PROTEOMIC ANALYSIS ON SHORT MONOLITHIC COLUMNS

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DESCRIPTION OF THE STUDY

Highly crosslinked monolithic capillary columns with inner diameter of 320 µm were prepared by radical polymerization of pentaerythritol tetraacrylate, polyhedral oligomeric silsesquioxane-methacrylate, and n-octadecyl methacrylate (RPLC) or sulfobetaine methacrylate (HILIC) in the presence of methanol, dodecyl alcohol, and polyethyleneglycol lauryl ether. The columns were evaluated in liquid chromatography. The HPLC analyses of peptides were performed on a portable chromatographic system assembled in our laboratory and connected to ESI-MS instrument (Bruker amaZon SL mass spectrometer - Bruker Daltonics, Bremen, Germany - equipped with an electrospray ion source).

MONOLITHIC COLUMNS

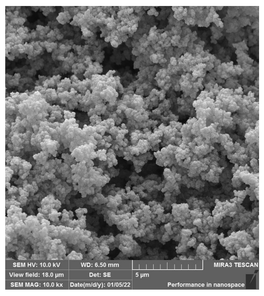
The monolithic columns were prepared in fused silica capillaries (320 µm i.d.) by radical polymerization (20 hrs at 65°C) of the mixture consisting of 60 mg POSS- methacrylate, 7.5 mg pentaerythritol tetraacrylate, 1 mg lauroyl peroxide, 10 mg BRIJ35, and 20 mg *n*-octadecyl methacrylate or sulfobetaine methacrylate in the presence of a porogenic mixture (**Table 1**).

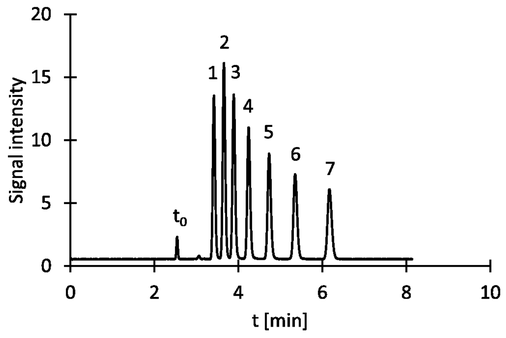
Column	Methanol/Dodecyl alcohol [µl]	Efficiency [N/m]	Pressure [MPa]
C18	330/50	110 000	2.8
HILIC	356/24	106 000	3.4

Table 1: Prepared monolithic columns

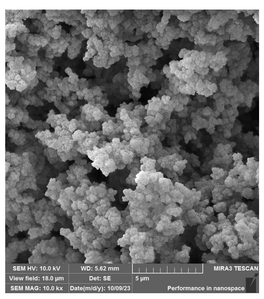
Efficiency [N/m] - the number of theoretical uracil plates per column length meter, flow rate 6 µl/min, mobile phase 90 ACN/10 water (v/v %).
Monomers: C18 – octadecylmethacrylate, HILIC - [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide.

RPLC





HILIC



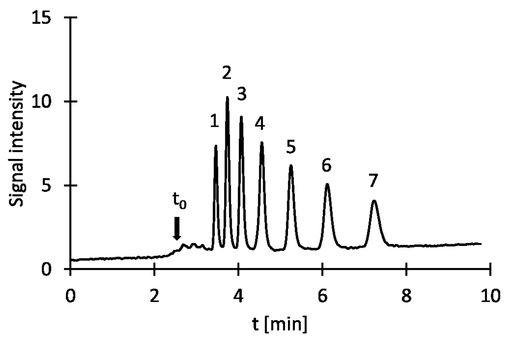


Figure 1: SEM photographs and separation of alkylbenzenes

Columns: 0.32 × 160 mm; Mobile phase: RPLC 80 ACN/20 water (v/v %), HILIC 60 ACN/40 5 mM ammonium acetate (v/v %); Linear velocity of mobile phase 1 mm/s. Peak identification: (t_0) uracil, (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene, (6) pentylbenzene, (7) hexylbenzene; Capillary LC system Agilent 1200, microflow mode - 10 µl/min, DAD 254 nm.

PORTABLE CHROMATOGRAPHIC SYSTEM

The portable chromatographic system has been assembled in the laboratory. It consists of a high-pressure syringe pump and a pair of low-pressure syringe pumps ensuring rapid and reproducible analyses; a high-pressure injector for accurate sample injection; a capillary column ensuring rapid and efficient gradient separation; and an optical detector.

Figure 2: The LC system

Overview	
Mobile phase flow	1–25 µL/min
Maximum pressure	100 MPa
Sample volume	0.4–5 µL
Capillary columns	0.32 × 50 mm or 160 mm
RSD of retention time	< 0.5 %
RSD of peak area	< 5 %
Column oven	up to 60 °C (±0.2°C)
Li-ion battery	> 8 hours of operation
Size	430 × 380 × 154 mm
Weight	9 kg

LC-ESI-MS ANALYSIS

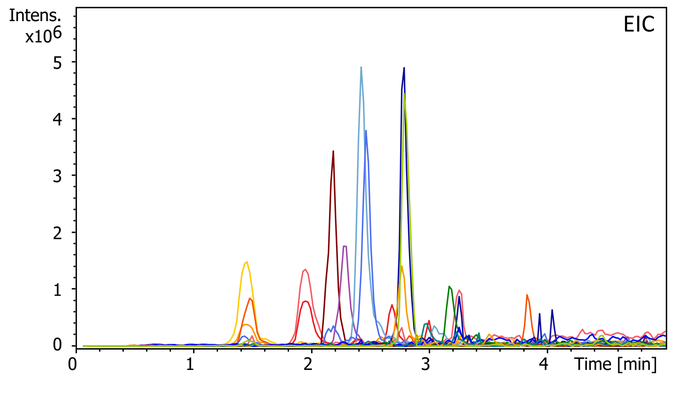


Figure 3: RPLC gradient separation of peptides from ovalbumin

Column: 0.32 × 160 mm; Mobile phase: solvent A - water + 0.1% TFA, solvent B - acetonitrile + 0.1% TFA; Gradient: RPLC 5-75% v/v B (40 µl), column regeneration 5% B (40 ul); Injection volume: 0.4 µl; Flow rate: 8 µl/min.

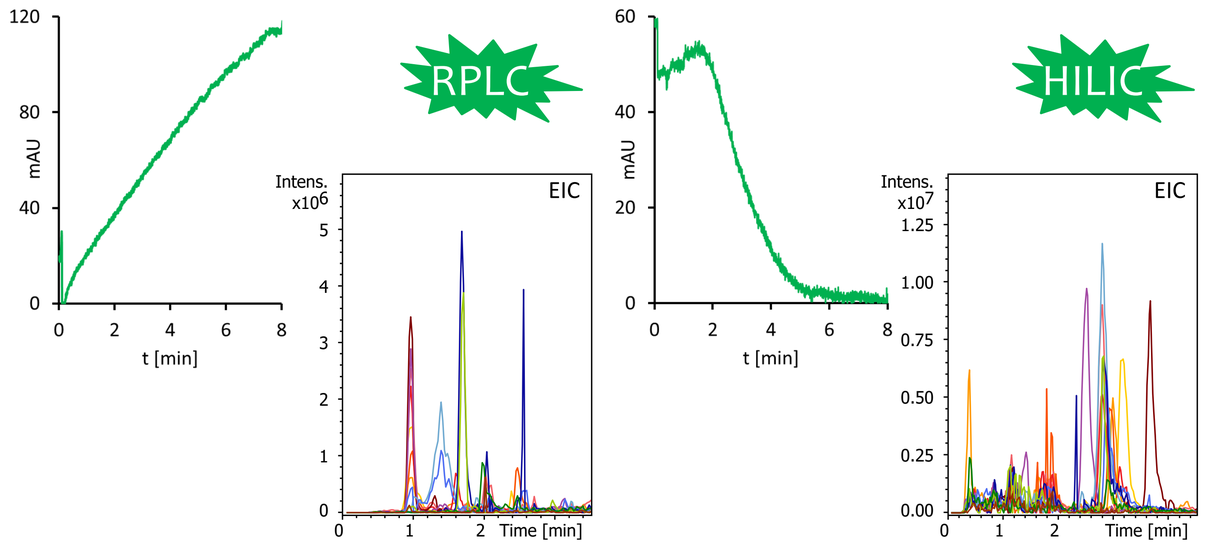


Figure 4: Gradient profiles and separation of peptides from ovalbumin

Columns: 0.32 × 50 mm; Mobile phase: solvent A - water + 0.1% TFA, solvent B - acetonitrile + 0.1% TFA; Gradient: RPLC 5-75% v/v B (80 µl), column regeneration 5% B (20 µl); HILIC 95 % B (10 µl), 95-60% B (20 µl), 60% B (10 µl), column regeneration 95% B (20 µl); Injection volume: 0.4 µl, Flow rate: 8 µl/min; Gradient tracking – 1% acetone in acetonitrile, UV detection 265 nm.

$[M+H]^+$ m/z	$[M+2H]^{2+}$ m/z	$[M+3H]^{3+}$ m/z	$[M+H]^+$ m/z	Position	Modification	Sequence
536.24			536.24	288-291		MEEK
580.31			580.31	124-127		ELYR
603.35			603.35	52-56		TQINK
632.34			632.34	183-187		GLWEK
647.39			647.39	281-285		VYLPR
780.39			780.39	106-111		LYAEER
822.40			822.40	220-227		VASMASEK
	605.28		1209.52	191-200		DEDTQAMPFR
	624.32		1247.62	191-200	Cys_CAM 368	DEDTQAMPFR
			1345.74	371-382		HIATNAVLFFGR
	791.36		1581.72	265-277		LTEWTSSNVMEER
	844.42	563.29	1687.84	128-143		GGLEPINFQTAADQAR
	887.45	591.97	1773.90	324-340		ISQAVHAAHAEINEAGR
	929.99	620.33	1858.97	144-159		ELINSWVESQTNGIIR
	1004.98	670.32	2008.95	341-360		EVVGSAEAGVDAASVSEEFR
	1044.96	696.98	2088.91	341-360	Phos 345	EVVGSAEAGVDAASVSEEFR

Table 2: The assignments of protein fragments obtained after trypsin digestion from ovalbumin (OVAL_CHICK (P01012)

$[M+H]^+$ m/z	$[M+2H]^{2+}$ m/z	$[M+3H]^{3+}$ m/z	$[M+H]^+$ m/z	Position	Sequence
500.25			500.25	25-28	DTHK
509.32			509.32	558-561	HKPK
517.30			517.30	281-285	ADLAK
537.28			537.28	157-160	FWGK
545.34			545.34	101-105	VASLR
609.29			609.29	524-528	AFDEK
649.33			649.33	223-228	CASIQK
658.32			658.32	118-122	QEPER
660.36			660.36	490-495	TPVSEK
712.37			712.37	29-34	SEIAHR
752.36			752.36	341-346	NYQEAK
789.47			789.47	257-263	LVTDLTK
818.43			818.43	562-568	ATEEQLK
886.42			886.42	131-138	DDSPDLPK
922.49			922.49	249-256	AEFVEVTK
927.49			927.49	161-167	YLYEIAR
974.46			974.46	37-44	DLGEEHFK
1002.58	501.80		1002.58	598-607	LVVSTQTALA
1014.62	507.81		1014.62	549-557	QTALVELLK
1015.49			1015.49	310-318	SHCIAEVEK
	582.32		1163.63	66-75	LVNELTEFAK
	642.36		1283.71	361-371	HPEYAVSVLLR
	653.36		1305.72	402-412	HLVDEPQNLIK
	700.35		1399.69	569-580	TVMENFVAFVDK
	756.43		1511.84	438-451	VPQVSTPTLVEVSR
	978.48	652.66	1955.96	319-336	DAIPENLPPLTADFAEDK

Table 3: The assignments of protein fragments obtained after trypsin digestion from bovine serum albumin (ALBU_BOVIN (P02769)

CONCLUSION

- The designed polymerization mixtures are suitable for preparing stable and efficient monolithic columns.
- The analysis and regeneration of the column require less than 500 µl of mobile phase.
- The fast gradient separations of tryptic peptides are achieved in less than 4.5 minutes.
- The number of detected peptides was comparable on both types of monolithic columns.

ACKNOWLEDGEMENT

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