Short communication

Monolithic columns based on a poly(styrene-divinylbenzene-methacrylic acid) copolymer for capillary liquid chromatography of small organic molecules

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ABSTRACT

A very simple and readily performed method is described for the preparation of poly(styrene-divinylbenzene-methacrylic acid) monolithic columns for capillary liquid chromatography. The effect of the methacrylic acid content on the morphological and chromatographic properties has been investigated. Methacrylic acid is shown to be essential for isocratic separations of small organic analytes by capillary liquid chromatography. Column efficiencies of about 28,000 theoretical plates/m have been obtained for all the test compounds. The batch-to-batch and run-to-run repeatability of the retention times is better than 1.5%.

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1. Introduction

"Single-piece" separation media, called monoliths, have become some of the most interesting topics in the search for new chromatographic stationary phases. Generally, there are two major monolith types – inorganic silica-based and organic polymer-based ones. Among organic polymers, monoliths based on polystyrenes, polymethacrylate esters and polyacrylamides have become most popular. This short communication deals with only the polystyrene-based monoliths. Up to now, a huge number of papers report on successful applications of polystyrene-based monoliths to separations of larger molecules, such as peptides, proteins and nucleic acids [1–5]. However, the number of studies applying these monoliths to separation of small molecules is limited.

Polystyrene-based monoliths have proven to be excellent stationary phases because of their high chemical stability across a wide pH range. In early 1990s, Wang et al. [6] described gradient HPLC separations of small aromatic molecules on polystyrene-based monolithic columns. However, the retention times were around 30 min and the column efficiency was about 13,000 theoretical plates/m. Further experiments have improved the separation performance of styrene-divinylbenzene copolymer for small organic molecules by postpolymerization modifications, such as Friedel–Crafts alkylation, a grafting procedure [7] or hyper-crosslinking [8,9]. Besides, novel monolithic separation media with excellent physical properties using 1,2-bis(p-vinylphenyl)ethane as the crosslinker were introduced by Bonn's group [10,11].

The current efforts are focused on the preparation of universal columns suitable for separations in both capillary liquid chromatography (CLC) and capillary electrochromatography (CEC). Therefore, the incorporation of methacrylic acid (MAA) in the polymerization mixture for monolith preparation was considered to be a possible way how to alter interactions between small analytes and the stationary phase [12,13] and how to generate electroosmotic flow (EOF) for the purpose of CEC [13–15]. The present work is the first comparison of the monolith preparations with and without MAA for application in CLC. The influence of MAA addition to the polymerization mixture on the morphology of monoliths, separation performance and selectivity of CLC columns was studied. An application of the newly designed polymeric separation media to separation of a set of typical low-molecular-weight organic compounds was also demonstrated.

2. Experimental

2.1. Materials

Polyimide-coated fused silica capillaries of 320 μm I.D. were obtained from Supelco (Bellefonte, USA). Styrene (for synthesis),

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divinylbenzene (for synthesis), toluene (extra pure), methacrylic acid (for synthesis) and α,α′-azobisisobutyronitrile (AIBN) (98%) were purchased from Merck (Darmstadt, Germany). Isooctane (p.a.) was supplied by Lach-Ner (Neratovice, Czech Republic). 3-(Trimethoxysilyl)-propyl methacrylate (99%) and ethylbenzene (p.a.) were provided by Fluka (Buchs, Switzerland). Sodium hydroxide (p.a.) was purchased from Lachema (Brno, Czech Republic). Thiourea (99%), phenol (99%), aniline (98%), benzene (99%), propylbenzene (99%) and butylbenzene (98%) were provided by Sigma–Aldrich (St. Louis, USA). Acetonitrile and methanol (gradient-grade for liquid chromatography) were supplied by Merck (Darmstadt, Germany). Deionized water used in this work was prepared with a Milli-Q water purification system (Millipore, USA). All the samples were dissolved in the mobile phases in concentrations ranging from 0.2 to 0.5 mg/ml.

2.2. Instrumentation

A UL 400 Memmert oven (Schwabach, Germany) was employed for thermostatting the capillaries during pretreatment. An ISCO syringe pump model 100 DM (Lincoln, NE, USA), a Valco International injection valve with a 100 nl internal loop (Schenkon, Switzerland) and a LINEAR UVIS-205 absorbance detector (San Jose, CA, USA) were used for all the chromatographic measurements. The inlet of monolithic column was installed in the injection valve using a PEEK sleeve and finger-tight fitting. The column outlet was connected to a 100 μm I.D. detection capillary by a piece of PTFE tubing. A detection window was burnt 80 mm from the outlet of monolith column. All the data were collected and evaluated using a Clarity software by DataApex (Prague, Czech Republic). The porous properties of the monoliths prepared were determined by mercury porosimeter Pascal 440 and Pascal 140 (Thermo Finigan, Rodano, Italy). The specific surface area was measured by Micromeritics Gemini VII (Micromeritics Instrument Corporation, Norcross, USA).

2.3. Preparation of monolithic columns

The preparation procedure for poly(styrene-divinylbenzene-methacrylic acid) monolithic capillary columns for CEC was developed and published by Jin et al. [14]. This procedure was modified in the present work in several aspects, to make it suitable for the preparation of styrene-based capillary monolithic columns for CLC in 320 μm I.D. fused silica capillaries.

Prior to the polymerization, the capillary was pretreated by the procedure of Courtois et al. [16]. After the etching and silanization, the preparation of monolithic columns followed this procedure: the polymerization mixture consisted of 50 μl of styrene, 100 μl of divinylbenzene and 50 μl of methacrylic acid in the monomeric form, 300 μl of toluene and 300 μl of isooctane as the porogen mixture and AIBN as the initiator (1%, w/w, with respect to the monomers). The mixture was sonicated for 15 min until it became homogeneous and then degassed with a stream of nitrogen for 10 min. The pretreated capillary was filled with the polymerization mixture, both its ends were sealed with rubber stoppers and the capillary was placed in a water bath of 60 °C for 6 h. The prepared columns were cut at both ends and flushed with methanol using the syringe pump to remove unreacted monomers and porogens. Prior to measurements, the columns were shortened to the desired length and washed with the appropriate mobile phase. A set of eight compounds differing in their hydrophobicity were used to investigate separation performance. The column permeability K was calculated according to equation:

$$K = \frac{F_c \eta L}{\Delta p \pi R_c^2} \qquad (1)$$

where F_c is the mobile phase flow rate, η the dynamic viscosity of eluent, L the column length, Δp the pressure drop across the column and R_c is the column inner radius. The value of the dynamic viscosity for mobile phase acetonitrile/water (65/35, v/v), $\eta = 0.65 \times 10^{-3}$ Pa s, was adopted from [17].

Samples for characterization of the polymer morphology were prepared in polypropylene vials in the same way as the columns, rinsed with mixture of toluene and isooctane at a volume ratio 1:1 to remove non-polymerized residues and dried under vacuum overnight. The porous properties of the monoliths were investigated by mercury intrusion porosimetry. The specific surface area was calculated from nitrogen adsorption/desorption isotherms.

3. Results and discussion

3.1. Influence of MAA addition on the structure and separation selectivity of the monolith

According to the literature [14,15], both large and small organic molecules can be separated using CEC on polystyrene-based monoliths. In order to generate EOF as the propelling force in CEC, a charge-bearing monomer, such as MAA, has been used [14,18,19]. Incorporation of this polar compound can highly affect the properties of the monolithic support. Two batches of the stationary phases, with and without MAA, were prepared (see Section 2.3). For columns without MAA, the proportion of styrene in the polymerization mixture was increased by the amount equal to that of MAA missing.

The morphological characterization of the both monolithic stationary phases revealed a significant difference between their structures. A globular structure that is required for monolithic stationary phases used in capillary liquid chromatography was characteristic for the monolith containing methacrylic acid. Mercury porosimetry found a total pore volume of about 3.85 ml/g, and the pore size distribution curve (not shown) was characterized by two maxima. In the range of the large through-pores, which allow flow of a mobile phase through the monolith, the most frequent pore radius was 5.8 μm. These pores markedly contribute to the permeability of the monolith. The backpressure did not exceed 40 bar and the column permeability was 2.5×10^{-14} m². The second local maximum of the pore size distribution curve was found at 233 nm, and, even in the range of mesopores (pores smaller than 50 nm) 0.3 ml of pores per 1 g of polymer was found. Results of the BET measurements corresponded with these findings. The specific surface area 261 m²/g provides sufficient space for interactions of the monolith with mobile phase carrying analytes. On the other hand, the material prepared from the polymerization mixture without methacrylic acid represented a compact rigid polymer almost without any interconnected pores. The total pore volume found was only 0.54 ml/g. No micrometer-size pores allowing a flow through the monolith were observed. The specific surface area determined by the BET measurements was 0.1 m²/g.

Surprisingly, the influence of MAA on the selectivity of monolithic columns is usually not considered in the literature. Since the charge-bearing monomer is essential for electrochromatographic measurements, it is difficult to assess this effect of MAA added to the polymerization mixture by CEC measurements but CLC can readily be used. A mixture of acetonitrile/water 65/35 (v/v) was chosen as the mobile phase for the separation of a set of eight low-molecular-weight organic compounds. The test mixture consisted of phenol, aniline, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene and thiourea as the dead time marker. No separation of the test compounds was achieved using the columns polymerized without MAA (Fig. 1A). All the test analytes eluted in a single broad peak because the monolith shows a very low specific surface area as
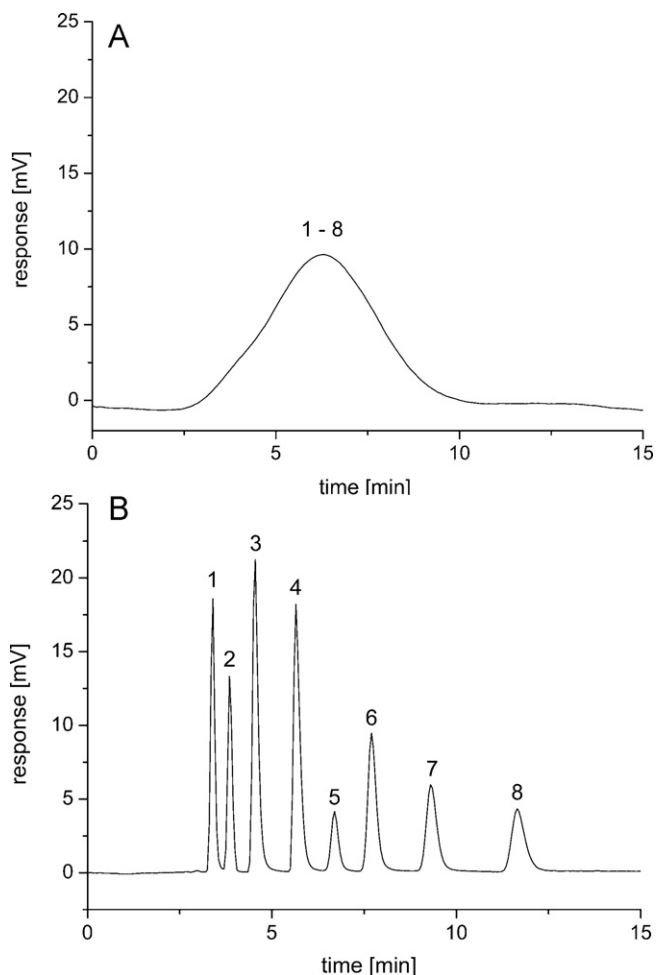
Fig. 1. A CLC separation of small organic molecules using poly(styrene-co-divinylbenzene) columns (A) without MAA and (B) with MAA. Conditions: mobile phase, acetonitrile/water (65/35, v/v); UV detection at 214 nm; flow rate, 4 μl/min; injection volume, 100 nl; effective column length, 170 mm. Peak identification: thiourea (1), phenol (2), aniline (3), benzene (4), toluene (5), ethylbenzene (6), propylbenzene (7) and butylbenzene (8). The concentrations of the analytes in the mixture were: 0.2 mg/ml for aniline and 0.5 mg/ml for all other solutes.

3.2. Repeatability of monolith preparation and column performance

Batch-to-batch and run-to-run repeatability were investigated in terms of the retention times (t_R) and the height equivalents to a theoretical plate (HETP) for the test mixture analytes. Chromatographic properties of three independently synthesised poly(styrene-divinylbenzene-methacrylic acid) columns in three independent batches were compared using the same test mixture and identical chromatographic conditions. The RSD values ranged from 1.0 to 1.5% for the retention times. Low variations of the separation efficiencies (RSD = 1.6–5.5% for HETP) were found to be more than satisfactory. Run-to-run repeatability of retention performance of the poly(styrene-divinylbenzene-methacrylic acid) monolith was assessed by repeated separations of the test mixture under constant chromatographic conditions. Fifteen runs were performed on the same column to evaluate the repeatability of the retention behavior. The RSD values for the retention times were found to be between 0.3 and 0.5%, which confirmed the high run-to-run repeatability of the prepared monolithic columns. These results clearly indicate that the presented process of preparation of the monolithic columns is highly reproducible.

4. Conclusions

Although most publications consider methacrylic acid as only a charge-bearing agent for generation of EOF in CEC, this work reveals the essential influence of this substance on the morphology of the monolith and its properties as a separation medium. The poly(styrene-co-divinylbenzene-co-methacrylic acid) monolith has also been shown to be a powerful stationary phase for CLC. To the best of our knowledge, the effect of the MAA presence in the monolith on the separation performance has not yet been studied. Efficient separations of low-molecular-weight organic compounds have been completed within less than 14 min. Comprehensive batch-to-batch and run-to-run repeatability studies have revealed high reliability of the separation media prepared. The preparation procedure is universal for both CLC and CEC methods and for capillaries with various inner diameters. However, the results obtained rise questions which will be studied and discussed in detail in our forthcoming work. These are, in particular, mechanical stability of the monoliths and ways to further improvement in their efficiency.

mentioned above. We suppose that in case of monolith prepared without methacrylic acid, the mobile phase did not flow through the material of monolith but through the disruptions which arose between monolith and the inner wall of capillary. In contrast, all the columns prepared with the additions of MAA provided baseline separation of all the eight test analytes with a column efficiency of about 28,000 theoretical plates/m (Fig. 1B).

These results clearly demonstrate the significant influence of MAA on the structure of the monolith and consequently the chromatographic performance. From the elution order of the test compounds, it can be deduced that the separation mechanism is similar to that in common reversed-phase systems – the more hydrophobic analyte, the higher retention. Based on this fact, we assume that electrostatic interactions possibly introduced by MAA carboxyl groups do not markedly contribute to separation mechanism. The same selectivity for separation of alkylbenzenes was observed by Jin et al. in CEC mode for separation of alkylbenzenes [14]. Based on the Jin's and our results, we can generalize that the procedure is robust and can be used for the preparation of monolithic columns in fused silica capillaries of various internal diameters without a need for changes in the composition of the polymerization mixture (320 μm I.D. for CLC and 100 μm I.D. for CEC).

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