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Large-bore particle-entrapped monolithic precolumns prepared by a sol–gel method for on-line peptides trapping and preconcentration in multidimensional liquid chromatography system for proteome analysis

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Abstract

The present report describes the preparation and characterization of large-bore particle-entrapped monolithic precolumns, which are suitable for incorporation into a two-dimensional liquid chromatography (2D-LC) system for proteome analysis. The fritless precolumns with different inner diameter (i.d.) (320 and 530 μm) were rapidly and successfully prepared by entrapping octadecylsilica (ODS) particles (5 μm, 300 Å) prepacked into fused silica capillaries with a sol–gel network, which was formed by hydrolysis and polycondensation of methyltriethoxysilane (MTES). By optimizing the composition of the sol solution, the resulting large-bore monolithic precolumns of 5 mm length allow a flow rate of 20 μL/min loading buffer at a reasonable low back pressure of 25 bar or less and are capable of withstanding up to 300 bar inlet pressure. Scanning electron micrograms of the precolumns profile showed that the evolving sol–gel network joined particles to each other and onto the column wall, and no cracking or shrinkage of the column bed was observed even in 530 μm-i.d. capillary. The performance of the particle-entrapped monolithic precolumns used for preconcentration and desalting of proteolytic digest was evaluated by on-line coupling the large-bore precolumns with a capillary reversed-phase liquid chromatographic (RPLC) column followed by UV detection. The laboratory-made monolithic precolumns with 320 and 530 μm i.d. were characterized by using BSA tryptic digest or peptide standards as the analytes with respect to sample loading capacity, linearity, recovery and reproducibility, etc. The results indicate that the large-bore and short precolumns (5 mm × 320 μm i.d. or 5 mm × 530 μm i.d.) allow sample fast loading at a flow rate of 30 or 60 μL/min. The precolumns also have a mass loading capacity for BSA peptides of about 70 μg and for standard peptides of about 80 μg. Good linear calibration curves ($R^2 > 0.99$) were obtained and the limits of detection (signal-to-noise ratio, S/N = 3) were improved by more than 60-fold and were between 0.53 and 1.32 ng/μL even with a UV absorbance detector. The total recovery was found to be approximately 90–100% for BSA digest and standard peptides. The day-to-day relative standard deviation (RSD) values for recoveries of BSA peptides on a single precolumn ranged from 4.66 to 7.56% and 2.68 to 3.05% for precolumn back pressure, while the column-to-column RSD values were 3.51–6.13% and 1.22–1.26% for recoveries of BSA peptides and precolumn back pressure, respectively. With good precolumn reproducibility, no significant degradation or decrease in precolumn performance was showed even after ∼150 preconcentration/desorption cycles. The precolumns also proved to be resistant to salt buffer with high concentration and low-pH mobile phase. The large-bore particle-entrapped monolithic precolumns will be further used in a high-throughput 2D-LC array system coupled with tandem matrix assisted laser desorption/ionization-time of flight–time of flight–mass spectrometry (MALDI-TOF–TOF–MS) detection for proteome analysis.

Keywords: Peptide preconcentration; Monolithic precolumns; Large-bore; Sol–gel; Capillary LC

1. Introduction

Chromatography-based methods for the analysis the components of complex protein mixtures have provided

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a promising alternative approach to two-dimensional gel electrophoresis (2D-gel) for proteome analysis [1]. Single-dimensional liquid chromatography (1D-LC) and two-dimensional liquid chromatography (2D-LC) coupled with tandem mass spectrometry (MS/MS) have been currently developed as powerful tools for characterizing proteomic mixtures. However, improving the sensitivity and robustness of methods remains a challenge [2].

On-line sample preconcentration by using a large-bore precolumn prior to separation is an important and widely used sample pretreatment technique, which offers both the enrichment of analytes and the reduction of interfering components. Its combination with electrophoretically based or chromatographically based techniques has gained increasing attention. On-line incorporation the precolumns into 1D- or 2D-LC platform has proven to be an attractive technique of allowing robust operation and facilitating achieving lower detection limits with increasing sample mass and solution volume processing capability in proteomics analyses [2–4]. Sample preconcentration was performed by using column-switching technology or not in different manners including preconcentration-reversed phase liquid chromatography (RPLC) [2,4–7], strong cation exchange (SCX)-preconcentration-RPLC [1,3,8] and preconcentration-SCX-RPLC [9]. In addition, this technique has also shown its versatility with the aid of different types of sorbent material in preconcentrator-cartridge. Not only RPLC packing materials, which was the most commonly used, but also many specific materials were employed as trapping material for any purpose, such as SCX resins [10], immunoaffinity resins [11], restricted access materials (RAM) [12,13] and molecularly imprinted polymers materials (MIP) [13], etc.

Most precolumns are commercially available products, especially those used for 2D-LC system. Certainly, there are several approaches of making precolumns in laboratory. The commonly used and laboratory-made trap is virtually a short slurry-packed capillary LC column, which means two frits or something similar to these (i.e., in-line filters) are required to retain the packed stationary phase [10,14–16]. Retaining frits are also known as a source of band-broadening, especially for short precolumns. In addition, the procedure of preparation is time-consuming and it is difficult to make the short packed columns have same characteristics. An alternative to avoid making frits is to making porous polymer and silica monolith, which have been prepared in capillaries [17–19] or even within the channel of a microfluidic device [20] for sample enrichment. However, one drawback associated with polymer monolith is its tendency of solvent swelling and pressure deformation of column bed, while silica monolith is prone to shrinking and cracking during column drying. Therefore, reproducibility and durability of the resulting columns could not be controllable precisely. Moreover, large-bore precolumn shows advantages on sample enrichment because of higher sample loading capacity, however, till now, the preparation of porous monolithic capillary columns are mainly designated for narrow-bore capillaries (50–200 μm). Although some publications [21–23] have already successfully demonstrated the possibility of making porous monolithic columns of large diameters to improve sample loadability, there are not many reports dealing with the preparation of these columns and their use for sample preconcentration.

Apart from porous polymer and silica monolith, there is a special particle-entrapped monolithic column which is prepared by entrapping the conventional LC packing materials inside a fused silica capillary using sol–gel technology [24–29]. Here, the sol–gel solution serves as a “glue” to create a bridge between adjacent particles, as well as the capillary wall and particles in its vicinity, thereby eliminating the need for retaining frits. The procedure of column making is so simple and reliable that the resulting columns provide good column-to-column reproducibility and remarkable stability. Another marked advantage of the “sol–gel-glued” monolithic column is that the presence of entrapped packing particles helps to largely alleviate the shrinking and cracking on the sol–gel matrix by decreasing the stress within the matrix during drying, and thus the procedure of column making has promising possibility of applying to large-bore capillaries. However, to our knowledge, this type of monolithic column is narrow-bore and till now, no attempt is cited in the literature for the fabrication of this kind of column with large inner diameter. Furthermore, it is mainly used in the range of capillary eletrochromatography (CEC) separations without having been employed as preconcentration column in multidimensional LC system.

Our aim of the work described in this report was to investigate the feasibility of preparation of large-bore particle-entrapped monolithic column and check the applicability of it as a preconcentration column for on-line peptides enrichment. In our previous study [30], we reported the on-column frit making by bonding bare silica gel or octadecylsilica (ODS) particles with sol–gel in the capillary in situ. The frits proved to be mechanically strong, permeable and reproducible. In the present study, firstly we have successfully prepared the ODS particle-entrapped monolithic columns with much larger inner diameter (320 and 530 μm) by similar use of methyltriethoxysilane (MTES) alone as precursor. And then, validations of the large-bore monolithic precolumns were performed by developing an on-line preconcentration of peptides system, in which the precolumn was followed by capillary RPLC–UV analysis via a switching valve. Various parameters affecting peptides preconcentration on the precolumns were investigated and optimized. The laboratory-made monolithic precolumns with 320 and 530 μm inner diameter (i.d.) are demonstrated to be able to enhance sample processing capabilities and separation efficiency in the analysis of peptides and both of them proved to be very suitable for incorporation into 2D-LC platform.