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Efficient separation of small molecules using a large surface area hypercrosslinked monolithic polymer capillary column

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Abstract

Monolithic poly(styrene-co-vinylbenzyl chloride-co-divinylbenzene) precursor capillary columns have been prepared and then hypercrosslinked to afford monolith containing an array of small pores. This monolithic column exhibited a surface area of 663 m²/g, or more than one order of magnitude larger than measured for the precursor column. Because it contains mesopores, this monolithic column affords good separation of uracil and alkylbenzenes in isocratic mobile phase mode and also proved useful for separations in size exclusion mode. A column efficiency as high as 73 000 plates/m was determined for uracil. In contrast, the presence of mesopores in this hypercrosslinked monolithic column had a detrimental effect on the separation of proteins.

Keywords

Porous polymer monolith; Surface area; Styrene-chloromethylstyrene-divinylbenzene; Reversed phase chromatography; Size exclusion chromatography; Small molecules

The introduction of monolithic chromatographic stationary phases in the 1990s^{1–4} marked a major advance in column technology.⁵ Current monolithic LC columns can be divided into two major categories, i.e. those prepared from silica and those from organic polymers. Typical silica-based monoliths include a significant volume of mesopores, which contribute most to their high surface areas reaching values as high as 300 m²/g.⁶ These monoliths functionalized with C18 chemistry are well suited for the rapid separations of small molecules but did not perform well in the separations of large molecules. In contrast, porous polymer monoliths exhibit much smaller surface areas reaching only a few tens of m²/g since they lack the mesopores.⁷ The absence of small pores makes them ideal for the fast separations of large molecules such as proteins, nucleic acids, and even synthetic polymers. Very early on,⁸ we ascertained that 8 mm I.D. columns of monolithic poly(styrene-co-divinylbenzene) having a surface area of 23 m²/g were not suitable for the efficient separation of small molecules such as alkylbenzenes. Several methacrylate-based monolithic capillary columns developed later only afforded 35,000–50,000 plates/m for the same analyte^{9–13} as a result of the lack of small pores. Numerous approaches were later explored to improve the separation performance of polymer-based monoliths for small molecules. These have included the copolymerization of dimethacrylates differing in the length and branching of the fragment connecting the polymerizable units;¹⁴ the termination of the polymerization reaction at an early stage^{15;16} to achieve large surface areas; and the use of high polymerization temperatures.^{17;18} However, it has always proven difficult to prepare polymer monoliths possessing both large through pores

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and a multiplicity of small pores in a single step and alternative approaches needed to be developed.

Hypercrosslinking, pioneered by Davankov several decades ago,^{19–22} enables the preparation of large surface area materials from pre-formed polymer precursors. The original implementation used linear polystyrene, which was crosslinked via Friedel-Crafts alkylation to afford materials containing mostly small pores.²³ Davankov's approach was later extended to crosslinked porous poly(styrene-divinylbenzene) particles and led to products containing both the original pores and an extensive network of additional micro- and meso-pores generated during hypercrosslinking.^{20;24–26} This letter demonstrates for the first time the use of in situ hypercrosslinking for the preparation of porous polymer monoliths with a large surface area and their subsequent use in capillary columns for the fast and efficient separation of small molecules as well as for rapid size exclusion chromatography.

EXPERIMENTAL SECTION

Preparation of monolithic capillary columns

Standard monoliths were prepared in capillaries *via* in-situ polymerization of a mixture comprising styrene (12wt.%), vinylbenzyl chloride (12 wt.%), and divinylbenzene (16 wt.%), with toluene (18 wt.%) and 1-dodecanol (42 wt.%) as porogens, and azobisisobutyronitrile (1% w/w with respect to monomers) as the initiator. After purging with nitrogen the mixture was used to fill vinylized capillaries. Both ends of the capillary were sealed and the capillary was placed in a thermostated water bath. Following polymerization at 70 °C for 20 h, both ends of the capillary were cut and the monolithic column was washed with acetonitrile.

Hypercrosslinking

The monolithic columns were flushed with 1,2-dichloroethane (DCE) at a flow rate of 0.25 $\mu\text{L}/\text{min}$ for 2 h. The filtered solution of 1 g of FeCl_3 in 20 mL of DCE was pumped through the columns at a flow rate of 0.25 $\mu\text{L}/\text{min}$ for 2 h and the columns were held in an ice bath for 2 h. The hypercrosslinking reaction was then carried out at 80 °C in water bath for 24 h followed by washing with water.

RESULTS AND DISCUSSION

Preparation and characterization of hypercrosslinked monoliths

The typical porous monolithic structure consisting of interconnected microglobules results from phase separation during polymerization of a mixture of monomers and porogens. The less than ideal reactivity ratios for monomers such as styrene, chloromethylstyrene, and divinylbenzene lead to polymer microglobules amenable to hypercrosslinking. The divinyl monomer polymerizes faster and the remaining monomer mixture becomes significantly richer in the monovinyl monomers as the polymerization reaction nears completion. This mixture then affords only slightly crosslinked chains attached to the surface of highly crosslinked microglobular scaffolds. When the pores are filled with a thermodynamically good solvent such as DCE, this surface polymer layer is solvated. The presence of such surface layers in crosslinked polymers was suggested long ago by Jerabek utilizing inverse size exclusion chromatography.²⁷ Once solvated, the layer containing chloromethyl groups can be crosslinked via Friedel-Crafts alkylation. During this process, the polymer chains become fixed in their solvated state thus forming small pores that persist even after the solvent is removed leading to a significant increase in the surface area of the monolith. Overall, the extent of hypercrosslinking depends on the percentage of divinylbenzene in the monomer mixture and the ratio of functional vinylbenzyl chloride and inert styrene, which controls the distance between reactive chloromethyl sites and the extent of hypercrosslinking and pore size.

Using a monomer mixture with an equal percentage of vinylbenzyl chloride and styrene led to a precursor monolith with a specific surface area of only 29 m²/g calculated from nitrogen adsorption/desorption in the dry state. Following hypercrosslinking, the monolith displayed both large through-pores and mesopores with a surface area increased by more than an order of magnitude to 663 m²/g. This value is almost twice as large as that found for silica-based monoliths developed by Tanaka for the separation of small molecules.²⁸

The surface area is a good indication of the presence of mesopores as measured in the dry state. Another useful metric is the total porosity ε_T that can be readily determined using chromatographic measurements on a column solvated with the mobile phase from the equation $\varepsilon_T = 100V_M/V_C$ where V_M is the elution volume of uracil in 80% aqueous acetonitrile, and V_C is the volume of an empty capillary column of the same length. After correction for the extra column volume, we found ε_T values of 61 and 59% for the precursor and hypercrosslinked monolith, respectively. As expected, hypercrosslinking does not lead to any appreciable change in porosity since the reaction does not add any significant mass to the original polymer, which would decrease the pore volume. While some of the chlorine atoms are eliminated, the new hydrocarbon crosslinks are included in the structure. The total pore volume determined using chromatographic measurement is similar to the volume percentage of porogens in the polymerization mixture which, in our case, is 63%.

Isocratic separation of small molecules

Experiments with uracil also enable the calculation of column efficiency with a value of 73 000 plates/m obtained at a flow velocity of 0.3 mm/s corresponding to the minimum of the van Deemter curve. The improvement in separation power resulting from hypercrosslinking of the monolithic stationary phase is best demonstrated in a comparison of isocratic separations of alkylbenzenes using the column before and after hypercrosslinking (Fig. 1). The precursor column performs poorly as all alkylbenzenes are less retained and eluted in a single broad peak. In contrast, baseline separation of all alkylbenzenes is obtained with the column after hypercrosslinking.

Gradient elution of proteins

From the very early days, a hallmark of porous polymer-based monolithic columns has been their excellent performance in the rapid separation of proteins.^{3;29;30} The reason for this success is the fast convective mass transport occurring in the large through-pores. Fig. 2 illustrates the effect of hypercrosslinking on the separation of five proteins achieved under identical conditions using both precursor and hypercrosslinked column. Clearly, the separation is better with the precursor column as expected from the negative effect of mesopores on the gradient elution of large molecules. The small size of the mesopores allows a protein such as albumin to probe the pores and the slow diffusional transport in and out of the entrance to the small pores reduces the separation performance and impairs resolution.³¹

Size exclusion chromatography

Providing the monolith with a multiplicity of small pores can make it suitable for separations involving the size-exclusion mode. Indeed, Fig. 3 shows the separation of toluene and two polystyrene standards with narrow polydispersity on the 17 cm long precursor column and its hypercrosslinked derivative using tetrahydrofuran as the mobile phase. The polystyrene standard with molecular weight 1.87×10^6 is excluded from the small pores and elutes from both columns at equal retention volumes. In contrast and despite the short column length, the hypercrosslinked column effects a much better separation of the polystyrene standard with a molecular weight of 1000 and toluene since it includes an array of smaller pores accessible to these analytes. It is worth noting that the separations shown in Fig. 3 only required 2.5 min of elution time.

CONCLUSIONS

While this report is only preliminary, it clearly demonstrates the capability of post-polymerization hypercrosslinking of the monolithic poly(styrene-*co*-vinylbenzenestyrene-*co*-divinylbenzene) stationary phase to afford columns that enables the highly efficient isocratic separation of small molecules in reversed phase and size exclusion modes. We are currently studying the effect of the composition of the polymerization mixture and refining the hypercrosslinking process to better control the formation of an optimal mesoporous structure and achieve a further increase in performance.

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References

1. Hjertén S, Liao JL, Zhang R. *J Chromatogr* 1989;473:273–75.
2. Tennikova TB, Svec F, Belenkii BG. *J Liquid Chromatogr* 1990;13:63–70.
3. Svec F, Fréchet MJ. *Anal Chem* 1992;54:820–22.
4. Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N. *Anal Chem* 1996;68:3498–501.
5. Al Bokari M, Cherrak D, Guiochon G. *J Chromatogr A* 2002;975:275–84. [PubMed: 12456082]
6. Cabrera K, Lubda D, Eggenweiler HM, Minakuchi H, Nakanishi K. *J High Resolut Chromatogr* 2000;23:93–99.
7. Smith NW, Jiang Z. *J Chromatogr A* 2008;1184:416–40. [PubMed: 17920615]
8. Wang Q, Svec F, Fréchet MJ. *J Chromatogr A* 1994;669:230–35. [PubMed: 8055104]
9. Coufal P, Cihak M, Suchankova J, Tesarova E, Bosakova Z, Stulik K. *J Chromatogr A* 2002;946:99–106. [PubMed: 11873988]
10. Moravcova D, Jandera P, Urban J, Planeta J. *J Sep Sci* 2004;27:789–800. [PubMed: 15354556]
11. Aoki H, Kubo T, Ikegami T, Tanaka N, Hosoya K, Tokuda D, Ishizuka N. *J Chromatogr A* 2006;1119:66–79. [PubMed: 16513125]
12. Huo Y, Schoenmakers PJ, Kok WT. *J Chromatogr A* 2007;1175:81–88. [PubMed: 18001748]
13. Hirano T, Kitagawa S, Ohtani H. *Anal Sci* 2009;25:1107–13. [PubMed: 19745538]
14. Xu Z, Yang L, Wang Q. *J Chromatogr A* 2009;1216:3098–106. [PubMed: 19215928]
15. Wang Q, Svec F, Fréchet MJ. *Anal Chem* 1995;67:670–74. [PubMed: 7893005]
16. Trojer L, Bisjak CP, Wieder W, Bonn GK. *J Chromatogr A* 2009;1216:6303–07. [PubMed: 19632682]
17. Peters EC, Svec F, Fréchet MJ. *Adv Mater* 1999;11:1169–81.
18. Meyer U, Svec F, Fréchet MJ, Hawker CJ, Irgum K. *Macromolecules* 2000;33:7769–75.
19. Davankov, VA.; Rogozhin, SV.; Tsyurupa, MP. US Patent. 3,729,457. 1971.
20. Pastukhov AV, Tsyurupa MP, Davankov VA. *J Polym Sci, Polym Phys* 1999;37:2324–33.
21. Davankov VA, Tsyurupa MP. *React Polym* 1990;13:27–42.
22. Davankov VA, Tsyurupa M, Ilyin M, Pavlova L. *J Chromatogr A* 2002;965:65–73. [PubMed: 12236538]
23. Tsyurupa MP, Davankov VA. *React Funct Polym* 2006;66:768–79.
24. Veverka P, Jerabek K. *React Funct Polym* 2004;59:71–79.
25. Ahn JH, Jang JE, Oh CG, Ihm SK, Cortez J, Sherrington DC. *Macromolecules* 2006;39:627–32.
26. Germain J, Hradil J, Svec F, Fréchet MJ. *Chem Mater* 2006;18:4430–35.
27. Jerabek K. *Anal Chem* 1985;57:1598–602.
28. Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N. *Anal Chem* 1996;68:3498–501.
29. Wang Q, Svec F, Fréchet MJ. *Anal Chem* 1993;65:2243–48. [PubMed: 8238925]
30. Svec F, Huber CG. *Anal Chem* 2006;78:2100–07.
31. Urban J, Moravcova D, Jandera P. *J Sep Sci* 2006;29:1064–73. [PubMed: 16830720]

Figure 1.

Separation of uracil and alkylbenzenes using precursor poly(styrene-co-vinylbenzyl chloride-co-divinylbenzene) column (A) and its hypercrosslinked counterpart (B). Conditions: Mobile phase 80% acetonitrile in water, UV detection at 254 nm, flow rate 1.5 $\mu\text{L}/\text{min}$. (A) column length 201 mm, back pressure 17.0 MPa; (B) column length 170 mm, back pressure 16.4 MPa. Peaks: uracil (1), benzene (2), toluene (3), ethylbenzene (4), propylbenzene (5), butylbenzene (6) and amylbenzene (7).

Figure 2.

Gradient elution of proteins using precursor poly(styrene-co-vinylbenzyl chloride-co-divinylbenzene) column (A) and its hypercrosslinked counterpart (B). Conditions: Column length 161 mm (A), 145 mm (B); Mobile phase gradient 5–80% acetonitrile in 0.1% aqueous formic acid in 10 min; flow rate 0.5 $\mu\text{L}/\text{min}$; UV detection at 214 nm. Peaks: ribonuclease (1), cytochrome C (2), myoglobin (3), α -chymotrypsin A (4), albumin (5).

Figure 3.

Size-exclusion separation of polystyrene standards with molecular masses of 1,870,000 (1) and 1,000 (2), and toluene (3) using precursor poly(styrene-co-vinylbenzyl chloride-co-divinylbenzene) column (A) and its hypercrosslinked counterpart (B). Conditions: Mobile phase 100% tetrahydrofuran, flow rate 0.5 $\mu\text{L}/\text{min}$, UV detection at 254 nm. (A) length 201 mm, back pressure 8 MPa, (B) length 170 mm, back pressure 8.2 MPa. V_e/V_C ratio of elution volume to geometrical volume of the column.