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Optimization of an innovative vinylimidazole-based monolithic stationary phase and its use for pressured capillary electrochromatography

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ABSTRACT

A novel polymer monolith based on the dicationic crosslinker 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide, the monomer 1-vinylimidazole and a ternary porogen mixture (1-propanol, decan-1-ol and water) was developed and optimized for capillary electrochromatography. This aim was accomplished by adjusting the composition of individual constituents in the polymerization mixture and monitored based on several relevant parameters (e.g. pore structure by scanning electron microscopy, generation of electroosmotic flow, or permeability of material). The ultimately selected composition yielded a monolithic phase which excellently resolved six methylxanthines (including caffeine, theobromine and theophylline) in 15 min. Key requirements concerning the utilized buffer were an acidic pH of 3 and the addition of 50% acetonitrile; additionally, a negative voltage (−25 kV) had to be applied during analyses. The proposed separation mechanism was mixed mode, i.e. the combination of electrostatic repulsion and hydrophobic interaction. Monolith fabrication as well as separation efficiency were found to be highly repeatable, the material was mechanically stable and useable for at least 150 injections. Thus the presented stationary phase is definitely a very promising option for CEC.

1. Introduction

Carrier-free electrokinetic separation techniques utilize the electroosmotic flow (EOF) to transport mobile phase and analytes instead of a pump. This driving force can be explained as “the flow of liquid in contact with a solid surface under the influence of a tangentially applied electric field” [1], and it is the basic principle of two separation methods, capillary electrophoresis (CE) and capillary electrochromatography (CEC). The latter, which is unique because it hyphenates chromatography (i.e. use of a stationary phase) with electrophoresis (i.e. EOF as driving force), has distinct (theoretical) advantages [2]. The number of possible formats and stationary phases seems endless [3], the EOF shows superior performance in view of the achievable plate count [4] and reduced band broadening [5], and a more even pore level velocity profile in comparison to its pressure driven counterpart [6]. As a consequence axial dispersion in porous beds is reduced and much higher separation efficiencies are possible.

Three CEC formats are differentiated; besides the rarely used open tubular column (OTC) variant [7], the capillaries are either packed with standard LC stationary phases (so called packed columns) or they contain monolithic material; the latter can be silica or polymer based [8]. Especially the last option is of great scientific interest because such materials are comparatively simple to prepare, they are extremely versatile in composition and thus chromatographic properties, and reported column efficiencies up to 270,000 plates/m are impressive [9]. Polymerization is carried out via a one-step molding process of a polymerization mixture containing monomer, crosslinker and initiator, in the presence of suitable porogenic solvents. The composition of this mixture is a key factor that highly influences the properties (physical, mechanical and chemical) of the pursued material [10–12]. To mention only a few of the many related publications, Yu et al. studied the impact of porogen composition on the porous properties of methacrylate based monoliths [13], Wouters and coworkers determined morphology changes of polystyrene type phases when different domain sizes are used [14], or Hoegger and Freitag investigated the influ-

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ence of reagent mixture composition on the CEC separation of aromatic compounds [15].

It is our continued interest to promote the use of CEC for natural products analysis [16–18], and in this manuscript we report on the development / optimization of a novel dicationic monolith material, which permitted the excellent separation of six, structurally similar methylxanthines including relevant natural products like caffeine or theophylline, by CEC.

2. Material and methods

2.1. Chemicals and standards

Standards (caffeine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine and 7-methylxanthine; purity always ≥ 98%) as well as chemicals for synthesis of the crosslinker were from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile and all chemicals (citric acid monohydrate, ammonium acetate and di-potassium hydrogenphosphate) required for CEC analysis had p.A. quality and were purchased from Merck Eurolab (Vienna, Austria). Ultra-pure water was prepared using a Sartorius Arium purification system (Göttingen, Germany).

Chemicals for the preparation of the monoliths (1-propanol, 1,4-butanediol, decan-1-ol, 3-(methoxysilyl) propyl methacrylate, 2,2-diphenyl-1-picryl-hydrazyl, dimethylformamide, 1-vinylimidazole, 1,6-dibromohexane and 2,2-dimethoxy-2-phenylacetophenone) were also purchased from Sigma Aldrich. The crosslinker 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide was synthetized as mentioned below. Relevant structures are shown in Figure S1, supplementary information.

2.2. Synthesis of 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide

The synthesis was carried out according to literature [19] with some adjustments for a larger batch size: 38.1 g (150.0 mM) of 1,6-dibromohexane (96%) and 28.3 g (300.0 mM) of 1-vinylimidazole (99%) were thoroughly mixed at room temperature and left to stand for 3 h. Due to the exothermic nature of the reaction, the mixture was cooled in an ice bath during this period, then allowed to return to room temperature and react for another 15 h. Afterwards the mixture was treated with a heat gun in order to maximize product formation, and the resulting block dissolved in 100 mL methanol by sonication. Precipitation of the product was accomplished by the addition of 350 mL diethyl ether, followed by filtration and washing the product with 100 mL of diethyl ether; the product was dried for 24 h under vacuum, resulting in 55.6 g (128.6 mM, 86% of theoretical yield) of pure 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide; IR and NMR spectra are provided as supplementary data (S2).

2.3. Preparation of monolith

Before synthesis of the monolith, the fused silica capillary (100 μm I.D. with UV transparent Teflon coating; Polymicro Technologies, Phoenix, USA) was silanized following an already described protocol [20]. In brief, the capillary was first filled with 0.1 M NaOH, sealed with silicon stoppers and heated to 100 °C for 1 h. Then it was rinsed with water, 0.1 M HCl and acetone for ten minutes each, and dried in a stream of nitrogen. The silanization mixture, consisting of diphenyl-1-picryl-hydrazyl and 3-(trimethoxysilyl) propyl methacrylate dissolved in dimethylformamide, was filled in the capillary; it was closed again and heated to 120 °C for 6 h. Finally, the capillary was rinsed with dimethylformamide, acetone and dichloromethane for one hour each, and dried again using a nitrogen stream.

The polymerization mixture for preparing the finally selected monolith (capillary 4) consisted of 16% (w/w) 1-vinylimidazole, 16% (w/w) 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide, 36% (w/w) 1-propanol, 8% (w/w) decan-1-ol and 24% (w/w) ultrapure water. To this mixture 2.4% (w/w, with respect to the total mixture) of the initiator 2,2-dimethoxy-2-phenylacetophenone was added, all constituents were thoroughly vortexed in a glass vial and degassed by sonication for 10 min. Afterwards, the mixture was filled into the silanized capillary with a syringe until the desired effective length was reached; the rest of the capillary was left empty. Both ends of the capillary were sealed with silicon septa, then the capillary was exposed to UV-light (254 nm, 8 W) for 30 min and flushed using a HPLC pump (200 bar) for the duration of 3 h with acetonitrile in order to remove porogens and unreacted monomers. Before actual use the capillaries were filled with the background electrolyte. The IR spectrum (Alpha FT-IR; Bruker, Bremen, Germany) of the monolith and its interpretation are presented in Figure S3.

2.4. SEM of monolith

For recording scanning electron microscopy (SEM) images of the different monoliths a Jeol JSM-6010LV instrument (Tokyo, Japan) was used; the applied acceleration voltage was 10 kV. Prior to measurement the capillaries were dried in an oven at 100 °C for 48 h, and pieces of 2–3 mm length cut with a razor blade. They were placed in a double sided carbon type sample holder and sputtered with gold prior to analysis.

2.5. Analytical method

All CEC experiments were performed on an HP 3D capillary electrophoresis system (Agilent, Waldbronn, Germany) equipped with air-cooled column cartridge, autosampler and diode array detector. The capillary columns installed always had a total length of 35 cm, with an effective length of 27.5 cm; UV-absorption was recorded on-column at 280 nm. The optimum mobile phase consisted of a 1:1 mixture of an aqueous 20 mM citric acid monohydrate solution, adjusted to pH 3 with an aqueous 20 mM di-potassium hydrogen phosphate solution (A) and acetonitrile (B). All buffers were membrane filtered (0.45 μm ProFill cellulose syringe filters, Bruckner, Linz, Austria) prior to use; they had to be changed after three injections to guarantee repeatability of the results. The temperature of the capillary cassette was maintained at 20 °C, and a voltage of −25 kV applied during analysis. Inlet as well as outlet vial were pressurized at 6 bar (N_2) to prevent air-bubble formation. For sample injection the high-flush mode was selected, i.e. for 3 s 6 bar (on the sample vial) and −20 kV were applied simultaneously.

2.6. Determination of column efficiency

The concept of Gu and Shamsi was followed in order to determine relevant parameters in this respect [21]. Under optimized separation conditions the retention factor, reflecting affinity to the stationary phase and the contribution of EOF, was calculated using the formula provided in this publication. Other indicators like resolution, peak symmetry or plate numbers were determined by the operating software (Chemstation from Agilent). Plate numbers are reported in plates/m.

3. Results and discussion

3.1. Composition of polymerization mixture

In this study the crosslinker 3,3′-(hexane-1,6-diyl)bis(1-vinylimidazolium) bromide was utilized for the preparation of a