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Short communication

Exploring the pressure resistance limits of monolithic silica capillary columns

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ABSTRACT

We report on an experimental approach to measure the pressure stability and mechanical strength of monolithic silica capillary columns with different diameters (50 and 100 μm i.d.) and considering two different domain sizes, typical for the second generation monoliths or smaller. The approach consists of exposing the capillaries to ultra-high pressures (gradually stepwise increased from 20 to 80 MPa), with intermediate measurements of the column efficiency, permeability and retention factors to check the mechanical stability of the bed. It was observed that all tested columns withstood the imposed pressure stress, i.e., all the tested parameters remained unaffected up till the maximal test pressure of 80 MPa. The applied pressure gradient corresponded to 320 MPa/m. The two 100 μm i.d.-capillary columns were also exposed to pressures between 80 and 90 MPa for a prolonged time (8 h), and this did not cause any damage either.

1. Introduction

The enormous success of ultra-high pressure LC (UHPLC) instruments, combined with the ability to produce highly efficient sub-2 μm particles, readily gives proof of the dominant role of the available inlet pressure on the kinetic performance of chromatographic systems [1–4]. Abundant literature reports are available clearly showing how applying high pressure conditions enhances the revolving power for the separation of small molecules, as well as peptides and proteins [5–8].

Theoretically, the importance of pressure can be understood by considering a series of packing materials with the same shape but a different size (e.g., packed beds of spheres with variable size but with the same packing quality), each operated at their optimal flow rate and exactly long enough to produce the maximal pressure at that flow rate. The well-known Knox&Saleem analysis shows that, for such a combination of systems, the minimal time needed to achieve a given number of theoretical plates is inversely proportional to the available column pressure drop [9–11]:

$$\text{time} \propto N^2/\Delta P \quad (1)$$

This rule holds for any type of chromatographic system and hence readily shows that, if silica monolithic columns are ever to be competitive with particulate columns, they will need to be able to produce and withstand the same high pressure drops. To produce similar pressure drops, recipes need to be found that produce monoliths with much smaller feature size (= through-pore and skeleton size) than currently achievable, without affecting the external-porosity and the intrinsic flow resistance.

Little is also known about how pressure-resistant silica monoliths actually are. Most of the data on the pressure stability of silica monoliths relate to so-called monolithic rod columns, where the weakest links are the adhesion between the rod and the cladding material that is needed to enclose the silica rod (for example polyether ether ketone, PEEK), and the cladding itself [12]. Several cladding techniques have been explored to improve the pressure resistance characteristics as well as the column efficiency [12–15], but to the best of our knowledge [14,16,17], there have been no reports using conventional-sized (i.e., with 1–4.6 mm i.d.) monolithic silica columns, at operating pressures higher than 30 MPa. For monolithic silica capillary columns with 100 μm i.d., where a cladding procedure is not required and the monolithic silica structure can connect to the fused-silica capillary wall with siloxane bonds [18], it was reported that the highest operating pressure was obtained in 5 m long column, which was shown to be capable to withstand inlet pressures up to 47 MPa [19]. As was demonstrated by Jorgenson, the fused-silica capillaries themselves remain

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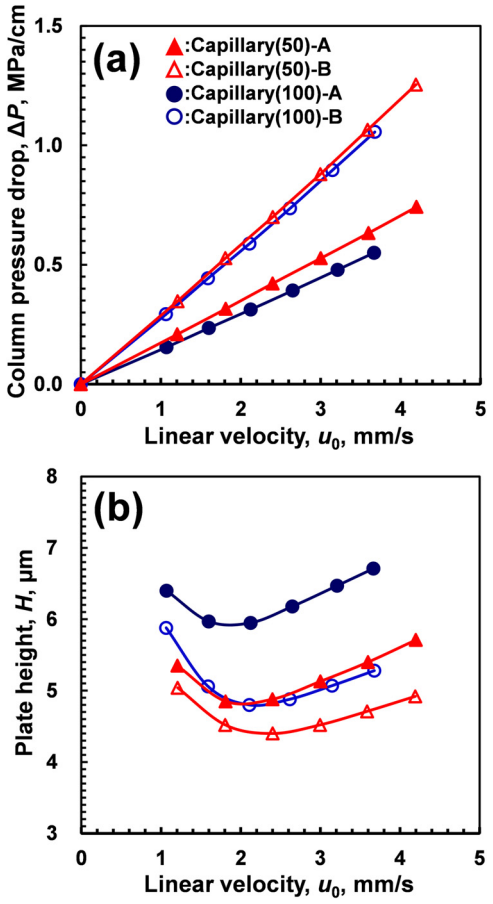

Fig. 1. Comparison of column performance with ODS-modified monolithic silica capillary columns. (a) Relationship between column pressure drop and linear velocities, (b) van-Deemter plots obtained for hexylbenzene against linear velocities. Symbol: Capillary(100)-A (●), Capillary(100)-B (○), Capillary(50)-A (▲), and Capillary(50)-B (△). Mobile phase: 70:30% (*v*/*v*) ACN:water. Temperature: 30°C. Detection: 210 nm. Sample: Thiourea + hexylbenzene. Plate height (*H*) was calculated for hexylbenzene, and linear velocities (u_0) for thiourea. Linear velocities are corrected because of the presence of the UV capillary flow path line after column outlet (i.d. 20 μm × ca. 30 cm).

mechanically stable up to 450 MPa [1], but, as already said, little is known about the mechanical strength of the silica backbone itself nor about its connection to the capillary wall.

The present contribution therefore reports on a dedicated experimental approach to assess the intrinsic pressure resistance of silica monolithic columns using conventionally available instrumentation. The study was conducted on 4 different silica monolithic columns (each 25 cm long) with two different i.d.'s (50 and 100 μm) and two different, small domain sizes. These columns were subjected to a series of successive pressure stress test runs (2 h per run, with increasing pressure for each new run) on a nano-flow HPLC pump, using a high-pressure capillary fitting to connect the capillary to the pump. Between two successive pressure stress run, the retention factor (*k*), plate height (*H*), and t_0-marker-based column permeability (K_{v0}) was evaluated near the optimal flow rate on a capillary LC instrument with flow splitting injection, and compared to the original performance.

2. Experimental

2.1. Chemicals and materials

Urea (>98%), thiourea (>98%), toluene (GC grade), tetramethoxysilane (TMOS), 1 M aqueous solution of acetic acid, polyethylene glycol (PEG) of molecular weight (MW) = 20,000 g/mol, and alkylbenzenes ($C_6H_5(CH_2)_nH$, with n = 0–6, GC grade) were purchased from Sigma-Aldrich Co. (Diegem, Belgium). Octadecyldimethyl-*N*,*N*-diethylaminosilane was gifted by Kyoto monotech Ltd (Kyoto, Japan). Acetonitrile (ACN, HPLC super-gradient grade) was obtained from Biosolve B.V. (Valkenswaard, NL). Deionized water was produced in-house with a Milli-Q water purification system Merck Millipore (Billerica, MA, USA). PTFE filters (0.20 μm × 25 mm) were purchased from Macherey-Nagel (Düren, DE). Fused-silica capillaries with an inner diameter (i.d.) of 50 μm and 100 μm and an outer diameter (o.d.) of 375 μm were purchased from Polymicro Technologies (Phoenix, AZ, USA). Stainless steel unions (JR-ZU1S6), ferrules (JR-ZF1S6-5), and nuts (JR-ZN1-5) were obtained from Valco (Schenkon, CH), and PEEK tubing sleeve (PM-2500-F) and stainless steel tubing (U-107) were purchased from IDEX Health & Science GmbH (Erlangen, DE) (Product No. are shown in the parenthesis).

2.2. Column preparation

Monolithic silica capillary columns with a length of 25 cm and an i.d. of 50 μm and 100 μm were fabricated following the same procedure as used in preceding studies [20,21] and using 5.6 mL TMOS, 0.09 g urea, 10 mL 0.01 M aqueous acetic acid solution, and a certain amount of PEG with MW = 20,000 g/mol (see end of paragraph for exact values). After the fabrication, in-situ functionalization of the bare silica surfaces was carried out with 20:80% (*v*/*v*) octadecyldimethyl-*N*,*N*-diethylaminosilane (ODS-DEA)/toluene mixture solution [20]. Two different domain sizes (type A and B) were prepared by changing the PEG amount in the feed solution (i.e., 1.39 g for type A and 1.40 g for type B).

2.3. Pressure stress test

Pressure stress runs were conducted at ambient temperature (ca. 25°C) with the UltiMate 3000 RSLCnano pump providing pressures up to 100 MPa (Thermo Fisher Scientific to pump high flow rates of mobile phase (40:60% (*v*/*v*) ACN:water) through the capillary. The stress test pressures were gradually increased in steps of 10 MPa between 20–80 MPa, keeping the pressure on for 2 h. Eventually, a final stress test was conducted for 8 h at a pressure between 80 and 90 MPa.

2.4. Determination of chromatographic properties

In between two successive pressure test runs, chromatographic performance tests were carried out using an Ultimate 3000 system (Thermo Fisher Scientific, Germering, DE) composed of a membrane degasser, LPG–3400 M pump, a forced-air column oven, and a VWD UV detector equipped with a 3 nL flow cell. UV detection was performed at a wavelength of 210 nm, applying a response time of 0.05–0.10 s. A 1 μL injection valve (Valco, Schenkon, CH) equipped with home-build split-injection flow system (e.g. a split ratio of ca. 1/700 for 100 μm i.d.-capillary columns) was used to minimize a column efficiency loss from injection [22]. Our preference to use split-flow injection also explains why we used the capillary LC system for the chromatographic testing: the split-flow ratios that can be achieved in the nano-LC system would be too low to sufficiently reduce the volumes injected onto the column. In addition, because of the lower flow rates, also the extra-column times are also larger on the nano-LC system. Isocratic LC measurements for ODS-modified capillary columns were conducted with alkylbenzenes in 70:30% (*v*/*v*) ACN:water and the column temperature was thermostatically controlled at 30°C. Thiourea was used to determine a mobile-phase linear velocity (u_0). Chromeleon software (6.80 SR14) was used for instrument control and data processing.