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In situ functionalization of *N*-acryloxysuccinimide-based monolith for reversed-phase electrochromatography

Mohamed Guerrouache, Benjamin Carbonnier, Claire Vidal-Madjar, Marie-Claude Millot *

Laboratoire de Recherche sur les Polymères, CNRS-Université Paris 12, 2 rue Henri Dunant, 94320 Thiais, France

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Abstract

Capillary electrochromatography (CEC) monolithic columns were prepared following a two-step synthetic pathway based on (i) UV-induced *in situ* radical polymerization of *N*-acryloxysuccinimide (NAS) and ethylene dimethacrylate (EDMA) and (ii) *in situ* functionalization of the NAS-containing monolithic matrix with various alkylamines. The first synthetic step was performed using toluene as a porogenic solvent. The successful grafting of the alkylamines onto the reactive matrix was confirmed on the basis of qualitative analysis of Raman spectra recorded before and after the chemical modification step. All the electrochromatographic results indicate a strong dependence of the retention, efficiency and selectivity of the monolithic columns on small variations of mobile phase composition and nature of the grafted aliphatic selector in agreement with the typical reversed-phase behaviour. Van Deemter plots for a series of alkylbenzene homologues injected on a column bearing hexyl-segments as side chains are also presented.

Keywords: UV polymerization; *N*-Acryloxysuccinimide; Functionalizable monolith; Reversed-phase electrochromatography; Alkylbenzenes

1. Introduction

Capillary electrochromatography (CEC) is an emerging "hybrid" separation method that combines features of both HPLC and capillary zone electrophoresis (CZE). This technique utilizes capillary columns containing solid stationary phases which interact with solutes. The application of an electric field across the capillary column generates an electroosmotic flow (EOF) with an almost uniform plug-like velocity profile which drives the mobile phase and analytes through the separation bed. Therefore, CEC affords significantly higher column efficiencies than methods in which the flow is driven by pressure leading to a parabolic flow profile with increased band broadening.

The most widely used columns in CEC are packed with alkyl-silica stationary phase [1,2]. This approach requires frits to prevent the loss of the stationary phase from the capillary. The main drawbacks of this technique are the packing of small diameter particles into narrow-bore tubes and the tedious fabrication of retaining frits within the capillary that is difficult to perform reproducibly usually leading to bubble formation. Open tubular CEC (OT-CEC) columns have been developed [3,4] but the relatively low phase ratio in OT-CEC restricts its further developments and applications.

As an alternative, monolithic stationary phases for CEC have attracted increasing attention. Advances in monolithic technology and their application for CEC have been reviewed in some recent articles [5,6]. Monolith may be defined as a continuous piece of highly porous material characterized with a well defined pore structure consisting of large flow-through pores for high permeability and small diffusion pores for desired surface area [7]. Monolithic CEC columns can be classified as either silica-based materials [8,9] or organic polymers [10,11]. Acrylamide- [12], styrene- [13], and methacrylate ester-based [11] monolithic columns have been developed and used as polymeric separation bed in CEC.

Two methods are commonly used to initiate polymerization in the preparation of organic monoliths: thermal- [10,14,15] and photo-initiation [16,17]. The largest advantage of monoliths is

* Corresponding author.
E-mail address: millot@glvt-cnrs.fr (M.-C. Millot).

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the ease of their preparation directly within the confines of a capillary or a microfluidic chip; this avoids problems encountered both with packing and frits formation.

The wide variety of commercially available functional monomers, allows for different surface chemistries to be envisioned. However, the polymerization conditions must be re-optimized for each polymerization mixture containing a new monomer in order to obtain the required stationary phase since the composition of the polymerization mixture controls the morphology of polymer monoliths [18,19].

Several alternatives have been developed to avoid tedious re-optimizations of the polymerization conditions. Photografting has been described by Svec and coworkers [20,21] where polymers bearing functional groups were grafted to the surface of a monolithic matrix by UV irradiation. The chemical modification approach allows for developing original chemistries for the preparation of monoliths with functionalities for which monomer precursors are not available. This approach is performed by the preparation of columns including two steps: (i) the preparation of a matrix with optimized reactivity and porous properties, (ii) *in situ* functionalization with a reagent to obtain the desired separation properties. The most common monomer used for preparation of monoliths is glycidyl methacrylate (GMA) containing epoxide as reactive group which reacts with a compound exhibiting nucleophilic properties [22,23]. One of the drawbacks of GMA-based monoliths is the sluggishness of immobilization reactions with nucleophilic reagents such as proteins [24]. Other reactive monomers were described in the literature giving a broad range of monoliths with various reactivities [25–28].

The purpose of the current work was to prepare and study a functionalizable acrylate-based monolithic column obtained from a mixture containing *N*-acryloxysuccinimide (NAS) as functional monomer. This monomer is well known for its reactivity towards proteins and was used to elaborate micro-reactors for proteomics applications [27,28]. To our knowledge, only thermal polymerization of NAS was reported for the design of micro-systems. In this study, monolithic columns were prepared by UV irradiation to avoid problems of inhomogeneity encountered in thermal initiation [29]. Moreover photoinitiation is fast and allows the spatial control of the polymerization to a restricted area using masks adapted to the development of microfluidic devices [7,21].

After optimization of the monolithic structure, columns were functionalized through reaction with various amine containing aliphatic chains and the chromatographic performances of the monolithic columns for CEC were investigated. The retention behaviour and the selectivity for a set of alkylbenzenes were studied.

2. Experimental

2.1. Instrumentation and materials

All the electrochromatographic experiments were performed on a P/ACE MDQ (Beckman, Fullerton, CA, USA) equipped with 32 Karat software (version 4.0) for data acquisition. Fused silica capillaries with a UV-transparent external coating (75 μm I.D. × 325 μm O.D.) were a kind gift from InnovaQuartz (Phoenix, ZA, USA). An HPLC pump (Shimadzu LC-10ATVP, Champs sur Marne, France) was used to flush monolithic columns with alkylamine solutions for the functionalization step or with mobile phase for conditioning. Spectrolinker XL-1500 UV crosslinker (Spectronics, Westburry, NY, USA) equipped with eight lamps (8 × 15 W, 365 nm) was used to photoinitiate the polymerization.

Morphology of the polymeric monoliths was evaluated using scanning electron microscopy with the help of a LEO Gemini 1530 SEM apparatus (Leo Elektronenmikroskopie, Oberkochen, Germany). Microscopic observations were performed for polymer samples as obtained after polymerization within capillaries and rinsing step with acetonitrile. Capillaries were cut at different places and small pieces were deposited on a SEM support with a silver-containing solution. The samples were dried under reduced pressure and coated with a thin layer of platinum (2 nm) with the help of a sputter coater Cressington 208 HR (Elektronen Optik Service, Dortmund, Germany).

Chemical structure of the monoliths was investigated using a Raman apparatus LabRAM HR from Horiba Jobin Yvon (Longjumeau, France) equipped with a laser emitting at 633 nm. Samples were investigated in different places to control the homogeneity. The acquisition time was fixed at 1 min.

2.2. Chemicals

N-acryloxysuccinimide (NAS), ethylene dimethacrylate (EDMA), 2,2′-azobisisobutyronitrile (AIBN), alkylamines (butylamine, amylamine, hexylamine, heptylamine, octylamine) and benzylamine were obtained from Acros Organics (Geel, Belgium). 3-(Trimethoxysilyl)propyl methacrylate, sodium hydroxyde (NaOH), hydrochloric acid (HCl), polycyclic aromatic hydrocarbons (PAHs; naphthalene, fluorene, anthracene, pyrene, chrysene), and alkylbenzenes (with size chains ranging from methyl to nonyl group) were purchased from Fluka (Isle-d'Abeau, France). HPLC grade acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF) and sodium phosphate were obtained from Sigma (Isle-d'Abeau, France). All the reagents were used without further purification. Buffers were prepared using 18.2 MΩ deionized water filtered through a Milli-Q plus purification pack (Millipore, Bedford, MA, USA). Hydro-organic mobile phases were prepared by mixing the appropriate amount of acetonitrile with the phosphate buffer of desired pH value.

2.3. Synthesis of the monolithic capillaries

The synthesis of the separative monolithic columns relies on a two-step process involving (i) the synthesis of the porous matrix and (ii) the chemical modification of the matrix with aliphatic side chains, as described in the following text and in Fig. 1.

2.3.1. Surface pretreatment of the capillaries

In order to ensure the stability of the monolithic column, the inner wall of the capillaries was submitted to a vinylization