New 3D Monolithic Architecture for Enhanced Analytical Performance

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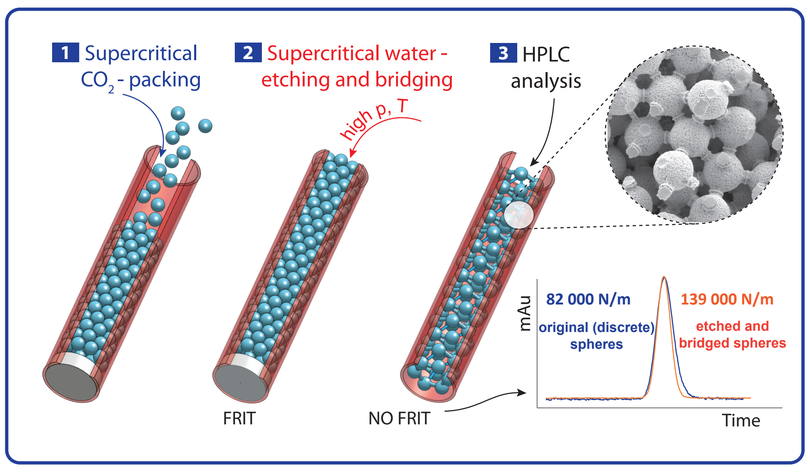
Abstract

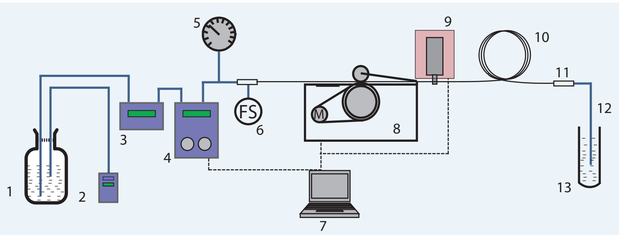
The initial aim of this work was to develop hybrid capillary chromatographic columns with high porosity, combining the advantages of both packed and monolithic formats. Such structures are particularly beneficial in high-pressure applications, where small particle sizes or high flow rates typically lead to elevated hydraulic resistance.
In the first phase of the study, we successfully fabricated a monolithic column within a 100 µm fused silica capillary using 5 µm silica microspheres. The process utilized the solvent properties of sub- and supercritical water to partially dissolve and fuse the particles into a stable, porous network. The resulting column exhibited good mechanical stability and promising chromatographic performance.
In the next phase, the focus will shift to using smaller input particles, ranging from 3 µm down to 1.5 µm. This is expected to yield more homogeneous structures with average particle sizes around 2 µm or even 1 µm, while maintaining high porosity and permeability. Such improvements could further enhance column efficiency and broaden their applicability in demanding analytical workflows.

Idea



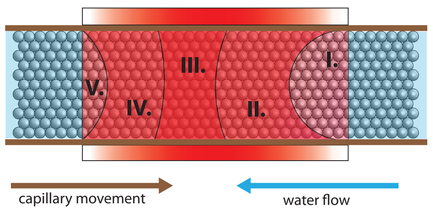
Experimental

Overall scheme of the experimental apparatus



1 - H_2O reservoir, 2 - oxymeter, 3 - degasser, 4 - high pressure pump, 5 - pressure sensor, 6 - flowmeter, 7 - process control and data collection, 8 - programmable moving device, 9 - heater (SCW generator), 10 - packed FS capillary, 11 - high pressure coupling, 12 - restrictor, 13 - waste

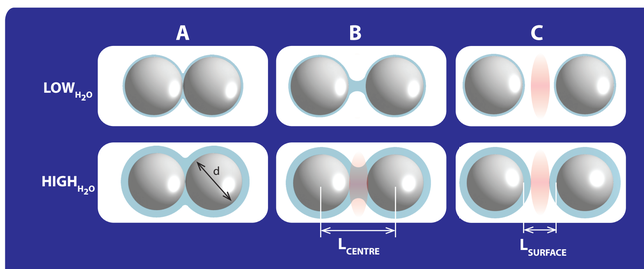
Scheme of the heater and continuous sintering process



I. water preheating II. etching of the formed 3D structure III. liquid to solid bridge transformation IV. Liquid bridge formation V. microspheres preheating

The regions I-V are schematically shown parts where the different steps of the sintering process occur at the steady state. The shape of the regions, their size, location and perimeters cannot be determined in any simple way, also because these parameters change with the flow rate, the capillary movement speed and the temperature.

Bridging Principle



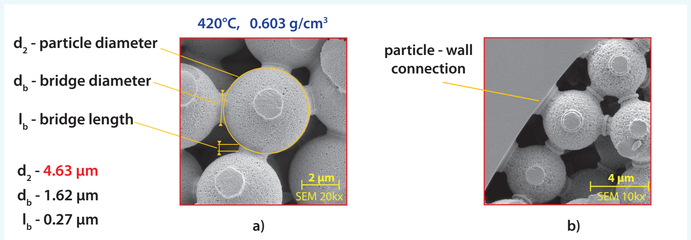
Wetting of differently spaced particles at low and high liquid content under the condition of mutual insolubility. The size and shape of the liquid bridge is determined by the distance between the particles and the thickness of the liquid layer.

A – in contact B – bridge forming distance C – too far for forming bridge

Formation of a bridge from the material removed from particles when the particle material dissolves. Assumption of the process : SCW is applied to the surface of the silica beads, a specific amount of SiO_2 dissolves, forms a silicic acid gel layer and reduces the diameter of the solid core of the beads from d_1 to d_2. This, in turn, creates a space between the surfaces of the beads $L_{SURFACE}>0$, which forms a liquid bridge subsequently while the distance between the centers L_{CENTRE} of the spheres remains constant.

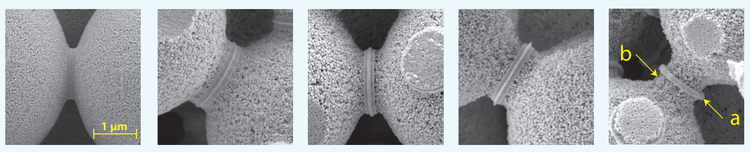
Scanning Electron Microscopy Analysis

1 Evaluation parameters and detail of particle-wall connection



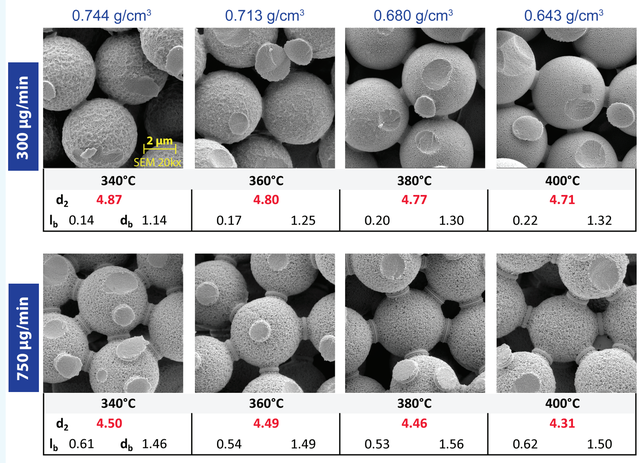
a) schematic view of the evaluated geometric parameters (measured on SEM Tescan Mira 3)
b) particles are not only connected to each other, but strong bridges are also formed between the particle and the capillary wall

SEM images of the connecting bridges produced under different conditions with visibly different centers



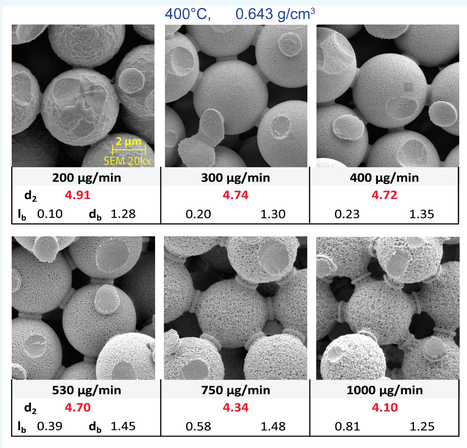
The rings in the middle of the bridges indicate a more complex process than was assumed on the left, the rings should be homogenous. The center white rings are the points of initial contact of solid particles where a strong connection is formed (a) and this structure is then further etched away as a whole by the SCW (b).

2 Column structures made at 725 bar and different temperatures



- at SCW flow rates of 300 µg/min and 340°C, water is already able to dissolve the surface of SiO_2 microspheres and the dissolved amount is even sufficient to form bonding bridges, but the surface is highly heterogeneous
- increasing the temperature to 360°C increases the dissolving power of water, the amount of SiO_2 etched increases but the microspheres still show residuals of the original surface
- the use of 380°C and more is already adequate for forming 'hybrid microcolumns' as the microsphere surface is already uniform and the bridges are solid and consistent
- increasing SCW flowrate to 750µg/min makes the process much more intense and homogeneous columns can be prepared already at lower temperatures (340°C).

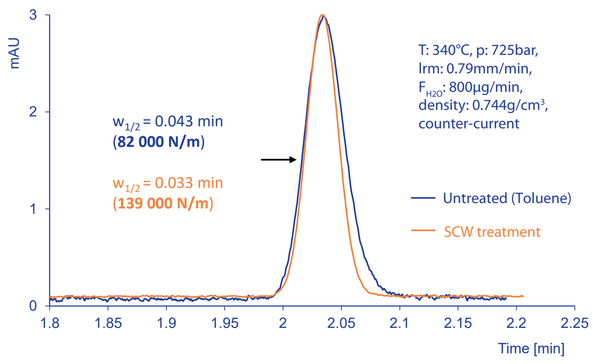
3 Effect of SCW flow rate change on column structures



- strong dependence of the column structure with SCW flowrate was observed
- except for 200 µg/min, all other structures produced are sufficiently homogeneous and suitable for use as a chromatographic column
- with a noticeable reduction in microsphere diameter, a significant increase in "surface roughness" can also be observed. The formation of these micropores increases the overall surface area of the 3D structure and can play a positive role in both chromatographic and, for example, catalytic processes.

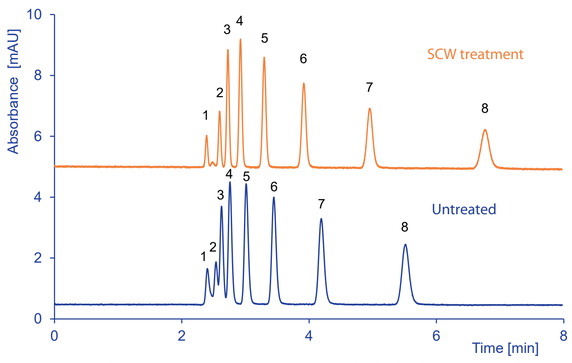
Results and Discussion

4 Peak profiles of unretained compound eluted from unmodified chromatographic column



Because of different total porosities of the columns (0.313 for the untreated one and 0.454 for the SCW-treated one), the amount of mobile phase in each column was different, and the difference resulted in different final flow rates (246 and 328 nL/min, respectively) to obtain the same retention times. We observed a change of efficiency from 82 000 to 139 000 plates/m when measured by width of the peak at half of its height. When efficiencies were calculated from statistical moments (by DataApex Clarity 5.02 software), values 98 000 for untreated column and 160 000 for SCW-treated column were obtained.

5 Chromatographic evaluation of SCW treated and untreated ODS modified columns



Measurements with non retained substance gave only indicative information; efficiency mainly reflects the homogeneity of the column bed. In the next step, SCW-treated column (800 µg/min) and column packed with untreated particles were submitted to ODS modifications. For SCW treated column, the performance for uracil and benzene confirmed values (130 000 plates/m), which we obtained for toluene (in 90% ACN). By comparing Tables S1 and S2, it is possible to determine the effect of treatment on the spectrum of analytes with higher retention factors.

Table S1: Chromatographic separation of alkylbenzenes on SCW-treated column and ODS-modified column

Peak	Reten. time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Compound Name
1	2.390	0.040	0.909	0.00	19833	**130483**		**Uracil**
2	2.593	0.043	1.182	0.08	19893	**130874**	2.879	**Benzene**
3	2.722	0.050	1.167	0.14	16475	**108390**	1.644	**Toluene**
4	2.922	0.053	1.000	0.22	16682	**109753**	2.284	**Ethylbenzene**
5	3.295	0.063	1.059	0.38	15041	**98953**	3.776	**Propylbenzene**
6	3.914	0.077	1.150	0.64	14483	**95285**	5.226	**Butylbenzene**
7	4.952	0.100	1.071	1.07	13629	**89666**	6.946	**Pentylbenzene**
8	6.766	0.146	1.075	1.83	11827	**77810**	8.691	**Hexylbenzene**

Column: i.d. = 0.1 mm, length = 152 mm, stationary phase: C18, mobile phase: 50% acetonitrile / water

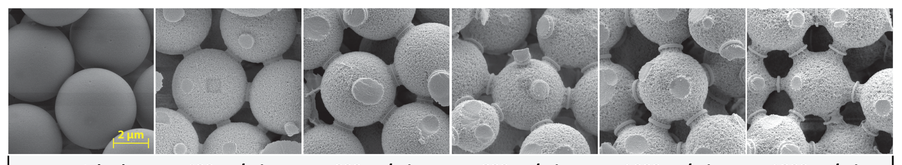
Table S2: Chromatographic separation of alkylbenzenes on SCW-untreated column and ODS-modified column

Peak	Reten. time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Compound Name
1	2.400	0.057	1.385	0.00	9937	**62109**		**Uracil**
2	2.537	0.053	0.917	0.06	12533	**78328**	1.466	**Benzene**
3	2.623	0.057	1.067	0.09	11873	**74206**	0.930	**Toluene**
4	2.757	0.063	1.200	0.15	10496	**65598**	1.311	**Ethylbenzene**
5	3.010	0.070	1.294	0.25	10243	**64022**	2.242	**Propylbenzene**
6	3.447	0.080	1.200	0.44	10283	**64270**	3.485	**Butylbenzene**
7	4.190	0.097	1.250	0.75	10408	**65053**	4.965	**Pentylbenzene**
8	5.517	0.127	1.088	1.30	10508	**65678**	7.010	**Hexylbenzene**

Column: i.d. = 0.1 mm, length = 160 mm, stationary phase: C18, mobile phase: 50% acetonitrile / water

6 Five fabricated chromatographic columns and comparison with nonetched column

340°C, 725bar, 0.744g/cm³



non etched	400 µg/min	600 µg/min	800 µg/min	1000 µg/min	1200 µg/min
d_2 5.01	4.69	4.63	4.44	4.28	3.95
l_b 0 d_b 0	0.22 1.15	0.37 1.42	0.48 1.39	0.69 1.50	0.80 1.30

counter-current mode, 100 µm i.d. fused silica capillary, capillary movement rate=0.79mm/min, P=725 bar, SCW flowrate=400-1200µg/min, SCW temperature=340°C, SCW density=0.744 g/cm³.

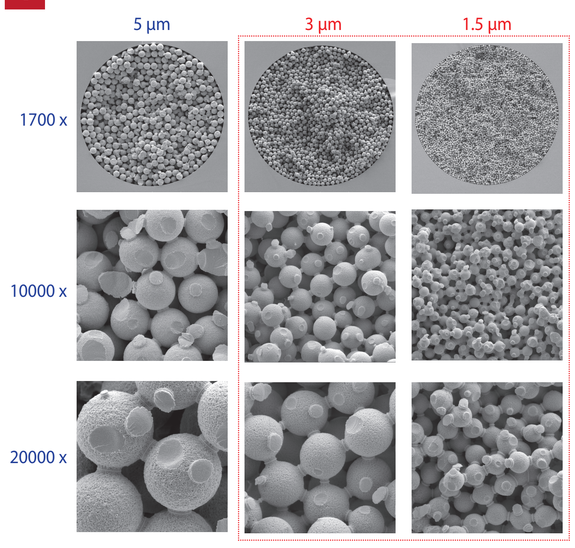
Table 1. Measured Chromatographic Parameters (Left Side), Average Values n = 3 (Right Side)

SCW flow	length[mm]	dp [MPa]	N/m $W_{50\%}$[a]	N/m stat.mom.[b]	ε total	k [× 10^{-14}m²]	E	N/m $W_{50\%}$[a]	N/m stat.mom.[b]	ε total	k [× 10^{-14}m²]	E
nonetched	153	3.1	96,000	96,000	0.313	1.13	9581	86,667	97,333	0.313	1.13	11931
	153	3.1	82,000	98,000	0.312	1.13	13,132					
	153	3.1	82,000	98,000	0.315	1.14	13,080					
400 µg/min	155	2.0	112,000	134,000	0.358	2.49	3205	116,667	128,000	0.360	2.51	2955
	155	2.0	113,000	125,000	0.364	2.51	3123					
	155	2.0	125,000	125,000	0.358	2.52	2538					
600 µg/min	155	1.3	137,000	173,000	0.394	2.91	1834	137,333	147,000	0.390	2.88	1840
	155	1.3	136,000	135,000	0.385	2.87	1883					
	155	1.3	139,000	133,000	0.390	2.87	1803					
800 µg/min	152	1.5	1,325,001	160,000	0.468	3.39	1679	133,500	146,000	0.454	3.32	1694
	152	1.5	138,000	138,000	0.454	3.28	1599					
	152	1.5	130,000	140,000	0.439	3.28	1802					
1000 µg/min	154	0.8	113,000	118,000	0.509	5.90	1327	114,333	125,333	0.509	5.87	1303
	154	0.8	115,000	128,000	0.509	5.87	1287					
	154	0.8	115,000	130,000	0.508	5.84	1294					
1200 µg/min	145	1.0	117,000	156,000	0.563	6.76	1080	121,333	141,333	0.563	6.88	990
	145	1.0	123,000	125,000	0.562	6.94	953					
	145	1.0	124,000	143,000	0.564	6.94	937					

[a]Calculated assuming Gaussian concentration profile. [b]Calculated from statistical moments by Data Apex clarity 5.02 software.

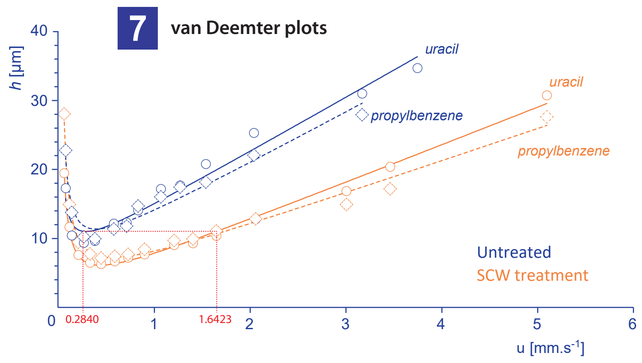
From Table 1, it can be seen that permeability k of particles after treatment grows linearly with SCW flow rate. When compared with non-etched particles, we can observe more than 6× increase of column permeability. From chromatographic point of view, higher permeability of the column is preferred because it reduces the pressure needed in the system. Also, a more viscous mobile phase or high flowrate can be used for separation.

? "Column Evolution: 5 µm Achieved, 3 µm and 1.5 µm Under Development"



The use of smaller particles (3 µm and 1.5 µm) represents our long-term primary objective, as it offers the potential to overcome limitations associated with high backpressure. These include restricted use of higher mobile phase velocities, limited compatibility with viscous solvents, and the need for ultra-high-pressure systems. The images show successful sintering of silica microspheres; however, this has so far been achieved only over a short section of the capillary. Achieving full-column homogeneity will require further optimization to address several physicochemical constraints.

7 van Deemter plots



Comparison of Van Deemter curve measurements for uracil and propylbenzene on the original and newly developed column types. The new column design shows a significantly lower height equivalent to a theoretical plate (HETP), allowing much higher linear velocities without loss of efficiency. For uracil, a velocity up to six times higher can be applied compared to the original column, resulting in a substantial reduction in analysis time—an advantage for high-throughput workflows.

Conclusion

This study presents a novel method for fabricating three-dimensional homogeneous silica structures using the solubilizing properties of supercritical water (SCW). The technique is highly tunable due to the broad range of applicable temperatures and pressures. Resulting chromatographic columns exhibit high separation efficiency and permeability. Microspheres are interconnected and anchored to the capillary wall, forming a rigid, frit-free monolith that supports bidirectional flow and can be trimmed as needed.
The 3D framework maintains structural integrity after repeated drying and rehydration, indicating excellent mechanical stability. A reduction in particle diameter from 5.04 to 3.95 µm decreases hydrodynamic resistance, especially with smaller microspheres. The use of ultrapure water and absence of surface heteroatoms enhance suitability for biochemical applications.
Performance evaluation using C18-functionalized columns and alkylbenzene mixtures confirmed the superior efficiency of the SCW-treated, bridged-microsphere column over conventional packed columns.

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