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Short Communication

Effect of ion adsorption on CEC separation of small molecules using hypercrosslinked porous polymer monolithic capillary columns

Both poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) and poly(4-methylstyrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) monolithic columns have been hypercrosslinked and for the first time used to achieve capillary electrochromatographic separations. Although these columns do not contain ionizable functionalities, electroosmotic flow was observed due to adsorption of ions from a buffer solution contained in the mobile phase on the surface of the hydrophobic polymer. An increase of more than one order of magnitude was observed with the use of both monolithic polymers. The hypercrosslinking reaction creates a large surface area thus enabling adsorption of a much larger number of ions. Alkylbenzenes were successfully separated using the hypercrosslinked monolithic columns.

Keywords: Capillary electrochromatography / Hypercrosslinking / Ion adsorption / Polymer monolith
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1 Introduction

Capillary electrochromatography (CEC) is a high-performance liquid phase separation technique carried out in packed capillary columns utilizing a flow driven by electroosmosis which results in significantly enhanced column efficiency compared with HPLC [1]. CEC features high selectivity typical of liquid chromatography based on specific interactions and high efficiency of capillary electrophoresis resulting from plug flow patterns typical of movement of solutes using voltage. Current CEC column designs may be characterized into three categories: (i) packed, (ii) open tubular, and (iii) monolithic formats [2].

Packed columns were widely used for CEC separation in the early days. However, tedious packing procedures and difficulty in reproducing fabrication of retaining frits were obstacles that led to the search for alternative approaches such as use of open tubular columns [3–5]. These columns, although easy to prepare and use, provide a small surface area resulting in a small phase ratio, limited sample loading capacity and retention, and slow electroosmotic flow (EOF). Monolithic CEC columns were introduced in the mid 1990s [6–8]. Organic polymer-based monolithic columns were used initially and rapidly attracted significant attention due to their ease of preparation, facile modification, and excellent stability over a wide pH range [1, 9–16]. Silica-based monolithic columns have also been demonstrated [2, 17–19].

EOF is the driving force which generates the flow through the column and is indispensable for the separation in CEC. The generation of EOF in typical CEC columns is associated with the formation of an electric double layer which results from the accumulation of counterions from the electrolyte in close vicinity to charges attached to the solid surface of the stationary phase. In columns containing silica-based packings, EOF is ascribed to the ionization of native silanol groups. In contrast, in polymer-based monolithic CEC columns monomers containing ionizable functionalities, such as acrylic and methacrylic acids, 2-acrylamido-2-methyl-1-propanesulfonic acid, and 3-methacryloyloxypropyl(trimethyl)ammonium chloride are typically copolymerized to obtain EOF.

EOF was also generated in monolithic columns that were void of fixed charges introduced by copolymerization. In one implementation, surfactants were added to the mobile phase and their dynamic adsorption enabled formation of the double layer and enhancement in EOF [20]. Several reports have also indicated that EOF in neutral methacrylate-based monoliths containing electron-rich ester groups capable of adsorbing cations monoliths results from adsorption of ions from the buffer solution in the mobile phase [20–28]. Most of these

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Abbreviations: poly(MST-VBC-DVB), poly(4-methylstyrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene); **poly(ST-VBC-DVB)**, poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene)

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Colour Online: See the article online to view Fig. 2 in colour.

attempts are summarized in an excellent review article by Karenga and El Rassi [29]. A much smaller number of studies have focused on generation of EOF in neutral poly(styrene-*co*-divinylbenzene) monoliths featuring plain hydrocarbon chemistry [11, 30, 31]. For example, Horvath's group claimed use of single phosphate buffer as the mobile phase and thiourea as a marker and found flow velocities up to about 2 mm/s [11]. Szumski et al. used buffers comprising organic components and acetonitrile (ACN) [30]. Based on experimental results and molecular modeling, Szumski et al. emphasize the role of water adsorbed at the aromatic rings of the polymer that then holds the adsorbed ionizable molecules of the buffer. Finally, Lav et al. prepared poly(styrene-*co*-divinylbenzene) monolith using ε-caprolactone oligomers as a porogen, and use it for the separation of alkylbenzenes in phosphate buffer/ACN mobile phase [31]. Although the effects of variables such as pH, voltage, composition of the mobile phase, and analytes was demonstrated in these reports, none of them considered the effect of the surface area of the monolith. Based on review of the preparation conditions and SEM micrographs, it is likely that all the monoliths mentioned above exhibited only a small surface area.

For the first time, we recently demonstrated the preparation of hypercrosslinked porous poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) [poly(ST-VBC-DVB)] monoliths exhibiting a large surface area and their use in capillary columns to facilitate the fast and efficient separation of small molecules using reversed phase liquid chromatography as well as for rapid size-exclusion chromatography [32, 33]. In this report we describe the preparation of monolithic poly(ST-VBC-DVB) and poly(4-methylstyrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) [poly(MST-VBC-DVB)] columns, their hypercrosslinking, and the use of these materials to achieve separation in CEC.

2 Materials and methods

2.1 Materials

Styrene (99%), vinylbenzyl chloride (mixture of 3- and 4-isomers, 97%), divinylbenzene (80%, technical grade), 4-methylstyrene (96%), 2,2'-azobisisobutyronitrile (98%), 1,2-dichloroethane, 1-dodecanol, thiourea, benzene, ethylbenzene, and butylbenzene were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Propylbenzene was purchased from Matheson Coleman & Bell (Los Angeles, CA, USA) and *n*-pentylbenzene was purchased from Alfa Aesar (Heysham, UK). ACN (HPLC grade) and toluene were obtained from EMD Chemicals (Gibbstown, NJ, USA). Ferric chloride was purchased from Fisher (New Jersey, NJ, USA). All monomers, i.e. styrene, 4-methylstyrene, vinylbenzyl chloride, and divinylbenzene shown in Fig. 1 were purified by passage through a bed of basic alumina to remove the inhibitors. Polyimide-coated 100 μm i.d. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

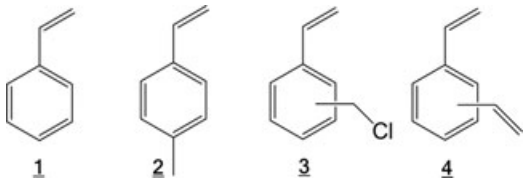

Figure 1. Chemical structures of the monomers used for the preparation of monoliths.

2.2 Preparation of generic monolithic capillary columns

The fused silica capillaries were rinsed with acetone, water, 200 mmol/L sodium hydroxide, water, 200 mmol/L hydrochloric acid, water, and ethanol. Then, a 20% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in ethanol with an apparent pH value of 5 adjusted using acetic acid, was pumped through the capillary for 1 h using a syringe pump. After this vinylization procedure, the capillaries were rinsed with acetone, and dried with a stream of nitrogen.

Generic poly(ST-VBC-DVB) monolithic columns were prepared according to the procedure reported by Urban et al. [32, 32]. The 40-cm-long vinylized capillary was filled by syringe with the deaerated polymerization mixture consisting of styrene (21 wt%), vinylbenzyl chloride (7 wt%), divinylbenzene (12 wt%), toluene (19 wt%), 1-dodecanol (41 wt%), and 2,2'-azobisisobutyronitrile (1% w/w with respect to monomers) to a length of 12 cm. Both ends of the capillary were sealed with rubber septa and the capillary was submerged into a water bath thermostated at 70°C for 20 h. Then the column was washed with ACN to remove unreacted components of the polymerization mixture that are mostly porogens. The preparation procedure of generic poly(MST-VBC-DVB) was similar to the procedure for poly(ST-VBC-DVB) except that the composition of polymerization mixture was 4-methylstyrene (21 wt%), vinylbenzyl chloride (7 wt%), divinylbenzene (12 wt%), toluene (13 wt%), 1-dodecanol (47 wt%), and 2,2'-azobisisobutyronitrile (1% w/w with respect to monomers).

2.3 Hypercrosslinking

The generic monolithic columns were flushed with 1,2-dichloroethane at a flow rate of 0.25 μL/min for 2 h, followed by pumping a filtered saturated solution of ferric chloride in 1,2-dichloroethane at a flow rate of 0.25 μL/min for 2 h. The columns were held in an ice bath for 1h and then placed in a water bath kept at 90°C for 2 h. The hypercrosslinked columns were washed thoroughly with ACN.

2.4 CEC

CEC separations were carried out using an Agilent^{3D} CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector and an external pressurization

system. A detection window was created at the end of the monolith by a MicroSolv window maker (MicroSolv Technology Corp., Eatontown, NJ, USA), and the column was cut to a total length of 33.5 cm with an effective length of 8.5 cm. An equal nitrogen pressure of 0.5 MPa was applied at both ends of the capillary column. The mobile phase was prepared from a 20 mmol/L phosphate buffer solution (pH 7.0) in a mixture of water and ACN. The sample solutions were injected electrokinetically using a voltage of 5 kV for 3 s, and the separations were performed at a voltage of 20 kV while the cassette compartment temperature was adjusted to 25°C. Thiourea was used as the EOF marker.

3 Results and discussion

Our original studies related to hypercrosslinked monoliths were carried out using poly(ST-VBC-DVB). The hydrophobic aromatic nature of this chemistry is not very reactive and, based on the lack of other functionalities, the hypercrosslinked capillary columns could be only used for the separations in reversed phase. In contrast, the methyl group of 4-methylstyrene, a monomer that we also used in the present study, can undergo a variety of chemical reactions that afford different functional groups. Due to similarity in structure of both styrene and 4-methylstyrene, their reactivity ratios are also similar and conditions optimized for the preparation of poly(ST-VBC-DVB) monoliths can be used for the preparation of their poly(MST-VBC-DVB) counterparts. Both types of monoliths were then successfully hypercrosslinked. Previous experiments we carried out with a bulk porous poly(ST-VBC-DVB) prepared in a glass vial indicated that the surface area of 27.3 found for the precursor monolith increased to 340 m^2/g after hypercrosslinking [33]. However, these values just illustrate the enormous increase but may not apply to the monoliths in capillary due to the confinement effects we observed previously [34]. Unfortunately, the amount of polymer in the capillary is too small for a direct measurement of the surface area.

3.1 EOF in the monolithic columns

If previous observations of EOF in CEC columns that did not contain any attached ionizable functionalities, and its attribution to the adsorption of ions were correct, an increase in the surface area of the monolithic stationary phase should provide more space for adsorption of a larger number of ions thus leading to an increase in the magnitude of EOF.

Indeed, Table 1 clearly demonstrates that EOF increased significantly in hypercrosslinked monolithic columns compared to the parent monoliths. Specifically, a 34-fold increase was observed for hypercrosslinked poly(ST-VBC-DVB) monoliths and a 21-fold increase was achieved for hypercrosslinked poly(MST-VBC-DVB). These results confirm the positive effect of the enlarged surface area on the adsorption of ions and the generation of EOF. It is worth noting that EOF remains stable over a long period of time as demonstrated with numerous separations using the same column. This finding indicates that the number of adsorbed ions remain constant.

Table 1. EOF mobility of generic and hypercrosslinked monolithic columns

Columns[a]	Electroosmotic mobility [b] μ_{EOF}, $m^2/(Vs)$
Poly(ST-VBC-DVB)	
Generic	6.78×10^{-10}
Hypercrosslinked	2.36×10^{-8}
Poly(MST-VBC-DVB)	
Generic	1.24×10^{-9}
Hypercrosslinked	2.57×10^{-8}

a)Column dimensions: total length 33.5 cm, length to detector 8.5 cm, length of the monolith 8.5 cm, 100 μm id.
b)Marker, thiourea; mobile phase, 5 mmol/L phosphate buffer solution (pH 7.0), 60% acetonitrile; voltage, 20 kV; temperature, 25°C.

The direction of the EOF in all the monoliths in this study is from anode to cathode. This cathodal EOF suggests that under the conditions used in our experiments, negatively charged phosphate anions are adsorbed. Mechanism of this interaction has not been elucidated in detail yet. However, both the parent monolith and its hypercrosslinked counterpart contain significant percentage of chlorine atoms that may play a role in the adsorption.

3.2 Separation using hypercrosslinked monolithic columns

Since the hypercrosslinked monoliths generate strong EOF, these columns were used in CEC to demonstrate their ability to separate small molecules. Figure 2 shows the separation of six alkylbenzenes using both poly(ST-VBC-DVB) and poly(MST-VBC-DVB) hypercrosslinked monolithic columns that was achieved in less than 8 min in a mobile phase composed of phosphate buffer and ACN.

4 Concluding remarks

This is the first demonstration of hypercrosslinked porous poly(ST-VBC-DVB) and poly(MST-VBC-DVB) monolithic columns used for CEC separation of small molecules. Despite the absence of fixed charges on the surface of the stationary phase, strong EOF was generated due to the increased number of anions that are adsorbed onto the significantly increased surface area. Our current efforts focus on the preparation of hypercrosslinked monoliths with properties optimized for highly efficient CEC separations. We are also exploring new routes to selected functionalities. For example, oxidation of the methyl group in the polymerized 4-methylstyrene units results in carboxylic acid groups. These monoliths can be used directly or serve as an intermediate for the

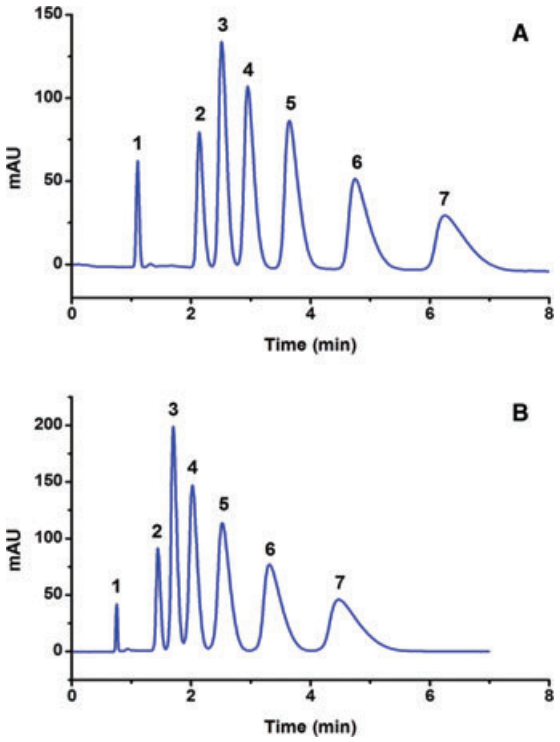

Figure 2. Electrochromatograms of thiourea and alkylbenzenes using (A) poly(ST-VBC-DVB) and (B) poly(MST-VBC-DVB) hypercrosslinked monolithic columns. (Conditions: column: total length 33.5 cm, length to detector 8.5 cm, length of the monolith 8.5 cm, 100 μm i.d.; mobile phase: 20% 5 mmol/L phosphate (pH 7.0), 80% acetonitrile; voltage: 20 kV; temperature: 25°C; electrokinetic injection: 5 kV for 3 s; detection: 210 nm). Peaks: 1, thiourea; 2, benzene; 3, toluene; 4, ethylbenzene; 5, propylbenzene; 6, butylbenzene; 7, pentylbenzene.

attachment of more complex moieties such as chiral ligands for enantioseparations.

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