octavinyloctasilasesquioxane (OVS) as a monomer, benzoyl peroxide/dimethylacetamide (BPO/DMA) as initiator, ethylene glycol dimethacrylate (EDMA) as cross-linker, 1-dodecanol as porogenic agent and dimethylbenzene as cosolvent. A tidy skeleton, much bigger specific surface area (22.4 m^2/g) and lower swelling property of the monolithic column with OVS added than the one without OVS added were determined with Scanning Electron Microscopy (SEM), Nitrogen adsorption/desorption measurements (BET) and swelling test with elute of different concentration of acetonitrile in water. Fourier-transform infrared spectra (FTIR) was taken to characterize the composition of groups. Moreover, a better separation performance for benzene series compounds under reversed phase liquid chromatography (RPLC) mode was obtained using the monolithic columns with OVS added than those without.

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Introduction:

Emerged in 1990s[1], porous monolithic materials have been confirmed to be a good stationary phase for their straightforward fabrication, excellent permeability, high separation efficiency and other unique advantages to be used for separation of small molecules and biological matrix[2-4], especially. Different from short-comings of traditional monolithic columns, the merits of hybrid monolithic columns including wide pH range tolerance, good mechanical stability, easy functionalization and high permeability enhanced its application[5-7]. That's because of the inorganic and organic components in hybrid polymers being linked together by covalent bonds. Such polymers are in fact molecular composites and are produced most often by the copolymerization of a monomer with an organically functionalized inorganic compound [8]. With those merits mentioned above, hybrid monoliths have been applied in both online separation fields including capillary liquid chromatography (CLC)[9], capillary electro-chromatography (CEC)[4,19], etc[10]. However, the process to prepare hybrid monolithic, the tediously condition-sensitive sol-gel and comprehensive polymerization, encumber their development and application [9].

Polyhedral oligomeric silsesquioxanes (POSS), a series of cage-like three-dimensional oligomeric, organosilicon compounds, were used for the preparation of hybrid monoliths with free radical polymerization via "one-pot" process[11,12] instead of the tedious and uncontrollable sol-gel procedure[13,14] in recent years. The periphery consisting of cage frameworks is covalently surrounded by organic groups, such as alkyl, vinyl, ester group, phenyl etc[8]. Many papers about the preparation and application of hybrid monoliths with POSS added have been published. Peng and his coworkers[1], for example, prepared two kinds of monolithic capillary columns with POSS added via photo- and thermally-initiated polymerization. And the POSS had been used to prepare hybrid monolith by Yao and his team members for high performance capillary electro-chromatography (CEC) [4]. Zou et al., prepared several kinds of hybrid monolithic capillary columns with POSS via click reaction, thermal- and photo-initiation for the separation of small molecules [1, 9,12,14,15].

In this study, a facile approach for preparation of hybrid monolithic columns with and without OVS added was adopted via thermal-initiation. Chromatographic assessments and applications were carried out to determine the chromatographic performance of the obtained monolithic columns.

2. Experimental section

2.1. Materials and Instruments

Materials used in the work were AR grade except methanol and acetonitrile, which was HPLC grade. Octavinyloctasilasesquioxane (OVS) used in this study was produced from Aladdin Industrial Corporation (Shanghai, China). The reagent including acetonitrile, methanol, dimethylbenzene and 1-dodecanol were bought from Tianjin kermel chemical reagent co., Ltd. (Tianjin, China). Ethylene glycol dimethacrylate (EDMA) was purchased from Acros (New Jersey, USA). Benzoyl peroxide (BPO) and dimethylacetamide (DMA) were bought from Tianjin Guangfu Fine Chemical Research Institute

All chromatography experiments were conducted on two Thermo Unitmate 3000 system (Thermo scientific, USA) equipped with solvent delivery pumps, pump mixers, well-plate autosamplers and UV detectors.

Ultrasonic cleaning machine was bought from Kun Shan Ultrasonic Instruments Co., Ltd (Jiangsu, China). Deionized water with an electrical resistivity of 18.25 mΩ/cm was prepared by a Up-pure Deionizer (Chengdu, China), and an automatic potentiometric titrator, which had been calibrated before used, was purchased from Shanghai Grows Precision Instrument Co., Ltd. (Shanghai, China). The freeze dryer was a product of Gold Sim (Gold Sim, USA). The thermostat water bath was tailor-made from Nanjing Startlab Co., Ltd. (Jiangsu, China). The vacuum drying oven with an oil pump was purchased by Shanghai Boxun Industry &Commerce Co., Ltd. (Shanghai, China)

2.2. Preparation of poly(OVS-co-EDMA) monolith column

The preparation was carried out with the method of in-situ polymerization in stainless steel columns (50x4.6 mm i.d.). A certain amount of OVS was first dissolved in a certain amount of dimethylbenzene (0.30 mL), and followed with the addition of desired amount of crosslinker, porogenic solvent (EDMA and 1-dodecanol). Then with BPO (0.0045 g) added, the mixture was sonicated for 15 min under nitrogen below 30 ℃ to obtain a homogeneous solution. And finally, with addition of DMA (40 □L) and vortex for 10 seconds, the polymerization mixture was poured in a stainless steel column with stoppers at the both sides to let the polymerization carry on. After being synthesized for 2.5 h at 35 ℃ in an oven, the obtained monolithic column was connected with the HPLC system and washed with ethanol (0.20 mL/min for 30 min) and methanol(10.00 mL/min for 60 min) to remove 1-dodecanol, dimethylbenzene and other soluble compounds present in the polymeric rod.

2.3. Characterization

2.3.1. Scanning electron microscopy (SEM)

The poly(OVS-co-EDMA) monolith was firstly eluted with methanol for 5h and dried under freezing for 48h. Then it was observed on a JEOL SEM 6700 microscope operating at 10kV

2.3.2. Fourier-transform infrared spectra (FTIR)

Fourier-transform infrared spectra (FTIR) were recorded by a Varian 640-IR instrument (Varian America) with a resolution of 4 cm^{-1} for 32 scans over a wave number range of 4000–400 cm^{-1}. The tablets were prepared with the mixture of KBr and samples, both of which were dried under 80 ℃ for 48h in a vacuum oven.

2.3.3. Nitrogen adsorption/desorption measurements

The detection of specific surface area of the dry bulk monoliths was performed by nitrogen adsorption-desorption on a MicromeriticsTristarⅡ3020 (Micromeritics, USA). The samples with different amount of OVS were dried under freezing for 48h and then purged on a Micromeritics flow prep 060 (Micromeritics, USA) with nitrogen for 6 h under 50 ℃. .

3. Result and Discussion

3.1. Optimization for the preparation conditions of monolithic columns

Single factor experiment was introduced for the optimization conditions to prepare poly(OVS-co-EDMA) hybrid monolithic column. With the consideration that the ratio between crosslinker, monomer and porogenic

Table 1. Conditions optimization for the preparation of poly(OVS-co-EDMA) hybrid monolithic columns

Column	EDMA (ml)	1-Dodecanol (ml)	Dimethylbenzene (ml)	OVPOSS (mg)	BPO/DMA (mg/µl)	Mechanical/ Physical properties (s[a],n[b],h[c],d[d])	Backpressure[e] (bar)
A	0.40	1.20	0.30	20	4.5/40	d	x
B	0.50	1.10	0.30	20		s	x
C	0.60	1.00	0.30	20		n	7
D	0.70	0.90	0.30	20		hh	14
E	0.80	0.80	0.30	20		hhh	19
F	0.55	1.05	0.30	30		d	x
G	0.55	1.05	0.30	20		n	8
H	0.55	1.05	0.30	10		n	7
I	0.55	1.05	0.30	0		s	x
J	0.6	1.00	0.30	40		d	x
K	0.6	1.00	0.30	30		h	9
L	0.6	1.00	0.30	10		n	7
M	0.6	1.00	0.30	0		s	x
N	0.65	0.95	0.30	40		hhh	20
O	0.65	0.95	0.30	30		hh	15
P	0.65	0.95	0.30	20		h	11
Q	0.65	0.95	0.30	10		n	8
R	0.65	0.95	0.30	0		n	12

[a] the Mechanical/physical properties of monolith is soft.

[b] the Mechanical/physical properties of monolith is not soft or hard.

[c] the Mechanical/physical properties of monolith is hard.

[d] deposition of OVS from mixture happened.

[e] Back pressure was obtained with methanol as the mobile phase at 1.00 mL/min and temperature at 25℃.

agent representing great affection to the polymer skeleton and also its chromatographic behaviors, several columns were prepared under different ratios between EDMA, OVS and 1-dodecanol illustrated in **Table 1**. Back pressure was adopted to compare the chromatographic performance of the monolithic column. Firstly, experiments from column A to column E were used to optimize the ratios between crosslinker and porogenic agent (EDMA and 1-dodecanol) ranging from 1:3 to 1:1 with constant bulk volume (1.60 mL). It could be seen that the more 1-dodecanol added, the softer material was obtained. Even though the mixture of column B could polymerize, the OVS precipitated from the mixture leading to an uneven monolith which is useless. This can be explained by much lower solubility of OVS in 1-dodecanol. Under the proper amount of crosslinker and porogenic agent, different amount of OVS (ranging from 0 mg to 40 mg) was added to investigate the effect of OVS to the columns formation. While, comparing the results from column R to column N, the mechanical strength of composite was getting stronger and the back pressure decreased at the beginning and then increased when more and more OVS was added. The phenomenon may be due to the inorganic material enhancing the mechanical properties of hybrid monoliths. The reduction of pressure along column with 20 mg OVS (column P) may because of the disordered agglomerated organic globules (as seen for column R) turned into innerframe structure. In **figure 1**, the column P possessed not only higher permeability ($1.2305\text{x}10^{-12}$ cm^2 for column P, $0.9829\ \text{x}10^{-12}$ cm^2 for column R) but also higher pressure endurance than that of column R. While, except the role in skeleton structure, another function of OVS during polymerization was nucleation.

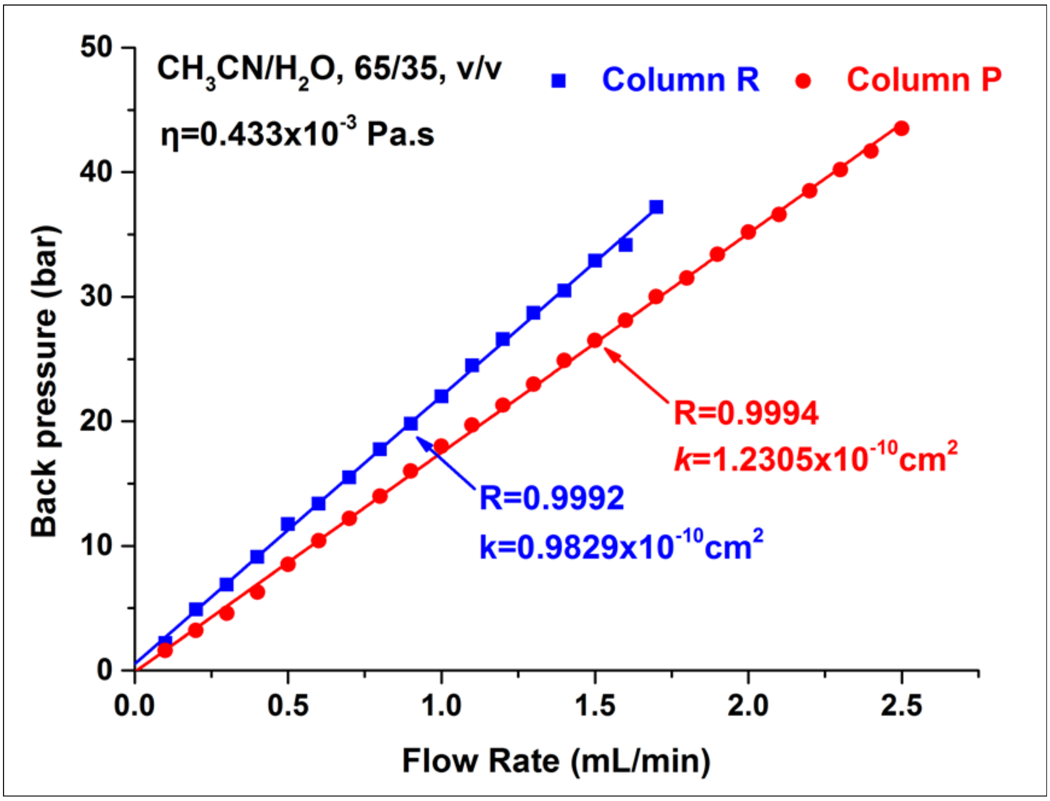

Figure 1. Plot of the pressure drop along the home-made monolithic column. The mobile phase was a mixture of acetonitrile and water (65/35, v/v) and temperature was 25±0.1℃ at flow rate between 0.10 and 2.50mL/min.The backpressure removed the viscosity influence of elutes.

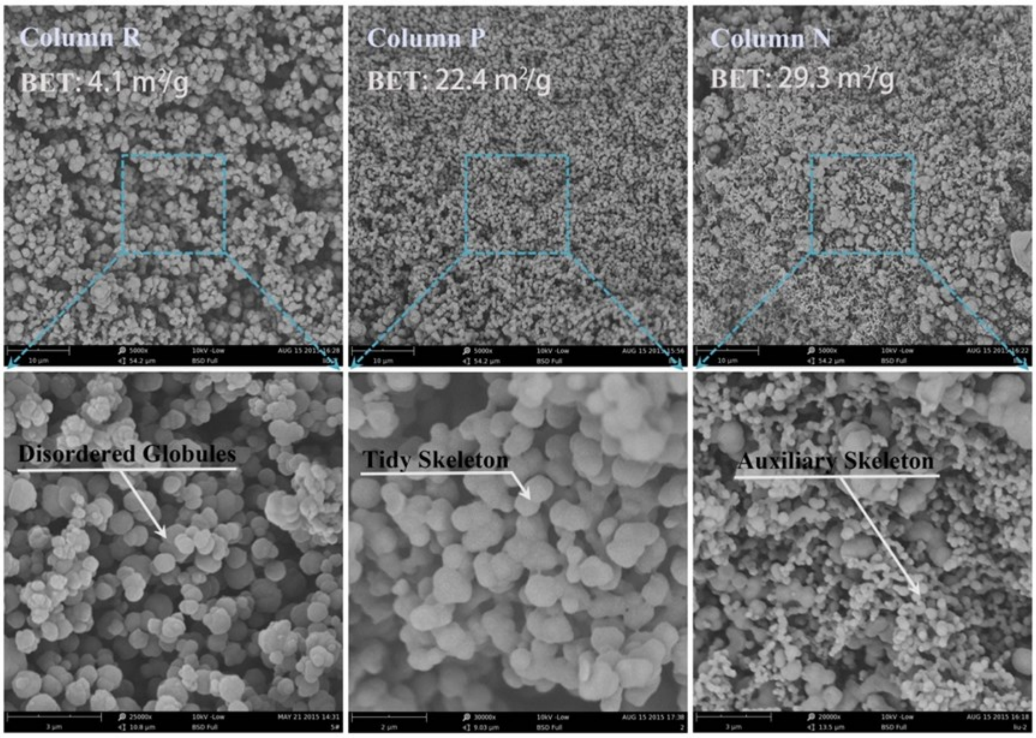

Figure 2. Polymer skeleton and specific surface area of monolithic columns with different amount of OVS tested with SEM and BET.

When the amount of OVS added being more than 30mg, the nucleation of OVS created smaller size of skeleton and more pyknotic accumulation of monolithic polymer in microscopic size.

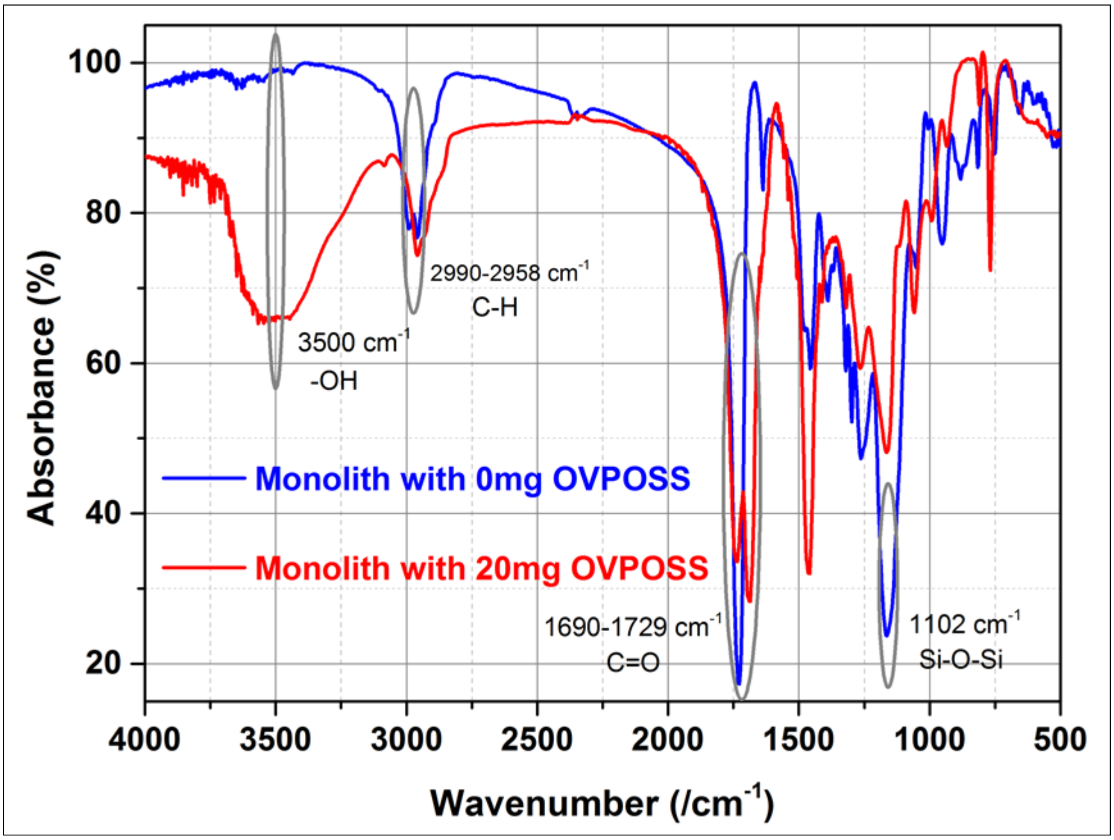

Figure 3. Difference between monolith with OVS (redline) and without OVS (blue-line) added based on FT-IR spectra.

Opinions above could be illustrated in **figure 2**. The effect of increasing the amount of OVS (column R, P, N) was observed using SEM and BET. More morphology was obtained for monolith with OVS (20 mg) than without. Even though larger specific surface area could be observed in column N, the tidy skeleton in proper size of column P was much more suitable for preparation of efficient monolithic column. The auxiliary skeleton observed for column N decreases the uniformity and controllability during the polymerization leading to lower chromatographic performance. The condition of column P will be used for further experiment.

FTIR was used to confirm the group composition in the monolith with and without OVS added. The result was shown in **figure 3**. The peaks associated with groups are C-H (2990-2958 cm^{-1}), C=O (1690-1729 cm^{-1}), Si-O-Si (1102 cm^{-1}) and –OH (3500 cm^{-1})[18].

3.2. Swelling properties

In liquid chromatography, the mobile phase can diffuse into and even swell the polymer monolith. It means that the volume and morphology of monolithic column can be different between the monolith in dry and wet state[16].

The swelling properties of the obtained monolithic column with and without OVS added was measured using different acetonitrile-water mixtures as the mobile phase. Thiourea, a non-retained compound for reversed-phase liquid chromatography, was injected to measure the porosity of monolith[18]. The elution volumes of thiourea under different concentration of acetonitrile in water for column P and R were illustrated in **figure 4 I curve A and curve B**, respectively. The result demonstrated that the elution volumes of thiourea were almost the same (0.5789 mL for column R and 0.5741 mL for column P) when the concentration

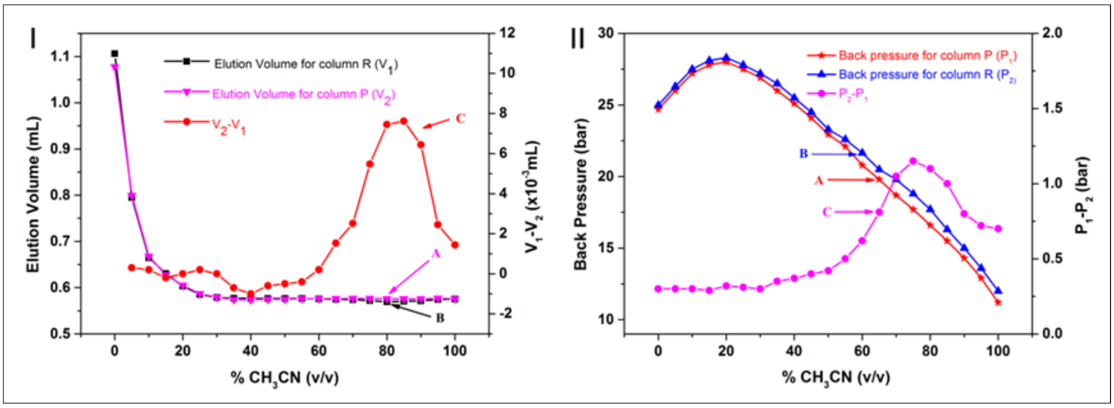

Figure 4. Top graph: changes in elution volume with changing composition of aqueous acetonitrile mobile phase. Curve A: the elution volume of thiourea for column P, curve B: the elution volume of thiourea for column R and curve C: calculated by subtracting curve B from curve A. Bottom graph: changes in backpressure with changing composition of aqueous acetonitrile mobile phase. Curve A: the pressure drop for column P, curve B: the pressure drop for column R and curve C: calculated by subtracting curve B from curve A. Experiment conditions were: elute was mixed from different concentration of acetonitrile and water, the temperature was 25±0.1℃ and flow rate was 1.00mL/min at the wavelength of 262 nm.

of acetonitrile was above 25%, during which a classical reverse phase liquid chromatography (RPLC) has been proved because of the non-retention property of thiourea along the monolith. The porosity of both monolith (69.7% for column R and 69.1% for column P) could be calculated from the elution volume of thiourea. While, a bigger swelling property of column R than column P could be seen from the sharp raise and drop appearance in **curve C**, which was calculated by subtracting **curve B** from **curve A**. The trend of **curve C** in **figure 4**Ⅱwas also calculated from **curve A** and **curve B**, which were the plots of back pressure with the concentration of acetonitrile in water raising from 0% to 100%. The tendency of both column P and column R were almost the same. The highest back pressure detected for both column R and column P were taken place with 80% acetonitrile in water as the elution. The elution volume of thiourea using 80% acetonitrile was smaller (about 4.1% and 1.5%) than 100% acetonitrile for both column R and column P (the dead volume of the chromatography system was removed). The result means that both columns swelled and the swelling of column P was smaller than the column R. The phenomenon above could be explained by the stronger rigidity of monolith skeleton with OVS than without OVS limited the swelling of polymers

3.3. Plate height along the flow rate

Plate height of the obtained monolithic column along flow rate was examined with the elute of acetonitrile

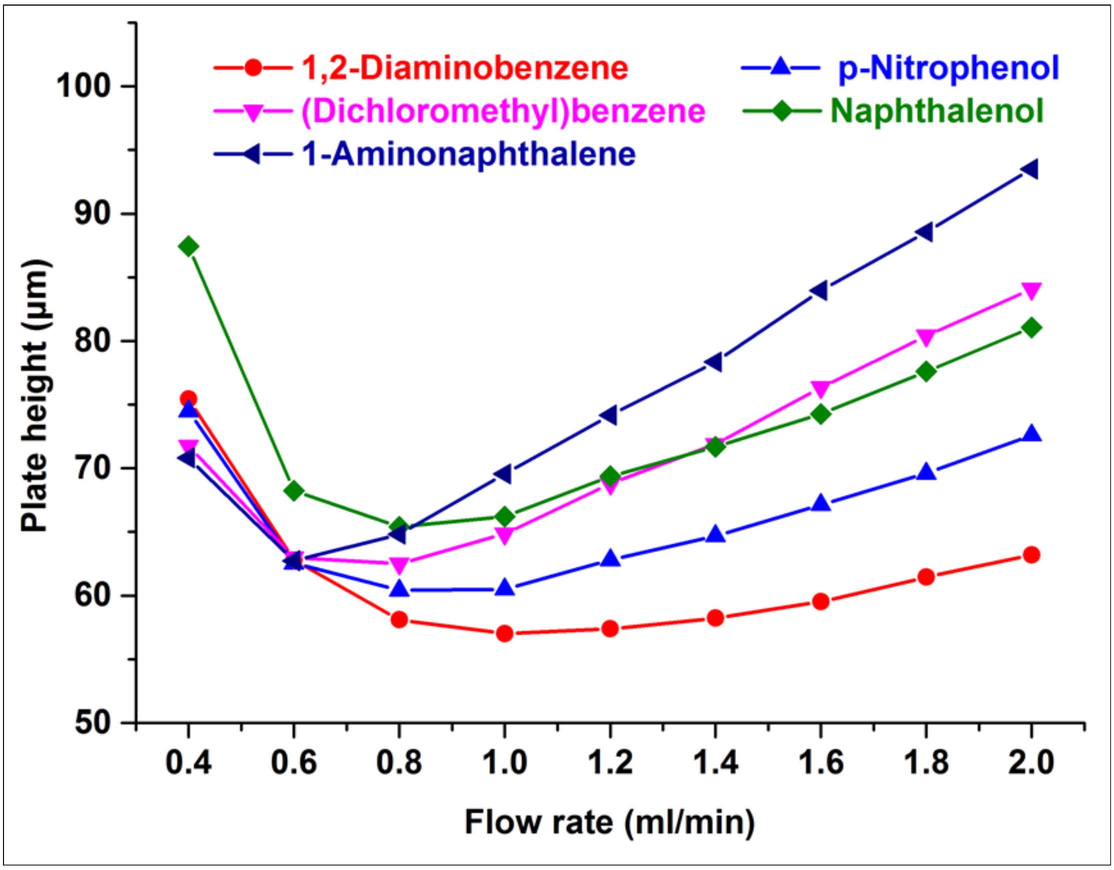

Figure 5. Plate height of different compounds recorded from column P under different flow rate. Experiment conditions were: the elute was a mixture of acetonitrile and water (75/25, v/v) and temperature was 25℃.

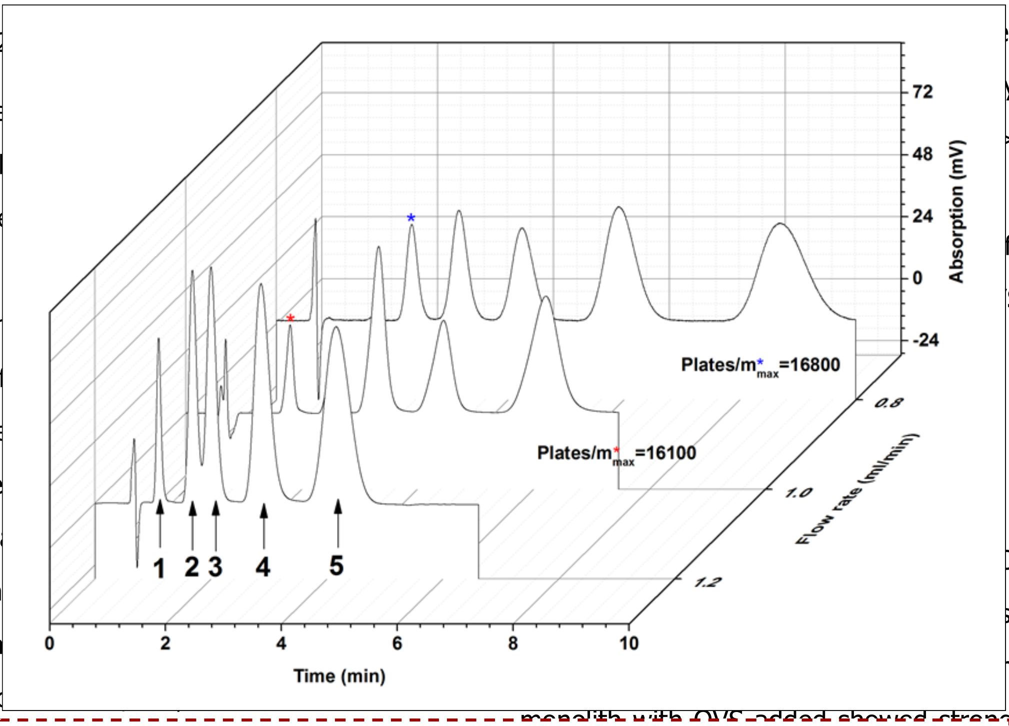

Figure 6. Chromatographic performance of column P under different flow rates (0.80, 1.00 and 1.20mL/min). The compounds are: 1: 1,2-Diaminobenzene, 2: p-Nitrophenol, 3: (Dichloromethyl)benzene, 4: Naphthalenol, 5: 1-Aminonaphthalene.

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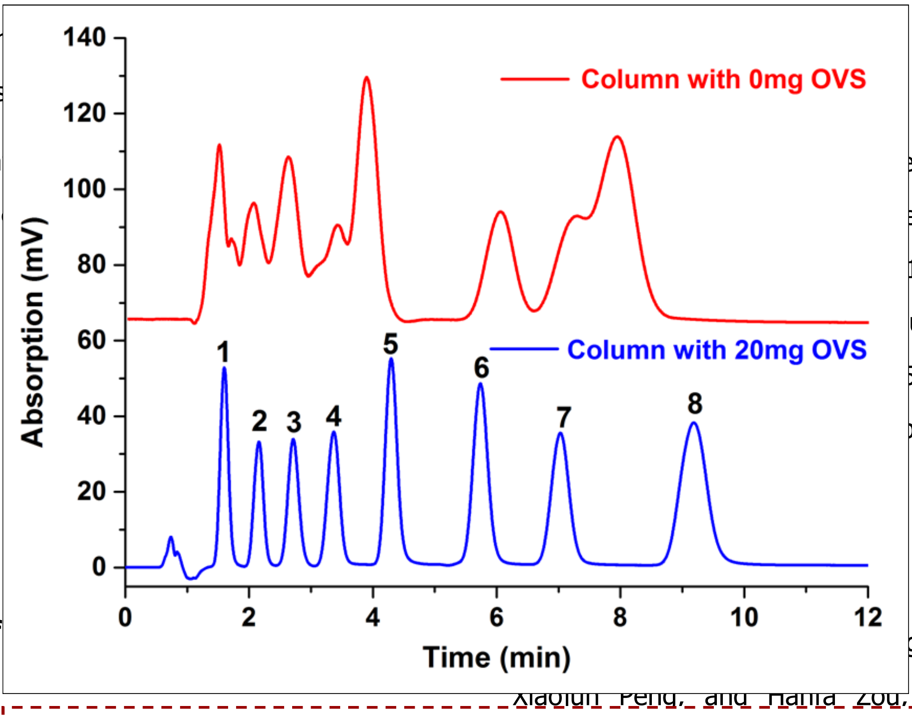

Figure 7. Small molecules separated via monolithic column with and without OVS added. Compounds were: 1:1,2-Diaminobenzene, 2:p-Nitroaniline, 3:Benzene, 4:1-Naphthylamine, 5:4-Chloronitrobenzene, 6:Naphthalene, 7:Diphenyl, 8:Phenanthrene. Experiment conditions were: the elute was a mixture of acetonitrile and water (65/35, v/v), temperature was 25℃ and flow rate was 1.00mL/min.

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