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Performance evaluation of thick film open tubular silica capillary by reversed phase liquid chromatography

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ABSTRACT

The unquestioned potential of open tubular column design for miniaturized liquid chromatography systems has been assessed recently. Instrument limitation and insufficient mass loadability of the columns, however, have prevented experimental breakthrough in this field, the latter requiring new methods for the deposition of thick films as the stationary phase. In this work, a previously proposed synthesis strategy has been applied for the preparation of a C_8 modified 15 μm inner diameter variant of 3 m length, providing a silica porous layer with a thickness of approximately 500 nm. The capillary column has been evaluated in terms of usability, permeability and chromatographic efficiency in reversed phase mode. Data was compared to both a monolithic and a particulate, commercially available C_{18} capillary column. High theoretical plate numbers have been generated in a test mixture separation composed of small molecules and the applicability of this new type of column was demonstrated by two reversed phase applications.

1. Introduction

The concept of open tubular (OT) column technology was transferred from gas to liquid chromatography (LC) for the first time by Tsuda et al. in the late 1970s [1]. The characteristic feature of this long and narrow column design comprises a large flow through pore, which results from the remaining void within a film coated or otherwise treated capillary. Although column efficiency gain compared to packed beds was calculated to be theoretically superior [2,3], pressure-driven OT column design has not been established practically until today. 30 years ago, this was mainly due to instrument limitation concerning appropriate detection of nano flow rates [4]. But even today, with suitable nano LC instrumentation being available, development of OT capillary technology is still lacking behind. Particularly in the academic field there is continuous interest in this technology, as the OT column design was considered to be one of the drivers for LC miniaturization in a recently published review by Desmet and Eeltink [5]. As a result of the high permeability, values for the separation impedance E_{min} are expected to be 100 times lower compared to packed bed columns. Even considering a thick film stationary phase, a significant reduction of analysis time for separations requiring high theoretical plate numbers can be achieved with an OT capillary of 5 μm ID [6].

The second driver for the implementation of miniaturized LC systems like OT capillaries is detection sensitivity. In case of limited sample volume available, intensity at the detector is higher using very small volume capillaries compared to standard bore columns. A corresponding major disadvantage in this regard is the necessity to use nanoscale detector cells which again challenge the sensitivity gain due to a reduced signal-to-noise ratio [5]. When applying nano flow electrospray ionization-mass spectrometry (ESI-MS) detection, volumetric flow rates originating from OT columns are most suitable for high detection sensitivity [7].

One major obstacle in the fabrication of OT capillaries has been the very low mass loadability due to an insufficient layer thickness or low porosity of the stationary phase [8]. In this regard, chemical modifications of the capillary inner wall, hence creating monolayers, give insufficient results. Acceptable retention can only be achieved by depositing porous thick films, therefore allowing the injection of larger sample volumes [9]. Compared to OT gas chromatography, thicker films can be used in LC as diffusion coefficients of solutes in liquid phase greatly differ from those in gas phase. Accordingly, phase ratios (layer thickness divided by the resulting flow through pore) can be 0.5–1 without a significant loss of efficiency caused by the stationary phase mass transfer limitation. Corresponding column IDs necessarily would be very narrow (<5 μm), which causes serious technological challenges in manufacturing columns featuring lengths up to several meters. Therefore, in practice, a compromise between theoretical OT column efficiency and experimental feasibility is always made.

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While porous layer open tubular (PLOT) stationary phases comprising an organic polymeric layer were used in LC/MS proteomics throughout the last five years by the Karger group [10–12], experimental progress on silica film deposition for pressure-driven OT capillary chromatography has stagnated. Referring to this, first research was carried out by the Poppe group utilizing a pre-gelled polyethoxysilane solution in a static [13] and dynamic [14] coating procedure. Similarly to the work by Crego et al. [15], capillaries were lacking sufficient retention factors, although the latter already offered high efficiencies. In recent years, the Desmet group applied silica sol-gel chemistry for the formation of a porous layer on μm-equidistant pillars arranged on a chip [16–18]. This technology is based on monolith formation in any kind of confined space providing a very high wall-to-volume ratio [19]. The coral-like monolithic structure, which would be expected from a bulk synthesis, is suppressed by a "surface directed spinodal decomposition" mechanism [20]. This results in a preferred wetting of the surface and the subsequent formation of a homogeneous silica layer. The utilization of this micro-scale phenomenon for the fabrication of thick film OTLC capillaries has been published previously [21].

In this work, we describe the application of a 15 μm ID capillary, synthesized according to the method mentioned above in reversed phase liquid chromatography. Usability, permeability and chromatographic efficiency of this new generation of OT capillary is assessed and compared to commercially available C_{18} capillary columns using standard LC instrumentation.

2. Experimental

2.1. Chemicals

Tetramethoxysilane, urea, poly(ethylene oxide) (MW 10,000), *N*-*N*-dimethylamino-dimethyl-octylsilane and ethanol were purchased from Merck KGaA (Darmstadt, Germany). Deionized water was further purified with a MilliQ Academic 0.22 μm Water System (Merck Millipore, Darmstadt, Germany). For the production of 0.01 M acetic acid a Titrisol pack (Merck KGaA) was diluted with water to obtain 5 L of solution. HPLC solvent acetonitrile and samples (uracil, alkylbenzenes and 32 pesticides) were purchased from Merck KGaA and used as received without any further purification.

2.2. Preparation of the capillary column

The manufacturing process has been described elsewhere recently [21]. Briefly, a fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with an ID of 15 μm was cut to a length of 3 m and used as received. A mixture of 4.3 g poly(ethylene oxide) and 8.0 g urea was dissolved in 100 mL 0.01 M acetic acid and cooled to 5 °C in a waterbath. 50 mL tetramethoxysilane (TMOS) were added and the solution was stirred at 5 °C for 30 min. The capillary was filled with the sol by means of an LC-pump operated at 400 bar in constant-pressure mode at room temperature. Subsequently, the ends were sealed with a rubber and then stored at 25 °C for 20 h. Fine-tuning of mesoporosity was performed using a hydrothermal treatment at elevated temperature. The column was flushed with water (1 h) to remove in situ generated ammonia and then with toluene for at least 3 h. A solution of 20% *N*-*N*-dimethylamino-dimethyl-octylsilane (Merck KGaA) in toluene was used for reversed phase modification (C_8) of the column at room temperature for 24 h. Finally, the capillary was flushed with toluene again and with acetonitrile for reversed phase equilibration.

2.3. LC instrumentation

Isocratic and gradient mode applications were performed using a Dionex Ultimate 3000 nano LC system (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a 3 nL UV detector cell. In order to avoid band broadening caused by pre-column dead volume, a flow split between the OT capillary and the injection port was assembled (JR-BPR2, Vici, Switzerland). Pre-column flow rate was varied from 200 μL/min to 2.5 mL/min, depending on the column pressure applied. A high flow rate was used for high pressure isocratic separations. The capillary end was directly connected to the UV cell capillary using a teflon sleeve (250 μm ID, Thermo Fisher Scientific). Reversed phase chromatography was conducted with acetonitrile/water as eluents in isocratic and gradient mode. Acquisition and post-run analysis was carried out with Chromeleon software (version 6.8, Thermo Fisher Scientific).

3. Results and discussion

3.1. Connection of OT capillaries to LC instrumentation

Reduction of extra column volume is crucial for the investigation of the separation efficiency of narrow micro-bore columns as it instantly will result in undesired band broadening. The inner volume of the 3 m OT capillary utilized in this work with an ID of 15 μm is only 460 nL, assuming a homogeneous silica layer of 500 nm thickness. Post-column dead volume originating from the UV cell cannot be eliminated completely, but its fused silica inlet capillary should be kept as short as possible. In this work, the capillary running through the detector flow cell (10 cm, 20 μm ID) contributes to post-column dead volume with approximately 30 nL. In contrast to commercially available fused silica capillaries comprising a packed or monolithic bed and a length of usually 15 cm, the typical length of OT columns with several meters is advantageous in terms of connecting it to any instrument without secondary fused silica tubings. The connection of the column outlet to the nano-cell UV detector inlet capillary also can have a strong impact on overall efficiency. Lowest dead volumes and therefore best results are obtained by using a simple 250 μm ID teflon sleeve clamping both ends directly to each other. Nano-tight unions will always give lower plate counts in this setup. Pre-column dead time (as well as instrument dwell time) can be minimized via an elevated pre-column flow rate compared to the mobile phase flow rate inside the OT capillary. The interplay between pre-column flow rate and the regulation of column backpressure (by manual split adjustment) also determines the actual injection volume. Only a fraction of the analyte solution in the 1 μL loop used in this work passes the capillary column inlet. For isocratic separations at high pressure – resulting in an elution time of the unretained solute below 1 min – the pre-column flow rate should be set as high as possible to give the best efficiency (2.5 mL/min in this work). This is of course due to the sample concentration prior to the capillary inlet, which accordingly is least diluted within the mobile phase. For medium pressure separations (below 100 bar) and particularly for gradient separations, there was no need for this wasteful mobile phase consumption and the pre-column flow rate was set to 200 μL/min without a significant performance drop. The modification of the standard LC instrument described above was optimized toward very low flow rates. The solvent issue certainly can be improved by the use of specifically designed nano-LC equipment with split injection.

3.2. Column permeability

One of the characteristic features that comes with OT column design is a very low backpressure (compared to packed and monolith beds). This enables the application of very long capillaries by still using 400 bar standard instruments. Column backpressure was recorded within a range of 2–264 bar using the OT capillary