Silica-based narrow inner diameter open tubular liquid chromatography columns for proteomics

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Background

In liquid chromatography (LC), narrow inner diameter (ID) columns are beneficial when high sensitivity is needed. High sensitivity is achieved due to less radial dilution which gives a higher signal with a concentration sensitive detector, such as the electrospray ionization mass spectrometer (ESI-MS) (**figure 1**) [1].

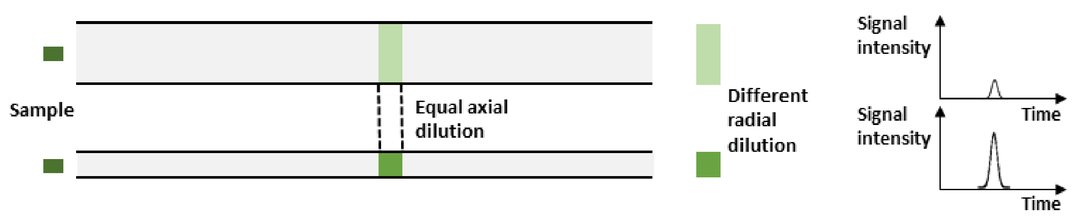

Figure 1 – Illustration of radial dilution and the effect on signal intensity, on a conventional sized column (above) and miniaturized column (below).

Such narrow columns can be silica-based porous layer open tubular (PLOT) columns with a stationary phase chemically bonded in the porous layer [2]. Such columns can be prepared from a silicon alkoxide by sol-gel synthesis. The "sol" consists of particles dispersed in a liquid. A series of hydrolysis and condensation reactions (**figure 2A**) link the monomers in the sol together and form a network. If this process is slow a gel can be formed [3]. The sol-gel process may take place inside a fused silica capillary, and with the right conditions a porous layer attached to the capillary wall can be created. Functionalization with e.g. reversed-phase stationary phase is possible (**figure 2B**).

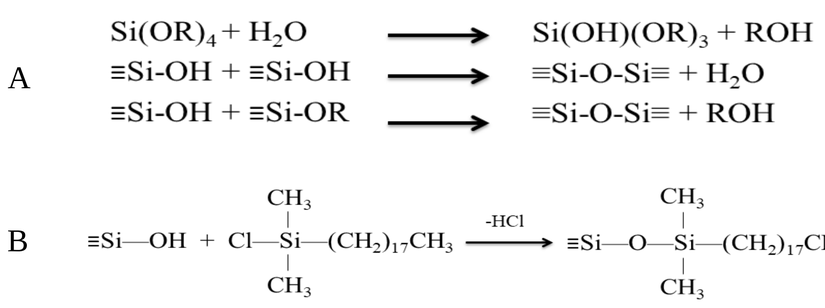

Figure 2 – A: Overall reactions of the sol-gel process when an silicon alkoxide is used as monomer. B: A typical functionalization process where a C18 stationary phase is attached to silica.

Aim of study

The goal of this study was to prepare silica-based C18 PLOT columns with 5 and 10 µm ID, which are needed for analysing ultra-small samples.

Experimental

PLOT column preparation

The PLOT columns have been prepared according to the method from Hara *et al.* [2] with some modifications. The functionalization process is based on Ortiz-Villanueva *et al.* [4]. The details of the preparation process is shown in **figure 3**.

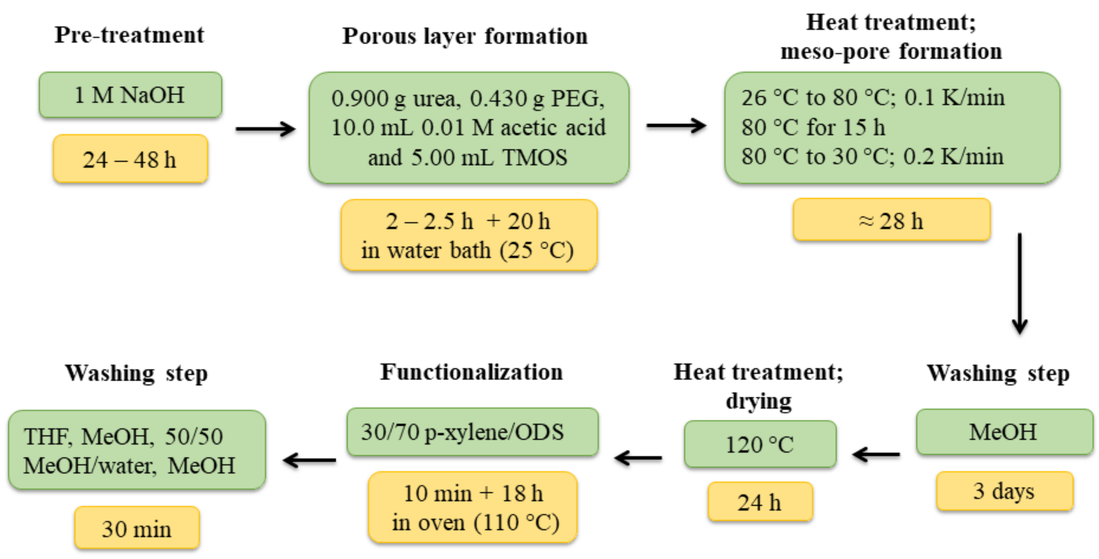

Figure 3 – Overview of the PLOT column preparation process. TMOS: tetramethyl orthosilicate, PEG: polyethylene glycol, ODS: chloro(dimethyl)octadecylsilane

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Chromatographic system

The chromatographic system used with PLOT column as analytical column is presented in **figure 4**. As detector a Q-Exactive™ Orbitrap was used.

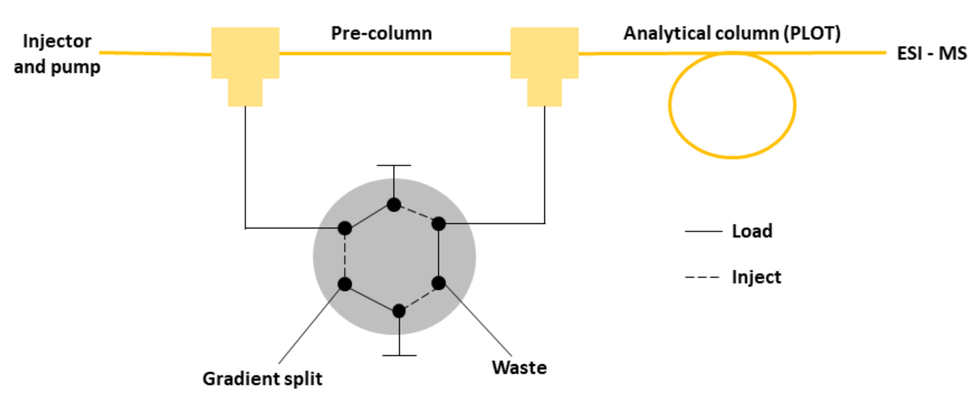

Figure 4 – Schematic drawing of the chromatographic system used with PLOT column as analytical column. For analysis of a cytochrome C digest, an in-house made silica-based monolithic pre-column (C18, 50 µm x 4 cm) was used, together with a 10 µm ID silica-based C18 PLOT column (length between 115 and 120 cm). Mobile phase flow rate through PLOT column was 60 nL/min.

Results

PLOT column preparation

Both 5 and 10 µm ID PLOT columns have been successfully made. Scanning electron micrographs of the capillaries before functionalization show that a porous layer has been formed inside (**figure 5).** The thickness of the porous layer of the 5 µm ID capillary (**figure 5A**) was roughly estimated to be between 290 and 340 nm at the inlet and between 240 and 290 nm at the outlet. The estimates are in agreement with the measurements made by Hara *et al.*, where the thickness was determined to be 306 nm [2]. However, occasionally the capillaries got clogged during the preparation process, and the success rate of the PLOT column preparation is still too low.

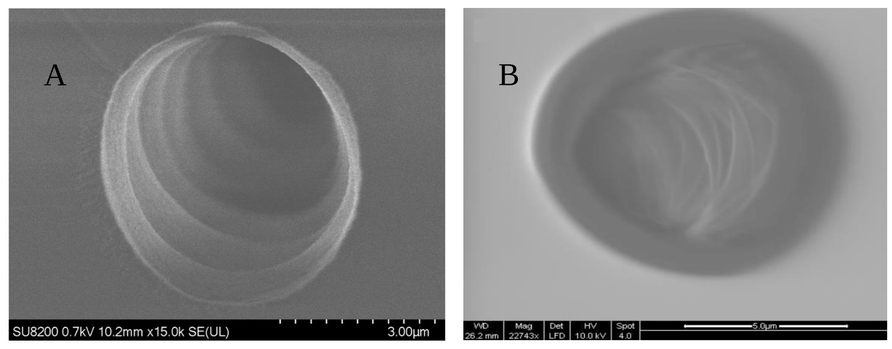

Figure 5 – Scanning electron micrographs of a fused silica capillaries with successfully formed porous layer with 5 µm ID (**A**) and 10 µm ID (**B**).

Application

The silica-based C18 PLOT columns can be used to separate proteins or peptides. An extracted ion chromatogram for three peptides from a cytochrome C digest, separated by a 10 µm ID silica-based PLOT column, is shown in **figure 6**.

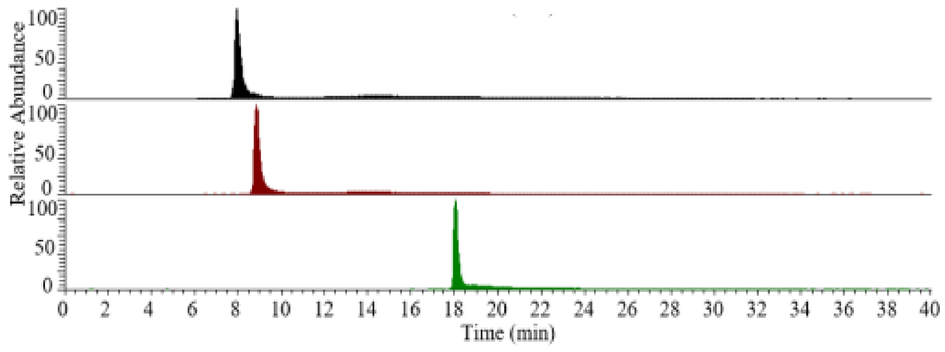

Figure 6 – Extracted ion chromatogram for three different peptides from a cytochrome C digest, using a 10 µm ID PLOT column with C18 stationary phase. The chromatographic system used is shown in **figure 4**. Mobile phase solution A: 0.1 % formic acid in water, B: 10% water and 0.1% formic acid in acetonitrile. The gradient used was 3-50 % B in 30 minutes.

Conclusion and further work

Silica-based PLOT columns (5 and 10 µm ID) have been successfully made by using the method of Hara *et al.*, with some modifications, and used in proteomics. Further work will consist of optimization of the preparation procedure to avoid clogging during the preparation of the porous layer, and applying both 5 and 10 µm ID PLOT columns for protein and peptide separation in ultra-small samples, e.g. organoids.

References

[1] Wilson, S.R., C. Olsen, and E. Lundanes, *Nano liquid chromatography columns*. Analyst, 144 (2019) 7090-7104.
[2] Hara, T., S. Futagami, S. Eeltink, W. De Malsche, G.V. Baron, and G. Desmet, *Very high efficiency porous silica layer open-tubular capillary columns produced via in-column sol–gel processing*. Analytical Chemistry, 88 (**2016**) 10158-10166
[3] Schubert, U. and N. Hüsing, *Synthesis of inorganic materials*. (2012): John Wiley & Sons
[4] Ortiz-Villanueva, E., F. Benavente, E. Giménez, F. Yilmaz, and V. Sanz-Nebot, *Preparation and evaluation of open tubular C18-silica monolithic microcartridges for preconcentration of peptides by on-line solid phase extraction capillary electrophoresis*. Analytica Chimica Acta, 846 (2014) 51-59