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Short communication

Deactivation of frits for use in capillary high-performance liquid chromatography and capillary electrochromatography with characterization by imaging with laser-induced fluorescence

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Abstract

Residual adsorptive activity of frits prepared by sintering silica gel particles used in capillary HPLC and electrochromatography can be significantly reduced by silanization. The adsorption of polar analytes on frits consisting of sintered normal-phase or reversed-phase silica gel before and after deactivation has been characterized by imaging with laser-induced fluorescence in combination with a charge coupled device camera.

Keywords: Frits; Silica frits; Capillary columns

1. Introduction

Liquid chromatography in packed capillary columns is a fast developing area with an increasing number of applications in capillary HPLC [1–3] and capillary electrochromatography (CEC) [3–10]. The columns used in these microseparation techniques consist generally of fused-silica capillaries of 20–300 μm inner diameter with frits prepared by in situ sintering of silica gel particles. Two approaches have been employed for frit preparation. First, frits made of sintered normal-phase silica gel can be used for containing the stationary phase. Using these frits many nonsilicious stationary phases e.g. polystyrene particles can be packed which are not suitable for frit formation. Unfortunately, the preparation of such columns is a tedious several-step packing process [3,4]. Alternatively, the frits of many reversed-phase silica gels can be sintered directly from the stationary phase in a one step process [5–10].

In the sintering process, the silica gel particles are connected to each other at their contact points assumedly in a hydrothermal process [8,9] and their surface is activated by the formation of unmasked silanol groups so that the frit can strongly bind certain components of eluent or the sample. The increased adsorption of sodium and potassium ions has caused problems in the coupling of such capillary columns to an electrospray mass spectrometer resulting in a decreased sensitivity [4]. Further, the

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adsorption of polar analytes on silica frits has recently been detected by imaging with laser-induced fluorescence (LIF) and detection with a charge coupled device (CCD) camera [11].

In this communication, we show that these problems can be solved by the appropriate deactivation of the frits. We have adopted a method [12] used for silanization in packed capillary column supercritical fluid chromatography (SFC) and tested the adsorption properties of the silanized frits with LIF/CCD detection.

2. Materials and methods

Fused-silica capillaries of 100 μm I.D.×360 μm O.D. (Polymicro Technology, Phoenix, AZ, USA) were packed with octadecyl silica (Gromsil ODS-2, d_p=5 μm; Grom, Herrenberg, Germany) as described before [3]. Removal of the protective polyimide coating with fuming nitric acid was carried out prior to fluorescence measurements. The frits consisted of sintered reversed-phase silica gel or normal-phase silica gel (Gromsil Si, d_p=5 μm) and were prepared by heating the capillary over a length of ca. 2 mm with an electrical wire. The frits made of octadecyl silica were sintered while flushing the column at 200 bar with water. The temperature of the electrical wire was adjusted in such a way that it was high enough to evaporate the solvent inside the capillary. The steam formation is recognizible by a reversible whitening of the packing at the heated position. However, the temperature was low enough to avoid irreversible blackening of the polyimide coating. The frits were formed by heating the capillary five times for ca. 10 s. Deactivation of the frits with diphenyltetramethyldisilazane (DPTMDS) was carried out as described in the literature [12]. In this process the capillary column is first purged by CO_2 at 200°C in order to dry it before 100 nl DPTMDS is injected at the same temperature to silanize the silica surface.

A Model 480 HPLC system (Gynkotek, Germering, Germany) was operated at a flow-rate of 0.3 ml/min. Splitting of the eluent in a ratio of ca. 1:200 was achieved by a stainless steel T-piece connected to a fused-silica capillary of 20 cm×50 μm I.D. Injection of 50 nl was accomplished by filling the injection device (Model CEC100, Grom) with 2 μl of sample and pressurizing for 10 s at 100 bar. The mobile phase was acetonitrile–water (30:70, v/v). The concentration of dansylated leucine (Sigma, St. Louis, MO, USA) was 10 μg/ml.

The instrumental set-up for LIF/CCD detection consisting of a XeCl eximer laser (Estonian Academy of Science, ELI-76E, Tallin, Estonia) and image-intensified CCD detector (Spectroscopy Instruments, CCD-576R, Gilching, Germany) equipped with a camera objective (Nikkor 50 mm f1.8, Nikon, Tokyo, Japan) has been described in detail in Refs. [13,14]. The laser had an output pulse energy of 200 μJ and was used at 308 nm. Fluorescence images were recorded at a rate of five frames per second. For better representation only four frames of 400 and 225 frames are shown in Fig. 1A and B, respectively.

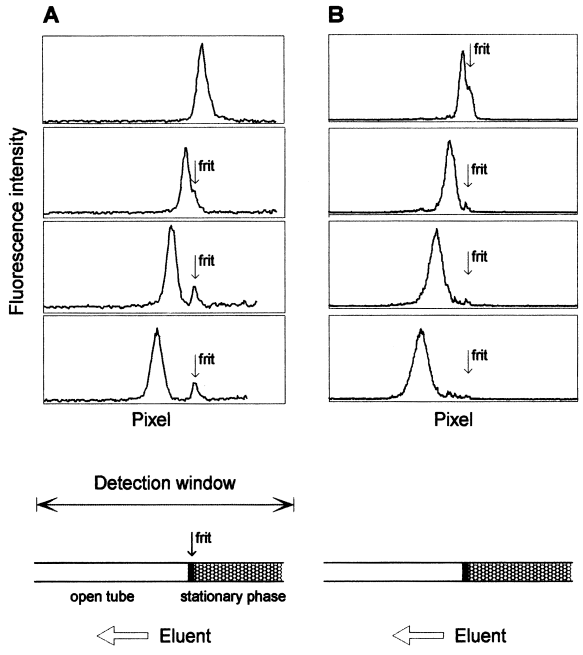

Fig. 1. Fluorescence imaging of the elution of dansylated leucine in a packed capillary column with an outlet frit made of sintered reversed phase particles. (A) Column before and (B) after deactivation. The position of the frits is indicated by arrows. Whereas in the untreated column (A) the dansyl leucine is strongly adsorbed on the frit as indicated by a fluorescence signal remaining on the frit, significantly reduced adsorption is observed in the deactivated column (B).

3. Results and discussion

A wide range of techniques has been developed to efficiently mask the silanol groups of chromatographic packings by organic functions [15–17]. We have chosen a method for silanization which is used in packed capillary SFC to improve the adsorptive characteristics of the columns [12]. The effectiveness of the in situ silanization is demonstrated in Fig. 1, where the fluorescence images representing the elution of dansyl leucine on two capillary columns are depicted. The images show 10 cm of the packed columns, focusing on the transition from the stationary phase retained by the frit to the open tubular area of the column as illustrated in Fig. 1. The transition of the chromatographic band from the stationary phase to the open tubular area is marked by increased band spreading in the open tubular area. When the analyte is transported across an untreated frit prepared from normal-phase silica gel or octadecyl silica packing (Fig. 1A) a fluorescence signal of the adsorbed dansyl leucin remains at the position of the frit. As the adsorbed dansyl leucine molecules are eluted, the signal vanishes within several minutes. It is noted that the adsorption of dansyl leucine on the frit did not result in tailing of the eluted band. With basic compounds, especially basic proteins, which are adsorbed easily on acidic binding sites, however, tailing is expected with the concomitant deterioration of the efficiency of the separation.

With a sintered reversed-phase frit treated with DPTMDS (Fig. 1B) significantly reduced absorption of dansyl leucine was observable thus indicating the deactivation of the silanol surface newly formed in the sintering process. These columns did not show any alteration in the chromatographic properties in agreement with the expectation that the endcapped stationary phase offers no binding sites for the silanization agent.

It has been demonstrated previously that columns packed with this endcapped stationary phase but untreated frits can be successfully applied in electrochromatography [3,4]. Future research will focus on the effects of the derivatization reagent in electrochromatography. It will be of particular interest to differentiate the influence of the silanization of the frit and that of the wall of the fused-silica capillary, respectively, on the chromatographic properties and on the electroosmotic flow velocity.

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