Use of polyhedral oligomeric silsesquioxane for fabrication of monolithic columns of different polarities and diameters

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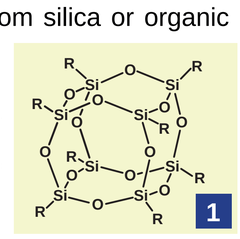
Introduction

Monolithic columns are a great alternative to common “particle packed” columns. Their nature is completely different, because the column is formed by a single monolithic solid block with bimodal inner structure. This results in good permeability and mass transfer kinetics, which offer higher separation efficiency at a similar pressure drop. Another positive attribute of capillary monolithic columns is the absence of column-end frits that are a frequent source of various issues (unwanted sorption of analytes, peak broadening). Typically, monolithic columns are prepared from silica or organic polymers (methacrylates, polystyrenes).

Polyhedral oligomeric silsesquioxane (POSS) are hybrid materials, popular in various fields of polymer industry. They consist of an inert and hard core structure with Si–O–Si bonds, surrounded by organic groups of polymers (e.g. R = methacrylate), **Fig.1**. Chemical stability of POSS, its nanoscale-architecture and possibility of surface modification is a challenge to use POSS as a chromatographic materials.

In our work, we studied the possibility to prepare monolithic chromatographic columns of wide range of diameters (50 – 530 µm) so that they could be used in a variety of analytical instruments and applications. Our goal was to find out the composition of polymerization mixtures when the best efficiency for nonpolar and polar stationary phases can be achieved.



Experimental

Monolithic capillary columns were prepared according to optimized formula, presented in **Fig.2**. The mixture consisted from POSS-methacrylate precursor, lauroyl peroxide initiator, crosslinker and porogens. For nonpolar stationary phase (C18), stearyl methacrylate was used for surface modification.

In the case of polar column synthesis, modification was performed by sulfobetaine zwitterions. We used commercially available DMAPS (also known as MEDSA) or prepared DMABS, **Fig. 3**. Synthesis of DMABS was performed according to **Fig. 4**.

Fused silica capillaries (50 - 530 µm i.d.) were filled with 1 M NaOH (2 hrs at 60°C) then rinsed with water, filled with 1 M HCl (0.5 hr at 25°C), and again rinsed with water, methanol, and finally dried with a stream of nitrogen at 25°C. Pre-treated silica capillaries were modified with a solution containing 20% v/v of γ-MAPS in toluene (20 hrs at 80°C), rinsed with toluene, methanol, and dried with the stream of nitrogen at 25°C.

These capillaries were used for preparation of the monolithic columns according of the mixture presented in **Fig. 2** for 20 hrs at 65 ºC.

Prepared columns were evaluated by liquid chromatography employing a water-rich or organic-rich mobile phases. Pore distribution was measured by ISEC in THF mobile phase by elution of polystyrene samples with molecular mass in the range 500 - 2M.

60 mg	POSS - methacrylate
1 mg	lauroyl peroxide
7.5 mg	pentaerythritol tetraacrylate
10 mg	BRIJ-35
20 mg	stearyl methacrylate (C18) (or DMAPS or DMABS)
330 µL	/ 356 µL methanol
50 µL	/ 24 µL dodecanol

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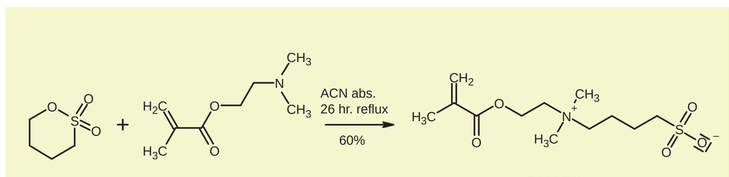
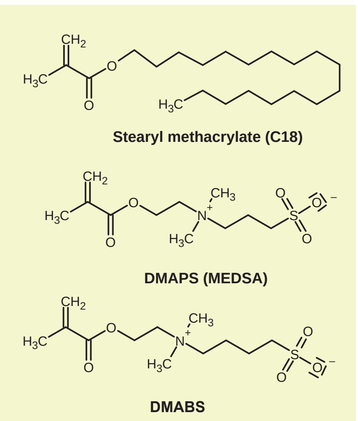
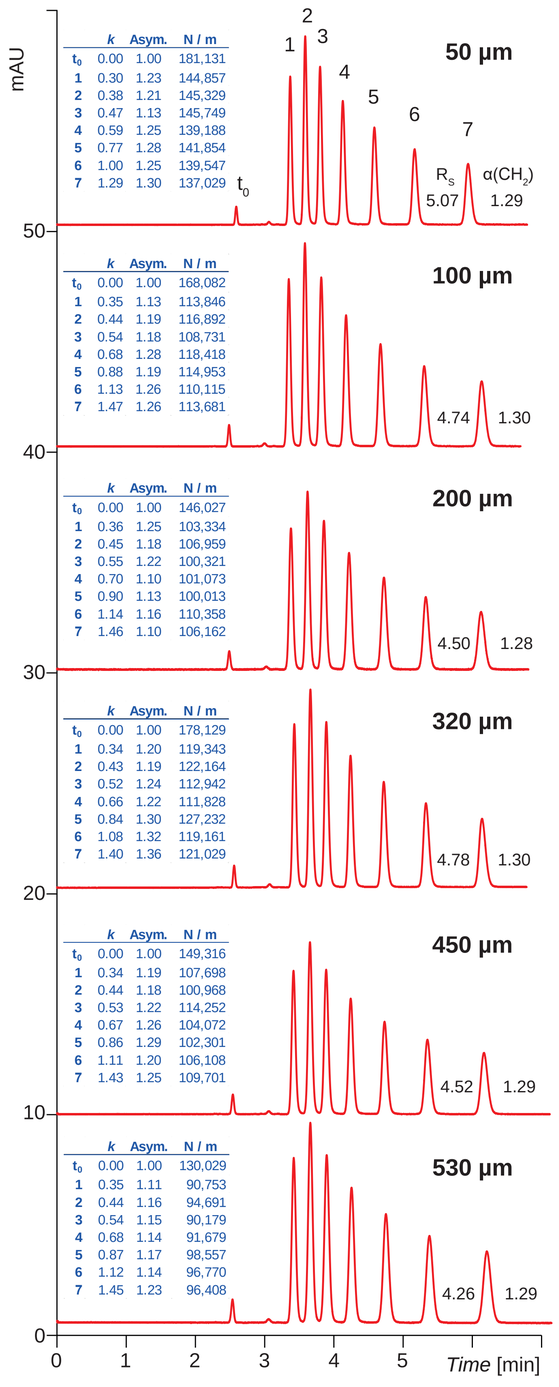
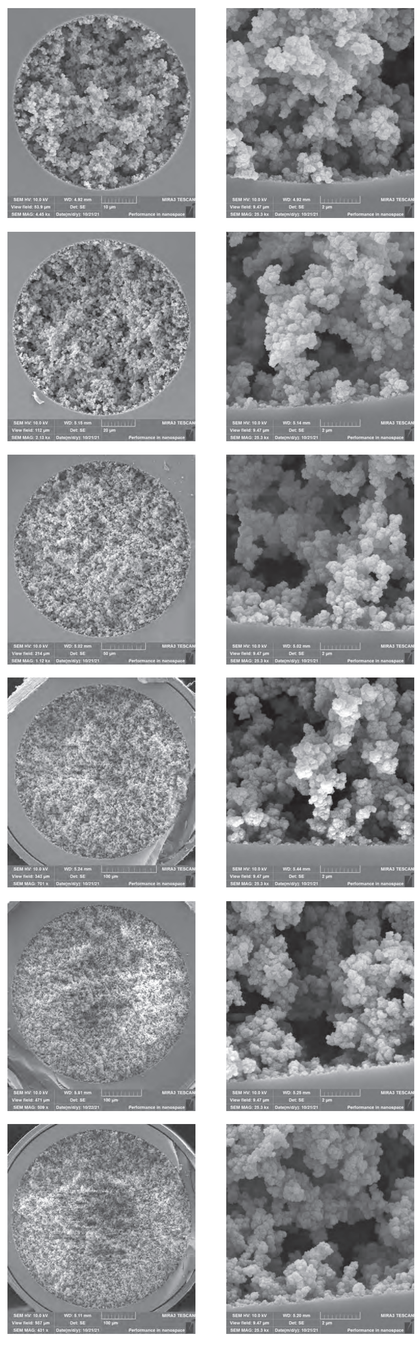




Results





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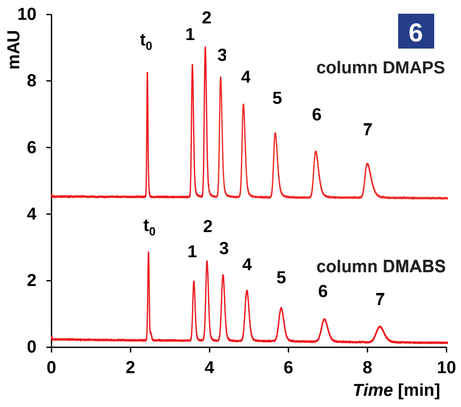
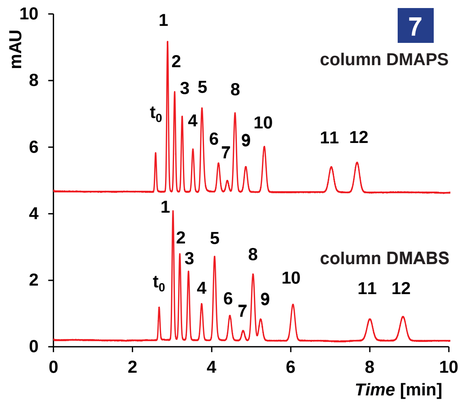
Separation of alkylbenzenes on prepared C18 monolithic columns (50 – 530 µm i.d.) and SEM photographs. Peak identification: (t_0) uracil; (1) benzene; (2) toluene; (3) ethylbenzene; (4) propylbenzene; (5) butylbenzene; (6) pentylbenzene; (7) hexylbenzene; k – the retention factor, Asym. – the peak asymmetry, N/m - the number of theoretical plates per meter of column length, RS – resolution between adjacent peaks, α(CH2) – methylene selectivity. Running conditions: column length 150 mm; mobile phase: 80 ACN/20 water (v/v %); linear velocity of mobile phase 1 mm/s; UV detection at 210 nm

Separation of alkylbenzenes

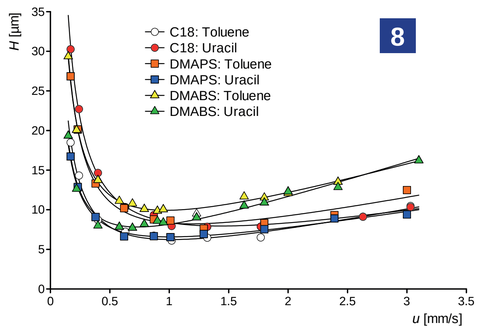


Running conditions: Split 1:20, column 160 mm × 0.45 mm; mobile phase: 60ACN/40 5 mM ammonium acetate (v/v %); UV 210 nm
Peak identification: (t_0) uracil, (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene, (6) pentylbenzene, (7) hexylbenzene

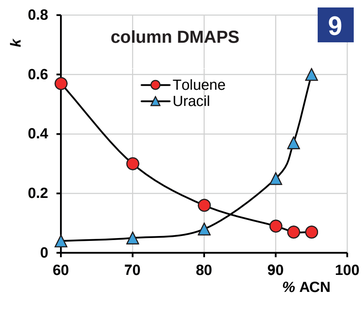
Separation of purine-, pyrimidine-bases and nucleosides



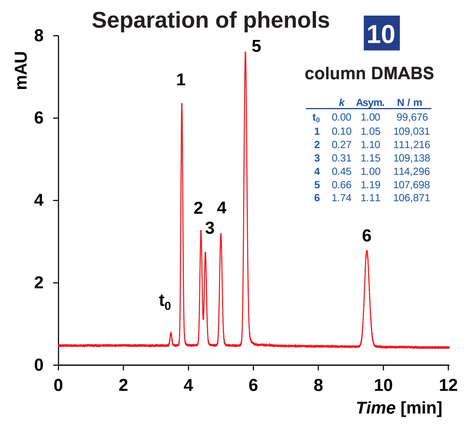
Running conditions: Split 1:20, column 160 mm × 0.45 mm; mobile phase: 5 mM ammonium acetate in 92.5% ACN (v/v); UV 210 nm
Peak identification: (t_0) acetone, (1) xanthine, (2) uracil, (3) 2-deoxyuridine, (4) adenine, (5) 5-methyluridine, (6) cytosine, (7) uridine, (8) 2-deoxycytidine, (9) inosine, (10) cytidine, (11) guanine, (12) guanosine



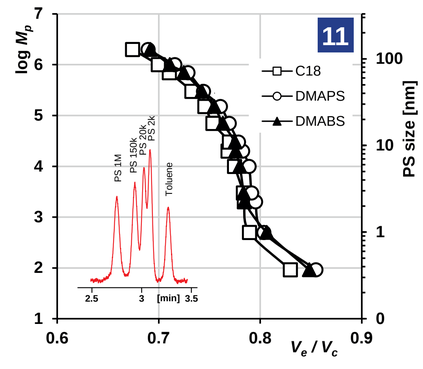
The van Deemter plots obtained with columns C18, DMAPS and DMABS. Split 1:20, column 160 mm × 0.45 mm; mobile phase: 5 mM ammonium acetate in 92.5% ACN (v/v); UV 210 nm



Retention factor of toluene and uracil as a function of % ACN for DMAPS column. Running conditions: columns 0.45 × 160 mm; mobile phase: 5 mM ammonium formate in ACN (v/v)



HILIC separation of phenolic compounds on DMABS monolithic column. Split 1:20, column 160 mm × 0.1 mm; mobile phase: 92.5% ACN; flowrate = 360 nL/min; p=3.7 MPa; detection UV 210 nm. Peak identification: (t_0) toluene, (1) phenol, (2) catechol, (3) hydroquinone, (4) resorcinol, (5) pyrogallol, (6) phloroglucinol



ISEC calibration curves for the tested capillary columns and the example of PS standards separation. V_e/V_c is the ratio of the elution volume of toluene and narrow-distribution polystyrene standards with different molar masses and the geometrical volume of the empty cylindrical column. The right axis shows the equivalent size of the PS molecule. Mobile phase: tetrahydrofuran (THF), columns: length 160 mm, the linear velocity of mobile phase 0.8 mm/s, UV detection at 210 nm.

Conclusions

► Highly crosslinked monolithic capillary columns with inner diameters in the range of 50–530 µm were prepared. The designed polymerization mixtures (**Figure 2**) are suitable for the preparation of stable and efficient monolithic columns. All columns exhibits high efficiency (more than 100 000 theoretic plates) - **Figure 5, 8**.

► The transition from HILIC to RP mode for DMAPS monolithic column occurs at composition about 84 % of ACN. This confirms that this phase has predominantly nonpolar nature, especially with combination with non-polar POSS-methacrylate- **Figure 9**.

► For polar columns, two zwitterionic sulfobetaine monomers were individually incorporated to the monolithic network. One of them was commercially available DMAPS (MEDSA), the other was synthesized (DMABS) - **Figure 3, 4**.

► Separation ability of the columns were demonstrated by separation of hydrophobic compounds (alkylbenzenes) as well as hydrophilic mixtures (purine-, pyrimidine-bases, nucleosides and phenolic compounds) - **Figure 6, 7, 10**.

► The shape of the ISEC curves indicates that pores of 10-100 nm in the stationary phase are more abundant than the smaller pores in the range 1-10 nm (a higher slope of ISEC) - **Figure 11**.

► Efficiency of prepared columns decreased by less than 3% during two months of active use (approx. 3500 analyses).

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