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Stage-frit: A straightforward sub-2 μm nano-liquid chromatography column fabrication for proteomic analysis

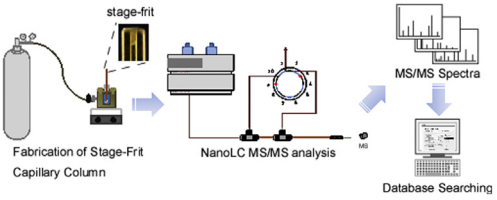
Ming-Yueh Hsieh, He-Hsuan Hsiao*

Department of Chemistry, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung 402, Taiwan

HIGHLIGHTS

- Fabrication of an inexpensive, highly reproducible and durable capillary frit.
- Simple preparation of the stage-frit by a C18 disk with a sol–gel polymerization.
- Operation of sub-2 μm capillary column under a normal pressure nanoLC system.
- Preferable separation efficiency in terms of average full width at half maximum.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work we demonstrated a facile method for the fabrication of C18 coordination polymer gel in a capillary, called stage-frit, which was efficiently applied to pack sub-2 μm C18 beads into the capillary by a high pressure bomb for the online separation of proteolytic peptides. The back pressure of the column with 10 cm × 75 μm i.d. is regularly lower than 170 bar at a flow rate of 300 nl/min, which could be operated on a common nanoLC system instead of nanoUPLC system due to the good permeability, low back pressure and high mechanical stress of the frit that will totally reduce the cost for the purchase of instrument. The stage-frit allows long-term continuous flow of the solvent and no significant beads loss or pressure instability was observed during the period. The repeatability of retention time for fifteen BSA tryptic peaks was found to be less than 1.08% (RSD) in six time nanoLC-ESI-MS/MS experiments. The average full width at half maximum (FWHM) of peptide peaks is 5.87 s. The sub-2 μm stage-frit nanoLC column showed better sensitivity than the commercial available for large scale proteomic analysis of total tissue proteins from human spleen. The number of identified peptides is approximately 0.4-fold and 0.2-fold higher than that obtained by utilizing commercial columns packed with 3 μm and 1.8 μm C18 materials, respectively. In the field of analytical chemistry, particularly the use of nanoLC systems, stage-frit nanoLC column offers a great potential for the separation of complex mixtures.

1. Introduction

Over the last decade, nanoscale liquid chromatography (nanoLC) coupling electrospray ionization tandem mass spectrometry (ESI-MS/MS) has become an important technique in proteomic research [1–4]. The performance of nanoLC separation has been a crucial factor for the protein qualification and quantification. NanoLC

* Corresponding author. Tel.: +886 4 22840411; fax: +866 4 22862547.
E-mail address: hhhsiao@dragon.nchu.edu.tw (H.-H. Hsiao).

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utilizes small inner diameter (i.d.) capillary column, typically 10 cm in length and 50–100 μm i.d., corresponding to a flow rate at hundreds of nanoliters per minute. Analytes eluting from the nanoLC column are then introduced to the mass spectrometer via a nano-ESI emitter with a high voltage of 1–3 kV.

The current nanoLC columns are made by packing materials into a tapered tip or frit capillary [5–8]. Tapered tip column is used to eliminate the band broadening due to longitudinal diffusion, where the tip also acts as nano-ESI emitter. The packing materials at the tapered tip spontaneously accumulate and form a keystone which can retain the packing materials in the capillary. However, removing the protective polyimide coating of capillary makes the tapered tip fragile. As the tip is damaged or contaminated, the whole column has to be replaced with a new one to maintain the ionization efficiency. In addition, the tapered tip cannot be applied for the fabrication of trap column due to the reduced outer diameter of capillary. The frit capillary could be fabricated by sintering of silica particles within a capillary [9–11]. However, it is hard to prepare the reproducible frits due to the fast sintering reaction, usually resulting in changeable liquid permeability and pressure resistance. Moreover, during the sintering process, the protective polyimide coating of fused-silica capillaries is burnt off causing the capillary very fragile at the capillary end. Up to now, a variety of sol–gel techniques for the preparation of column frits in fused-silica capillaries have been reported [5,7,12,13]. The column frit has to be sufficiently powerful to retain the packing materials and to resist the high pressure employed for packing and flushing the column. The column frit also needs to be extremely permeable for different solvents. In any LC applications, sol–gel frits have been shown to contribute the possible sources of adsorption and band broadening, especially for nanoLC columns. Besides, it is very difficult to control the length of frit and may produce significantly high back pressure during liquid flow, which is very important to minimize post-column band broadening. There has been an effort to fabricate frit columns for the alleviation of these problems.

In this study, a simple and fast method for the fabrication of a stable and permeable frit was developed by using potassium silicate cross-linked the C18 disk to the inner wall of the capillary. The stage-frit capillary was fabricated without sintering and thus preserved the polyimide coating that could be applied for the preparation of trap and analytical nanoLC columns for the proteomic analysis. The frits did not generate excessive back pressure and were more tolerant to the mechanical stress during chromatography. The stage-frit nanoLC columns were further applied to separate proteolytic peptides for the evaluation of the column efficiency, sensitivity and reproducibility.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), total tissue protein from human spleen, chloroacetamide (CAA), dithiothreitol (DTT), formic acid, formamide and ammonium bicarbonate were obtained from Sigma–Aldrich (St. Louis, MO). Sequencing grade, modified trypsin was obtained from Promega (Madison, WI). Acetonitrile (ACN) and acetone were obtained from Merck (Darmstadt, Germany). Kasil 1 potassium silicate was obtained from PQ Corporation (Malvern, PA). RapiGest™ SF was obtained from Waters Corporation (Manchester, UK). NUCLEOSIL® C18 (100 Å, 3 μm) was obtained from Macherey–Nagel, GmbH & Co. KG (Düren, Germany). Spherical C18 (120 Å, 1.8 μm) was obtained from SiliCycle Inc. (Quebec, Canada).

2.2. Fabrication of stage-frit packed nanoLC column

The stage-frit is simply manufactured by a C18 disk with a sol–gel polymerization shown in Fig. 1A. In general, the fabrication of "stage-frit" consists of the following five steps: 1) A blunt end syringe needle is driven manually through a C18 disk (3 M Empore™). 2) The cut-off C18 disk is inserted into the bottom of a pipet tip. 3) 10 μl of sol–gel polymerized solution (80% Kasil 1 potassium silicate, 10% formamide and 10% H_2O) is added and passes through the pipet tip under pressure from an air-filled plastic syringe. 4) A gentle manual push is applied to the capillary, polymerization solution goes through the pipet tip and then the C18 materials are fixed on the inner surface of the capillary. 5) The resulting capillary

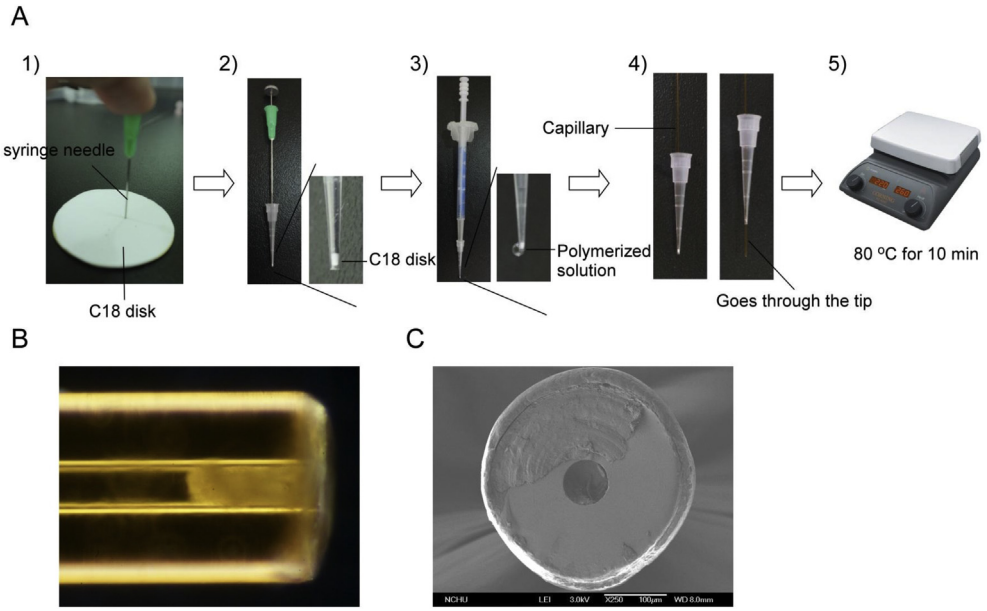

Fig. 1. Illustration of stage-frit assembly and photograph of stage-frit. (A) Workflow for the fabrication of a stage-frit capillary. (B) Micrograph shows an overview of the stage-frit. The inner diameter of the fused silica capillary is 75 μm and the length of stage-frit is approximately 250 μm. (C) Scanning electron microscopy scan of a stage-frit at 250× magnifications.