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Direct observation of frits and dynamic air bubble formation in capillary electrochromatography using confocal fluorescence microscopy

Gufeng Wang, Mark Lowry, Zhenming Zhong, Lei Geng*

Department of Chemistry, The Optical Science and Technology Center and The Center for Biocatalysis and Bioprocessing, University of Iowa, Iowa City, IA 52242, USA

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Abstract

Confocal fluorescence microscopy has been used to study the capillary electrochromatography (CEC) frits and dynamic air bubble formation under real chromatographic conditions. Confocal fluorescence microscopy provides a nondestructive way to view the three-dimensional structure of the frits with high spatial resolution. Frits prepared with four different procedures were studied: (1) sintering bare silica beads with sodium silicate; (2) sintering bare silica beads wetted with water; (3) sintering C18 beads wetted with water; and (4) sintering C18 beads wetted with water and then surfaced-recovered with C18. Frits prepared with sintering silicate-wetted beads have a high degree of heterogeneity, while the other three types of frits have similar, more homogeneous packing structures. Confocal fluorescence microscopy also provides sufficient temporal resolution for in situ observation of the dynamic processes in air bubble formation. In this study, air bubble formation is imaged during the reorganization process of the packing bed and is shown to occur close to the border between the packing bed and the outlet frit. Confocal fluorescence microscopy opens a new avenue in studying dynamic processes in situ in CEC separations.

Keywords: Confocal fluorescence microscopy; Capillary electrochromatography; Frits; Bubble formation

1. Introduction

In conventional packed-column capillary electrochromatography (CEC), the preparation of the frits is central to the making of a column. The quality of the frits affects separation efficiency and sensitivity of the column. Many problems in separations are associated with the frits, for example, tailing, column fragility and air bubble formation [1,2]. The initial frit preparation methods in pioneering work of CEC are either using sol–gel reactions [3,4] or by sintering silicate-wetted chromatographic beads [5]. Frits prepared with these two methods have the best mechanical stability and sufficient permeability. Subsequently, methods were pursued for improved reproducibility in the electric current and permeability of the columns. Behnke et al. reported that frit made from sintering beads soaked with pure water gives the column a stable condition of electroosmotic flow and a stable baseline, well suited for CEC [6]. Rebscher and Pyell's study confirmed that frits prepared with this method exhibit high permeability and the absence of bubble formation [7]. The most common method in preparing frits today is sintering a portion of packed beads [8,9]. Silica particles are connected to each other at their contact points in a hydrothermal process. Frits prepared this way also possess good permeability and a constant electroosmotic flow (EOF). A disadvantage exists that the surface of beads in the frit is reactivated in the sintering process. An improved method is to re-deactivate the frits by recoating the frit surface with silane. It was found that the adsorption on the re-deactivated frits could be significantly reduced [10]. Furthermore, it was shown that recoating the frits also reduces the bubble formation in the columns [11]. In recent years there is a renaissance of using the sol–gel method [12–15] and taking advantage of photopolymers in

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* Corresponding author. Tel.: +1 319 335 3167; fax: +1 319 335 1270.
E-mail address: lei-geng@uiowa.edu (L. Geng).

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frits preparation [16]. These new methods avoid destructive heating on both beads and column and also have been claimed to produce frits with reduced bubble formation. In a recent report, the performances of frits prepared with sol–gel, sintering, photopolymerization and modified sol–gel technologies are evaluated [17]. It is shown that the modified sol–gel and photopolymerized frits show improved day-to-day and column-to-column reproducibilities. However, the sintered frits are still associated with the least amount of peak dispersion [17]. The new methods are still in their developing stages while sintering a portion of packing material may still be the most widely adopted frit preparation method in packed-column capillary electrochromatography.

In preparing routinely used columns and inventing new methods for frit preparation, permeability, reproducibility and dispersion caused by the frits are important parameters that researchers are most interest in. The overall performance of a frit is usually characterized using traditional EOF method [10,11,16,17]. Furthermore, it is more recognized recently that bubble formation in the CEC capillary is highly related to the frits [1,2,7,12,18]. It is desirable to directly visualize the underlying properties, e.g., the packing quality, deformation of sintered beads, and the surface-adsorption of a frit, to correlate with separation parameters obtained in the traditional EOF method.

Microscopic imaging has received much attention to study the fundamentals of separation since it approaches microscopic phenomena directly [19–24]. Scanning electron microscopy (SEM) has been extensively used to study the packing of both the frits and column [6,9,12,15,16]. A column containing the frit is usually cut into halves in the middle and the cross-section is imaged. The spatial resolution of electron micrograph is high, allowing fine details of CEC packings to be examined. However, due to the restriction of the experimental conditions of SEM, the observation could only be made in dry environment and static conditions. Phenomena and properties related to the frits under real chromatographic conditions, e.g., analyte-stationary phase interaction, and air bubble formation, cannot be observed using SEM. Guiochon and co-workers used direct photographic imaging to study the separation phenomena in situ [21–24]. Considerable amount of new information on packing bed heterogeneity, sample injection, migration, diffusion, and separation-related phenomena in liquid chromatography was obtained by photographic imaging.

Another approach is reported by Lowry et al. to study the solute distribution in CEC using laser scanning confocal fluorescence microscopy [19]. It has been shown that confocal fluorescence microscopy provides not only high contrast between chromatographic beads and their surrounding environment, but also excellent spatial resolution to image thin slices of the interior of chromatographic beads in situ for the first time. More importantly, the confocal imaging system provides good temporal resolution to observe the dynamic processes under real chromatographic conditions. In this paper, we extend this technique to study the frits structure and frits-associated bubble formation in capillary electrochromatography.

2. Experimental

2.1. Chemicals

Rhodamine6G was purchased from Aldrich (Milwaukee, WI). HPLC grade acetonitrile was obtained from Fisher (Fair Lawn, NJ). Luna silica beads used in frit preparation and CEC packing were obtained from Phenomenex (Torrance, CA). The 10 μm beads are C18 modified, and have a surface coverage of 17.5%, an average particle size of 10 μm, and average pore size of 100 Å. The coverage of bonded phase is 3.00 μmol/m^2. The 7 μm particles are uncoated, and have an average pore size of 100 Å. Deionized water was purified with a MilliQ system (MilliQ-Plus, Millipore, Bedford, MA) and was used to prepare all aqueous solutions. Refractive index matching oil ($n = 1.51$) was obtained from Carl Zeiss Microimaging Inc. (Thornwood, NY). Polyimide-coated capillaries (50 μm i.d., 360 μm o.d.) and non-coated quartz tubing (400 μm i.d., 550 μm o.d.) were acquired from Polymicro Technologies (Phoenix, AZ).

2.2. Capillary electrochromatograhy

Frits are prepared with four different methods: (1) sintering packed bare silica beads wetted with silicate solution; (2) sintering packed bare silica beads wetted with water; (3) sintering packed C18 silica beads wetted with water; (4) frits made using method (3) and then the surface of the beads is recoated with C18.

For the first method, a procedure was developed similar to established procedures in the literature [5] and described elsewhere [19,25,26]. Briefly, a segment of 50 μm i.d. capillary tubing was conditioned with 0.1 M NaOH for 20 min. The preconditioned capillary was then cut into 10 cm pieces. The polyimide coating was burned off with a small flame of a microtorch. Five milligram 7 μm uncoated silica beads and 3 μL Na_2SiO_3:H_2O (v/v) = 1:1 solution are mixed and stirred to form a paste. One end of the capillary was pressed into the paste to take up a few millimeters of the mixed materials. A thermal wire stripper was used to heat the sodium silicate mixture to form a frit at the end of the capillary.

The frits made from methods 2–4 are prepared with modified procedures in the literature [6]. Briefly, a preconditioned 50 μm i.d. capillary was stripped off coating and then cut into 10 cm pieces. One end of the capillary was fritted using method 1 as mentioned above as a temporary frit. The capillary was then packed with chromatographic beads using a vacuum pump. In method 2, 7 μm uncoated beads are packed while in methods 3 and 4 the 10 μm C18 beads are packed. The packing was then wetted and compressed using a syringe pump by pumping acetonitrile toward the temporary frit. Acetonitrile was then rinsed off with water