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Adamantyl-group containing mixed-mode acrylamide-based continuous beds for capillary electrochromatography. Part III. Optimization of the chromatographic efficiency

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ABSTRACT

In a previous article we described the synthesis of amphiphilic monolithic stationary phases by in situ free radical copolymerization of cyclodextrin-solubilized N-adamantyl acrylamide, piperazinediacrylamide, methacrylamide and vinylsulfonic acid in aqueous medium in pre-treated fused silica capillaries of 100 μ.m I.D. In this work, a series of N-adamantyl-group containing acrylamide-based continuous beds is synthesized under variation of different synthesis parameters. The studied synthesis parameters are (i) concentration of the lyotropic salt ammonium sulfate, (ii) concentration of the initiator ammonium persulfate, and (iii) concentration of the negatively charged monomer vinylsulfonic acid in the polymerization mixture. The influence of the synthesis parameters on the chromatographic efficiency is studied under isocratic conditions for a homologues series of alkylphenones in the reversed-phase mode at constant composition of the mobile phase via capillary electrochromatography with varied electric field strength. With varied concentration of the lyotropic salt ammonium sulfate or varied concentration of the initiator ammonium persulfate in the polymerization mixture, a strong impact on the chromatographic efficiency is observed, while there is only a minor influence when varying the molar fraction of the charged monomer VSA. The absence of a significant influence of extra-column band broadening effects on the determined efficiency is confirmed. There is a good repeatability (with respect to capillary-to-capillary variation and run-to-run variation) reached for the theoretical plate heights obtained for DMF and selected alkylphenones in the reversed-phase mode.

1. Introduction

In capillary electrochromatography (CEC) polyacrylamide-based organic monolithic stationary phases synthesized in situ in fused-silica capillaries are an alternative to capillaries packed with particulate material [1–4]. Monolithic materials as stationary phases in chromatography have attracted a widespread interest during the last two decades which is documented in a large number of review articles [5–12]. In the last years the optimization of organic monoliths for the separation of small molecules has come into the focus of intense research activities [6,7,13–20].

The interest in polymer-based monolithic stationary phases stems from their chemical resistance toward a broad range of possible mobile phase compositions including pH, the possibility to fine-tune the chemical composition and the retention properties by variation of the mass fractions and the type(s) of monomer used in the polymerization mixture, and the ease of their preparation by an in situ free radical polymerization process. However, the actual situation must be characterized as follows: the separation efficiency observed for small molecules is often relatively poor (in comparison to particulate modified silica gel) and largely dependent on retention factors (and mobile phase composition) and even reported to be solute-specific [17,21,22]. The reason why the chromatographic performance shows these dependencies is far from being fully understood. The situation is also characterized by the observation that polymer-based monolithic stationary phases show a much better chromatographic efficiency when they are employed in CEC in comparison to the efficiency observed in capillary liquid chromatography (CLC) [23].

If the preparation of the organic monolith is performed by free radical copolymerization, the morphology of the macroporous polymer is formed spontaneously depending on the composition of the polymerization mixture and on the reaction conditions [10]. The morphological properties (such as average pore size and average microglobule size) of the produced organic monolith are usually controlled by following synthesis parameters: (i) type(s) and molar fraction(s) of monomer(s), (ii) type and molar fraction of crosslinker (%*C*), (iii) total monomer concentration (%*T*), (iv) type(s)

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and concentration(s) of porogen(s), (v) type, concentration and activation of initiator, (vi) reaction time, and (vii) reaction temperature [1–3,10,24–29]. There is a large number of investigations on the influence of these synthetic parameters on characteristics of the formed monolith. The mechanical and chemical stability, the permeability, and the domain size of the formed monolith defined as the combination of the mean flow-through pore size and the mean globule size [30] are influenced by the total monomer concentration (%*T*) [2–4,31] and the mass fraction of crosslinker (%*C*) [1,2,31]. The hydrodynamic permeability and the pore size distribution of the produced monolith are also affected by the concentration of the free radical initiator in the polymerization mixture [25].

Hoegger and Freitag [1] investigated the influence of various factors affecting the morphology and the chromatographic properties of hydrophilic polyacrylamide-based continuous beds polymerized form the water soluble co-monomers N,N-dimethylacrylamide (DMAA), piperazinediacrylamide (PDA), and vinylsulfonic acid (VSA). They observed that the mass fraction of crosslinker (%*C*), the type and the concentration of the pore forming agent, the type of solvent, and the type and molar fraction of the monomers in the polymerization mixture have a significant influence on the morphology and the chromatographic properties of the produced monolith. In a similar study, Ratautaite et al. [3] investigated the effect of buffer concentration and pH (buffer used as polymerization medium), and the total monomer concentration (%*T*) on the morphology and the chromatographic properties of amphiphilic polyacrylamide-based monoliths polymerized from the water soluble co-monomers methacrylamide (MA), piperazinediacrylamide (PDA), N-isopropylacrylamide (IPA), and vinylsulfonic acid (VSA). The authors confirmed that the hydrodynamic permeability is dependent on the ionic strength of the buffer. There is an increase in the volume fraction of flow-through pores in the monolithic scaffold with increasing buffer concentration.

In a previous publication [4], we reported the synthesis of a monolith which is based on the solubilization of N-adamantyl acrylamide in aqueous solution via a 1:1 complex formed with statistically methylated β-CD. Monoliths are prepared by in situ free radical copolymerization of this complex with PDA, MA and VSA in aqueous medium in pre-treated fused silica capillaries (100 μm I.D.). In the second paper of this series [32], we performed detailed investigations of these continuous beds (synthesized under variation of the concentration of the lyotropic salt ammonium sulfate in the polymerization mixture) regarding their morphology and porous properties by inverse size exclusion chromatography (ISEC) and scanning electron microscopy (SEM). In the present paper, we investigate the chromatographic efficiency of a series of N-adamantyl-group containing amphiphilic acrylamide-based continuous beds synthesized under variation of several synthetic parameters, which can be regarded to be decisive for the morphology (and chromatographic efficiency) of the produced macroporous polymer: (i) the concentration of the lyotropic salt ammonium sulfate in the polymerization mixture, (ii) the composition of the mixture of monomers (variation of the concentration of the charge-bearing monomer vinylsulfonic acid) and (iii) the concentration of the initiator. The impact of these varied parameters on the chromatographic efficiency obtained for a homologues series of alkylphenones is investigated by CEC in the reversed-phase mode at constant composition of the mobile phase under variation of the separation voltage. Special emphasis is placed on the confirmation of the absence of a significant influence of extra-column band broadening effects. The repeatability of the synthesis procedure is confirmed by comparison of data obtained for several capillaries regarding the plate heights reached for DMF and selected alkylphenones in the reversed-phase mode.

2. Experimental

2.1. Chemicals and instruments

All chemicals were used without further purification. *N*,*N*,*N*′,*N*′-Tetramethylethylendiamine (TEMED), 3-(trimethoxysilyl) propyl methacrylate (bind silane), di-sodium hydrogenphosphate dihydrate, N,N-dimethylformamide (DMF), ammonium sulfate (AS), and hydrochloric acid (37%, v/v) were from Fluka (Buchs, Switzerland). Propiophenone, butyrophenone, valerophenone, vinylsulfonic acid (VSA, 25%, w/v in aqueous solution), and ammonium persulfate (APS) were from Sigma Aldrich (Steinheim, Germany). Methacrylamide, acetic acid (AA), and sodium dihydrogen phosphate monohydrate were from Merck (Darmstadt, Germany). Acetophenone was from Riedel-de Haen (Seelze, Germany). Triethylamine (TEA) was from KMF Laborchemie (Lohmar, Germany). 1,4-Diacryloylpiperazine (PDA) was either from Alfa-Aesar (Karlsruhe, Germany) or from Molekula (Dorset, UK). Statistically methylated β-cyclodextrin (Me-β-CD) was either from ABCR (Karlsruhe, Germany) or from Sigma Aldrich (Louis, USA). N-(1-adamantyl)acrylamide was synthesized according to [33] (synthesis procedure and product characterization see [4]). Fused silica capillaries (100 μm I.D. × 360 μm O.D.) were from Polymicro Technologies (Phoenix, USA).

With all mobile phases the electric conductivity and the pH* value were controlled. The pH* value is the pH in the presence of organic solvent determined with a pH meter calibrated with an aqueous buffer. The pH* was determined with the mobile phase after addition of the organic solvent to the aqueous buffer. The pH meter Inolab pH 720 (WTW instruments, Weinheim, Germany), and the conductometer LF 191 (WTW, Weinheim, Germany) were used to measure the pH* and the electric conductivity of the prepared mobile phases. Mobile phases were prepared by buffering 100 mL of distilled water with 2–3 mL of a mixture of 0.2 mol L^{-1} TEA with 0.2 mol L^{-1} AA (1:1, v/v). Subsequently, the required volume of this solution is taken and mixed with the corresponding volume of organic solvent. The pH* and the electric conductivity of the final mixture were adjusted with 0.20 mol L^{-1} TEA or AA.

The mixed-mode monolithic stationary phases used in CEC measurements were synthesized as described in Ref. [4] by copolymerization of a mixture of N-(1-adamantyl)acrylamide (solubilized via 1:1 complex formation with Me-β-CD), methacrylamide (MA) as hydrophilic monomer, piperazinediacrylamide (PDA) as crosslinker, ammonium sulfate (AS) as lyotropic salt, vinylsulfonic acid (VSA) as charge-bearing monomer, ammonium persulfate (APS) as free radical polymerization initiator, and *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED) as catalyst (accelerator) in the redox initiator system in aqueous phosphate buffer (100 mM, pH 7.0) inside a 100 μm I.D. fused-silica capillary, which had been pre-treated with 3-(trimethoxysilyl)propyl methacrylate (bind silane). For monoliths prepared under varied concentration of ammonium sulfate (addition of 8, 12, 32, or 42 mg AS to a fixed volume of solution), all synthesis parameters were kept constant with exception of the concentration of ammonium sulfate present in the polymerization mixture (Table 1 – Monoliths A, B, C, and D). For monoliths with varied molar fraction of vinylsulfonic acid, different volumes of vinylsulfonic acid (7.5, 15, 25, and 35 μL, 25%, w/v) were added to the polymerization mixture (Table 1 – Monoliths E, F, G, and H). For monoliths prepared with varied concentration of the redox initiator, the synthesis procedure was altered by variation of the mass of ammonium persulfate (10 μL of 3.5, 7, 10, and 15%, w/v) present in the polymerization mixture (Table 1 – Monoliths I, J, K, and L).