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In situ synthesis of monolithic stationary phases for electrochromatographic separations: Study of polymerization conditions

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Abstract

Acrylate-based monolithic capillary columns were prepared from fused-silica capillaries using UV photopolymerization. The effect of the pretreatment of the capillary wall surface before polymerization was investigated and several procedures were compared. The columns were characterization by van Deemter curves and SEM imaging. The results indicated that a pre-silanization of the capillary wall in order to introduce methacrylate groups at the wall surface gave similar efficiencies but more homogeneous structures than when the silanization agent was introduced in the polymerization mixture. The conditioning of the capillary before silanization, especially the conditions of basic rinsing was also an important factor. The effect of the dose of UV light that was applied for the polymerization had also been investigated. The results demonstrated that the irradiation energy is a critical parameter. The minimum energy threshold required to obtain a suitable monolith was 3 J/cm^2 and the maximum was around 12 J/cm^2. A higher energy destroys the monolith. Within the convenient range of energy, the columns had the same efficiency and a good structure as seen by SEM imaging. Using the optimized procedure for the pretreatment and an adequate energy, the column-to-column repeatability was found good ($n = 12$). The repeatability was obtained for the plate height at two velocity values, the retention factor and the electroosmotic mobility with RSD values below 10.

Keywords: Acrylate-based monolithic columns; Electrochromatography; Pretreatment; Polymerization; Column reproducibility

1. Introduction

Capillary electrochromatography (CEC) is a hybrid separation technique that combines advantages of both liquid chromatography (LC) and capillary electrophoresis (CE). The stationary phase included in the capillary provides additional interaction especially for uncharged molecules while the mobile phase is driven through the capillary by the electroosmotic flow (EOF) characteristic of electrophoretic methods. High efficiencies can be expected due to the flat flow electroosmotic profile, even if it was pointed out that capillary packed with particles or monoliths might not have such a perfect plug profile [1].

Although the potential of CEC using packed capillary columns was demonstrated in the early 1980s, serious technical difficulties have delayed its development [2–5]. Most of the problems came from column preparation, especially particle packing, fabrication of frits, bubble formation due to frits and non-uniformed field strengths, limited stability and fragility of packed columns [6]. Recently, CE and CEC are also moving to the microchip format and are receiving much attention. As a consequence, alternative stationary phases are now under development to overcome the above problems. A first approach was given by the development of open-tubular (OT) coated capillaries and different chemistries and types of phases have been developed [7–10]. The advantage of this technique is to avoid the use of frits. However, some major drawbacks remain, such as limited surface area and low sample capacity due to the too low phase ratio [11]. The second approach consists in monolithic stationary phases – originally called continuous polymer beds by Hjertén, then polymer or silica rods by Svec and Tanaka – that are made of a continuous piece of highly cross-linked porous microstructure prepared by in situ polymerization [11–16]. They were shown to be very similar in behaviour to conventional packed columns with beads [17]. Monoliths can be sorted into silica-based monoliths, generally prepared by sol–gel technology [16,18–21] and rigid organic polymer-based mono-

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liths, including acrylamide- [22–24], styrene- [25], and acrylate- or methacrylate-based polymers [15,26–35]. The resulting network thus provides high surface area, tuneable functionality, low backpressure, and no need for frits, since their structure can be anchored to the inner wall. A specific advantage of polymer-based monoliths is their ease and flexibility of preparation.

Acrylate- and methacrylate-based polymers are well suited for microchips because the polymerization can be photoinduced, allowing thus an easy designed localization of the phase within the microchip [30,32,33]. As the channel dimensions of the separation microsystems are similar to those of capillary columns used in CE, several studies have also been conducted using modified capillaries with UV transparent external coating [26,29,31]. The majority of the publications dealing with the set-up of monolithic columns have optimized the composition of the polymerizing solution made usually of a porogenic solvent mixture, various monomers and a cross-linker which are critical parameters for controlling the porosity and obtaining efficient columns. Reproducibility and robustness of the synthesis are almost never described. Other parameters such as the capillary pretreatment before the in situ synthesis and the dose of UV light provided for the polymerization process have received much less attention.

The first continuous polymer beds made by Hjertén and his group were obtained after an extensive pretreament of the capillary that consisted of washing the capillary walls by basic and acidic solutions and then applying a pre-silanization step using 3-(trimethoxysilyl)propyl methacrylate (3-TMSM or γ-MAPS) in order to provide methacrylate functions for a better attachment of the polymer to the silica walls [22,23,36,37]. This procedure was then applied by several authors, sometimes with slight modifications [24,25,32–35]. On the other hand, the first molded rigid polymers monoliths for electrochromatography were prepared without any capillary pretreatment [24]. Other studies have removed the pre-silanization step but introduced the 3-TMSM directly in the polymerization mixture with good results [31,36].

The energy that is required for the polymerization should be also an important parameter. We know that a minimum energy is required, but also that a too high energy should destroy the monolith, as demonstrated by the fact that one very simple means for obtaining a detection window was to expose the monolith to the UV detection lamp during several hours [31,36,38]. The importance of this parameter on the resulting structure of the monolith and its electrochromatographic performances has never been described.

Obtaining reproducible, stable and efficient monolithic columns from one synthesis to another one is necessary. The objective of our work was to investigate the effect of the two parameters, pretreatment and dose of UV light for polymerization on both the resulting monolith structure and the electrophoretic performances. These studies were conducted using UV-transparent silica capillaries and the columns were tested using an electrophoresis apparatus.

2. Materials and methods

2.1. Chemicals and materials

Monobasic and dibasic sodium phosphate were purchased from Aldrich (St. Louis, MO, USA). Butyl acrylate, hexyl acrylate, 1–3-butanediol diacrylate, 3-(trimethoxysilyl)propyl methacrylate (3-TMSM), 2-acrylamido-2-methylpropanesulfonic acid (AMPS), 2,2′-azobis(2-methylpropionitrile) (AIBN) were obtained from Acros Organics (Geel, Belgium). They all were of the highest available purity grade and used without further purification. Analytical grade solvents such as ethanol and acetonitrile were from J.T. Baker (Paris, France) and Carlo Erba (Val de Reuil, France), respectively. Pesticides were obtained from Dr. Ehrenstorfer (Augsburg, Germany), naphtalene from Acros, fluorene, anthracene, and pyrene were from Prolabo (Fontenay-sous-Bois, France), alkylbenzenes were from Aldrich and formamide (99%) from Fluka (Buchs, Switzerland). Buffers were prepared using 18.2 MΩ deionized water filtered through a Milli-Q plus purification pack (Millipore, Bedford, MA, USA). Seventy-five and one-hundred micron I.D. of fused-silica capillaries with UV-transparent external coating were purchased from Polymicro Technologies, (Phoenix, AZ, USA).

2.2. Preparation of monolithic columns

2.2.1. Surface pretreatment of the capillaries

Four types of procedures were tested as described in Table 1. The capillary was rinsed with water or acetone between the different steps, and finally dried under air flow. In procedure A and C, the silanization solution was composed of 4 μL of 3-TMSM in 1 mL of acetic acid. In procedure B and C, the silanization solution was obtained by mixing 3-TMSM in DMF (1:1, v/v). The binding silane solutions were kept inside the capillary by closing each ends by septa.

Table 1
Description of the different pretreatment procedures studied

	Pretreatment procedures			
	A	B	C	D
Basic wash (NaOH 1 M)	30 min, 25 °C	30 min, 25 °C	90 min, 90 °C	90 min, 90 °C
Acidic wash (HCl 0.1 M)	None	30 min, 25 °C	None	45 min, 90 °C
Silanization with 3-TMSM	Acetic acid	DMF	Acetic acid	DMF
	Overnight	5 h	Overnight	5 h
	25 °C	70 °C	25 °C	70 °C