Published in final edited form as:
Anal Chem. 2009 September 1; 81(17): 7390–7396. doi:10.1021/ac901162x.

Downscaling limits and confinement effects in the miniaturization of porous polymer monoliths in narrow bore capillaries

Ivo Nischang[1], **Frantisek Svec**[*,2], and **Jean M.J. Fréchet**[*,1,2]

[1]Department of Chemistry, University of California, Berkeley, CA 94720, USA

[2]The Molecular Foundry, E. O. Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Abstract

Monolithic poly(butyl methacrylate-co-ethylene dimethacrylate) columns have been prepared in capillaries ranging in inner diameter from 5 to 75 μm using thermally initiated free-radical polymerization of a mixture of butyl methacrylate, ethylene dimethacrylate and porogens at different temperatures. Scanning electron microscopy and the measurement of hydrodynamic properties reveal that the downward scalability of the monolithic columns is greatly affected by the confinement effect of the capillary wall resulting from the decreased volume-to-surface ratio as the capillary diameter is decreased. The downscaling process is affected most by the polymerization temperature, the diffusion of the propagating radicals, and the density of coverage of polymerizable groups on the inner walls of the capillary. Optimization of all these factors enables the preparation of monolithic structures in capillaries with inner diameters as low as 5 μm while retaining the desirable properties of monoliths prepared in much larger capillaries. Under these conditions, the formation of undesired dense polymer layers attached to the capillary wall was minimized. The chromatographic performance of 10, 25 and 50 μm capillaries evaluated in the reversed phase gradient separation of three proteins showed no change in elution times at identical flow velocities and gradient times while peak elution width was the smallest with the narrowest capillary.

Keywords

Confinement; Porous polymer monolith; Scalability; Miniaturization; Nano-LC-ESI-MS; Gradient elution; Reversed phase chromatography; Proteins

The use of capillary HPLC columns with nanospray interface is required to further improve the quality of mass spectrometric detection as its sensitivity increases with the inverse square of the column diameter. Other advantages of the capillary column format include the small consumption of both sample and solvents, and a reduction in the peak broadening that results from radial diffusion. All these aspects have led recently to the development of HPLC instruments dedicated to separations in capillary columns. The current generation of commercial capillary columns packed with particulate stationary phases generally have inner diameters in the range of 75 to 100 μm. As better solvent delivery systems capable of pumping the mobile phase at a rate of few nanoliters per minute become available, the size of these columns is likely to decrease further.[1] Theory predicts that the plate height of very narrow-bore open-capillary columns operated in isocratic mode may be as small as the inner diameter of the column.[2] However, packing very small capillaries with particles may present significant

*To whom correspondence should be addressed: fsvec@lbl.gov frechet@berkeley.edu.

SUPPORTING INFORMATION AVAILABLE Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

challenges.[2–4] Therefore, polymer-based monolithic stationary phases, which are typically prepared *in situ* from liquid precursors provide a viable alternative to the more traditional packed columns.

Porous polymer monolithic columns have been developed in the early 1990s.[5,6] Capillary electrochromatography, emerging in the mid 1990s, has sparked the interest in capillary columns[7] and the monolith technology appeared well suited for the fabrication of these columns.[8–16] In subsequent years, monolithic capillaries 75–200 μm in diameter became widely used[17] in academic research and their application was extended to fields such as micro and nano HPLC,[17–25] solid phase extraction,[26] gas chromatography,[27] and enzyme immobilization.[28] For monolithic columns prepared using ring opening metathesis polymerization downscaling from 3 mm I.D. analytical size monolithic columns to capillaries 200–50 μm in diameter was reported to affect the reproducibility of their hydrodynamic properties.[29] A reduction in column diameter below 50 μm leads to a significant decrease in the volume-to-surface ratio, which translates into an increased difficulty in packing the columns with particles.[30] The change in volume-to-surface ratio upon reduction of capillary diameter also affects the preparation of monoliths in the confined space of the very small capillaries. For example, He et al. have recently reported that spatial confinement has a strong effect on the morphology of porous polymer monoliths.[31] They indicated that downscaling of porous polymer monoliths in narrow-bore capillaries and/or microfluidic chips can be difficult to implement in a way that enables the preparation of columns with morphology and performance similar to that of their larger diameter counterparts.

To circumvent this problem, several groups have used the porous layer open tubular (PLOT) column format in which a thin porous monolithic layer is attached to the wall of capillaries with I.D. as small as 10 μm.[32–36] The reduced lateral dimension enabling fast mass transfer from the mobile phase to the interacting functionalities in the thin layer were paramount to excellent performance in both pressure and electrodriven flow separations. Unfortunately, the small amount of the stationary phase in the capillary reduces the column sample loading capacity. However, as a result of their good permeability the length of PLOT columns may be extended to several meters, thus partly alleviating the loading problem.[33;35]

In this report, we address the preparation of polymer monoliths within narrow-bore capillaries with inner diameters in the range of 5 to 75 μm, developing polymerization conditions that preserve the desirable homogeneous morphology and porous properties which prevail in larger monolithic separation media. All polymerizations presented below were carried out using thermally initiated polymerization. Although photoinitiated polymerization is alternatively used for the preparation of monolithic capillary columns, the kinetics of polymerization, which is one of the most important variables controlling the morphology of the monolith, can be easily controlled by temperature during thermal initiation.

EXPERIMENTAL SECTION

Chemicals and materials

Ethylene dimethacrylate (EDMA), butyl methacrylate (BuMA), 1-propanol, 1,4-butanediol, azobisisobutyronitrile (AIBN) and 3-(trimethoxysilyl)propyl methacrylate were purchased from Aldrich (Milwaukee, WI, USA). Prior to use, the inhibitors were removed from the monomers EDMA and BuMA by passing them through a bed of basic alumina. MS-grade acetonitrile and MS-grade water, as well as proteins cytochrome *c*, myoglobin and ovalbumin were obtained from Sigma Aldrich (St. Louis, MO, USA). Typical concentration of the protein stock solutions was 1 μg/mL in MS-grade water containing 0.1% (v/v) formic acid. Polyimide coated 5–75 μm I.D. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Preparation of monoliths

The surface of inner walls of all capillaries was first vinylized as previously described.[37] Briefly, the capillaries were rinsed with acetone, water, 200 mmol/L sodium hydroxide, water, 200 mmol/L HCl, and ethanol. Then a solution of 20 %(v/v) 3-(trimethoxysilyl)propyl methacrylate in ethanol at an apparent pH value of 5, adjusted using acetic acid, was pumped through each capillary for 2 h using a syringe pump (KdScientific, New Hope, PA, USA). The reaction time was adjusted in some experiments to probe its effect on the formation of the porous polymer. After vinylization, the capillaries were rinsed with acetone and dried under a stream of nitrogen. The modified capillaries were filled using a syringe containing a previously optimized polymerization mixture[38] consisting of 24% BuMA, 16% EDMA, 34% 1-propanol, 26% 1,4-butanediol, and 1% AIBN (all w/w). After filling, the outlet and inlet ends of the capillaries were sealed with a rubber septum. Relatively long polymerization times of 72, 48, and 24 h were used for the thermally initiated polymerization at water bath temperatures of 50, 60, and 70 °C, respectively, to ensure total conversion of the monomers into porous polymer monoliths. Once the polymerization reaction was completed, the seals were removed, a short piece of the capillary (ca. 2 cm in length) was cut at each end, and the monoliths were washed with acetonitrile using a syringe pump. Finally, the columns were cut to the desired length and stored in 50% aqueous acetonitrile.

Equipment

All chromatographic measurements were carried out using a Dionex Ultimate 3000 HPLC system, including built in flow splitter, flow sensor, and flow control valve enabling to achieve exact flow rates. Tests with a nano-flow sensor (Upchurch Scientific, Oak Harbor, WA, USA) attached at the outlet of the columns confirmed the accuracy of the programmed flow. For permeability measurements, the monolithic capillaries were directly connected to the injector and inlet pressure at a given flow rate recorded. The actual column back pressure was obtained after substracting the system pressure monitored in the absence of the capillary column.

LC-ESI-MS experiments were carried out using a MicroTOF-Q mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The capillaries were connected with the Distal coated silica PicoTips (New Objective, Inc., Woburn, MA, USA) via zero dead volume connections (Upchurch Scientific, Inc., Oak Harbor, WA, USA). The PicoTip used with the 25 and 50 μm I.D. columns had a tubing internal diameter of 20 μm and a tip diameter of 10 μm, while custom-made PicoTips with an internal diameter of 10 μm and a tip diameter of 5 μm were used with the 10 μm I.D. monolithic capillary column. The electrospray voltage was optimized to a value of approximately 1300 V to obtain stable electrospray over a wide range of flow velocity. The instrument was operated in the positive ion mode. The tip was positioned in front of the orifice at an angle of 45° and a distance of 1.5 mm. Data were acquired in a mass-to-charge range of 500 to 1500 and total ion chromatograms were recorded at the same data acquisition rates for all columns. Scanning electron micrographs were obtained using an S-4300 SE/N scanning electron microscope (Hitachi, Pleasanton, CA, USA).

RESULTS AND DISCUSSION

Permeability to flow

Porous polymer monolith are well known for their high permeability to liquid flow[39] which enables their use even at flow rates that are typically not achievable with packed beds.[2] The superior permeability of monoliths results from their specific morphological structure that comprises clusters of microglobules intertwined with large pores. The permeability κ of a conduit of any dimension and shape is related to the superficial flow velocity as described by the following equation:[2;40–42]

$$K = \frac{L}{\Delta P} \eta u_{sf} \quad (1)$$

where L is the length of the conduit, ΔP is the pressure drop, η is the mobile phase viscosity, and u_{sf} is the superficial velocity. The value of u_{sf} is often referred to as the "empty tube" velocity and is obtained by dividing the volumetric flow rate F_V by the cross sectional area A of the conduit. The permeability can be measured experimentally by recording the pressure drop per unit of length of the tube at a given flow rate, or alternatively, by measuring the superficial velocity at a given pressure. The Hagen-Poiseuille equation describes the flow of liquids in circular tubes:[39;41]

$$u_{sf} = \frac{R_h^2}{2} \frac{\Delta P}{\eta L} \quad (2)$$

where R_h is the hydraulic radius of the tube defined as the ratio of cross sectional area A and the wetted perimeter U, the factor 2 is a shape factor derived for cylindrical conduits. Therefore R_h also reflects the volume-to-surface ratio for a tube of given length. A decrease in R_h, which, for capillaries, is simply achieved by decreasing their internal diameter, requires that the pressure be increased to retain a constant value for u_{sf}. Consequently, and according to equation 1 a decrease in R_h leads to a reduction in permeability.

Darcy's law defines the pressure drop ΔP per unit length generated by a conduit containing a porous medium that can be formed by a bed of packed particles or monolith:[2;40;41]

$$k_{p,f} = \frac{L}{\Delta P} \eta u_{sf} \quad (3)$$

where $k_{p,f}$ is the permeability of conduits for fluid flow through the porous bed and u_{sf} is again the superficial velocity, which has the same value as that for a generic "empty" tube. Due to the tortuosity of the individual pores within the three dimensional random porous architecture, as well as much smaller average channel size, the hydrodynamic permeability of a conduit containing a porous medium is significantly smaller than that of an open tube. Despite their simplicity, the above equations enable a comparison of the permeabilities of the various conduits.[2;37;41]

Figure 1 shows the pressure drop generated at various flow rates in 5–75 μm I.D. capillaries containing porous polymer monoliths prepared by polymerization at different temperatures. The pressure drop normalized to a 1 m length is always a linear function of the flow rate for all capillaries regardless of their inner diameter, thus confirming the validity of equation 3 for this system. These plots also confirm that the monoliths retain their rigidity since they are not compressed within the pressure range tested. It is worth noting that the flow rate of 0.25 μL/min in the 10 μm I.D. monolithic capillary translates to an extremely high superficial velocity of 5 cm/s. Use of these high flow velocities validate the excellent permeability of our monolithic columns and enable very fast separations. Also, monoliths prepared at a higher polymerization temperature of 70 °C, which feature smaller through pores[43] exhibit a drastic reduction in hydrodynamic permeability compared to monoliths prepared using a lower polymerization temperature. The high back pressures needed to achieve flow-through in columns prepared at 70 °C with I.D. smaller than 25 μm prevents their use in typical HPLC equipment.

The pressure drops found for the capillaries with larger dimensions correspond well with those we observed previously for monoliths prepared at the same polymerization temperature.[37] The calculated permeabilities $k_{p,f}$ for monoliths in the 75 and 50 μm I.D. capillaries are 9.78×10^{-14} and 9.30×10^{-14} m^2, respectively, which is well within the range of 9.15–10.13 m^2 we observed previously for monoliths in both cylindrical and non-cylindrical conduits.[37] The increase in pressure drop in monolithic capillaries prepared at higher temperatures results from the formation of smaller through pores, an effect we have documented previously.[43] The effect of the cross sectional area of the capillary on the slope of the back pressure vs. flow rate plots is seen in Figure 2. While the slope is an approximately linear function of the cross section for the monoliths prepared using polymerizations carried out at 60 and 70 °C, as expected, the situation is different for monolith prepared at 50 °C. Surprisingly, the plot reproducibly exhibits a double bend connecting two nearly linear parts at cross sections between about 300–1300 μm^2 representing capillaries with diameters of 20–50 μm and a volume-to-surface ratio of smaller than 12.5. These bends may indicate the significant effect of confinement during polymerizations characterized by slow kinetics resulting from the low temperature.

Electron micrographs of porous polymer monoliths in capillaries of various sizes prepared from the same polymerization mixture are shown in Figure 3. Close examination of all the micrographs of monoliths prepared at 50 °C reveals the presence of what appears to be a less porous layer of polymer located at the wall of the capillary. While the thickness of this layer is similar for both the 50 μm and the 10 μm capillaries, this layer represents a much larger fraction of the total cross section of the smaller capillary. Consequently, the proportion of monomers forming this layer, relative to the overall content of monomers in the capillary, is larger for the smaller size. As a result, a smaller amount of monomer is available to form the bulk porous structure. Indeed, the morphology of monoliths prepared in 50 and 10 μm capillary is quite different. While the monolith prepared in the 50 μm capillary exhibits typical aggregates of microglobules interspersed with large pores, the structure of monolith in the 10 μm capillary is much looser since the amount of monomers remaining to form the porous matrix is smaller than in the larger I.D. capillary.

In contrast, when the polymerization is carried out at a temperature of 60 °C, the thickness of the surface layer in the 10 μm capillary is thinner than that in the 50 μm capillary while the morphology of both monoliths remains essentially identical. This similarity of the porous structures correlates well with the measurements of back pressure vs. flow rate shown in Figure 1b where the permeability depends only on the cross sectional area. Figure 4 confirms that the back pressure is a function of the superficial flow velocity but not of the capillary diameter since the experimental points coincide for all 50, 25, and 10 μm capillaries. We believe that this is a direct result of the enhanced initiation rate of polymerization at 60 °C, which is faster than the rate of diffusion of monomers and propagating chains to the wall of the capillary. Therefore, most of the polymerizing monomers become immobilized within the crosslinked porous matrix before they have a chance to accumulate in the wall layer.

Effect of reaction kinetics

The significant difference between monoliths prepared at different temperatures reflects the effect of reaction kinetics. The rate of polymerization depends, among other variables, on the concentration of polymerizable double bonds. Our system includes double bonds originating both from the monomers present in the polymerization mixture and from the methacrylate functionalities attached to the capillary wall. These functionalities are used to create the covalent attachment of the monolith to the surface of the capillary in order to preclude the formation of undesired voids that could result from shrinkage during polymerization. Both the conditions for vinylization of the capillary walls and the composition of the polymerization mixture used were the same for all of the experiments presented in this report so far. The wall

surface in a given length of the 10 μm capillary is five times smaller than that for its 50 μm counterpart. Therefore, the number of attached vinyl functionalities is also 5 times lower in the 10 μm capillary. In contrast, the volume of the polymerization mixture in a given length of the 10 μm capillary is 25 times smaller than in the 50 μm capillary. This means that in the smaller capillary the importance of the vinyl functionalities located on the walls is magnified: a surface "confinement effect". A simple statistical analysis then suggests that polymerization at the walls of the capillary will be enhanced in the small capillaries in comparison to their larger analogs. In addition, the short distances within the tube also facilitate diffusional transfer of monomers and growing chains from the bulk solution to the wall. As a result of the enhanced probability of polymerization at the wall, formation of a dense polymer layer occurs at the capillary wall as observed in SEM micrographs. The polymerization mixture, which is relatively depleted of monomers, then affords monolith with a looser, more porous structure.

Wall surface vinylization

Given the significance of the formation of the dense layer at the capillary wall and its effect on the morphology and porous properties of the monolith, we explored the effect of the extent of wall vinylization on monolith formation. Clearly, as the vinylization time is increased, the number of available functionalities located at the wall of the capillary increases, thus favoring formation of a thick dense surface layer during monolith formation. To explore the effect of density of vinyl functionalities at the wall we varied the time of vinylization of the wall surface in the 10 μm I.D. capillary. First, we used a capillary without any wall functionalization for the preparation of a monolith using the standard mixture of monomers and porogens. The SEM micrographs of the cross section of this capillary shown in Figure 5 clearly illustrate that in the absence of anchoring moieties at the surface of the capillary, very poor adhesion of the monolith to the wall is obtained. In contrast a very short 5 min vinylization time is sufficient to provide the wall surface with a sufficient enough functionalities to enable good attachment of the monolith. An increase in the vinylization time to 1 and 2 h leads to the formation of a less porous surface layer with a thickness that increases with increasing vinylization time. Further increases in the extent of vinylization using longer reaction time and/or higher temperatures should ultimately lead to the formation of a thick surface layer leaving little or no monomer available to form a monolithic structure at the center of the capillary. Indeed, after vinylizing a capillary at 120 °C for 6 h Huang and Horváth did not obtain a "standard" monolith but obtained instead an interesting porous open tubular (PLOT) capillary column.[44] Similarly, Karger et al. also obtained a 10 μm I.D. PLOT capillary column after vinylizing the capillary wall for 12 h at room temperature.[34] These reported experiments support our findings on the importance of the extent of vinylization of the capillary walls.

The extent of wall surface modification (measured as the vinylization time) also affects the hydrodynamic properties of the column. Figure 6 shows the effect of the flow rate on the back pressure for monoliths prepared under identical polymerization conditions in 10 μm I.D. capillaries that were vinylized for different times. As expected, permeability increases with vinylization time. As was seen in Figure 3, a significant amount of monomers used for monolith formation is converted to the less porous layer. Therefore, a lower proportion of monomers remains available for formation of the porous monolith, leading to a highly permeable structure with large pores and high porosity at the center of the capillary. As the vinylization time is decreased, formation of the wall layer is less prevalent and more monomer is available leading to a denser porous monolith trough the entire cross section of the capillary. The lowest permeability is observed for the monolithic column prepared in a capillary vinylized for only 5 min for which surface layer formation is minimized while adhesion of the monolith to the wall of the capillary is preserved (Figure 7). This is in qualitative agreement with the permeability values calculated using equation 3, which show a rapid increase as vinylization times increase and level off after 2 hours. In this scenario the polymerization at the wall

dominates and the center of the capillary is only filled with a very loose monolithic structure. Therefore, the permeability approaches that of an empty capillary with a narrower bore. Permeability measurements in a narrow bore capillary constitute an excellent probe of the extent of vinylization which is difficult to obtain otherwise.[45] In contrast, Figure 7 also shows that this surface "confinement effect" is not observed for monoliths prepared in 50 μm I.D. capillary that have a significantly larger volume-to-surface ratio.

Separation of proteins

The experiments presented above provide guidance for the preparation of monolithic columns with identical hydrodynamic properties regardless of the capillary diameter. However, identical hydrodynamic properties may not translate into equal chromatographic performance. To test the effect of capillary size on the separation of proteins, we prepared monolithic columns in capillaries with diameters in the 10–50 μm I.D. range using a polymerization temperature of 60 °C in order to alleviate the surface confinement effects.

We used the gradient-volume concept[46] to compare the separation of proteins in gradient mode using columns of different I.D. According to this concept, the ratio of gradient volume V_g to the column hold-up volume V_m should remain constant to enable comparable separations. In other words, a change in column size must be compensated for by a change in flow rate according to:

$$\frac{V_m}{V_g}=\frac{V_m}{t_g F_v}=\text{const.} \quad (4)$$

Where t_g is the gradient time and F_v is the volumetric flow rate. The flow rate in experiments with all three capillary columns was adjusted to achieve the same superficial and linear flow velocity. As a result, the hold-up volume of the column is permeated in the same period of time. Since, according to Figure 4, the back pressure of porous polymer monolith is practically independent of the capillary size at comparable flow velocities, the equal back pressure values we found indicate that the flow velocity in all columns is the same.

Figure 8 shows the sample separation of three model proteins using 10, 20, and 50 μm I.D. capillary columns and a linear mobile phase gradient of acetonitrile in 0.1% aqueous formic acid. The respective flow rates were adjusted according to Equation 4 to achieve a superficial flow velocity of 13.6 mm/s in all columns that translates to a linear chromatographic velocity of more than 20 mm/s. The gradient time was kept constant at 3.1 min. This approach ensures that the separations are carried out at the same flow velocity inside the column and that the same relative volume of mobile phase is percolated through the column for a given gradient of acetonitrile. We also adjusted the amount of injected proteins as a function of column volume in order to compare separations obtained at a similar column load. The chromatograms demonstrate that the elution times in all three columns are very similar. However, the narrowest peaks are monitored in the 10 μm capillary as a result of limited radial diffusion and small volume of injected sample. Figure 9 shows the effect of capillary diameter on peak width of the eluted protein in a series of five columns with diameters of 10, 20, 30, 40, and 50 μm, respectively. The peak width is expressed in volume since different flow rates have to be used for each capillary size in order to obtain the same flow velocity. An additional benefit of the use of a narrow bore capillary column is the lower flow rate that leads to improved ionization efficiency in nano-ESI MS and thus increased sensitivity of detection.[36]

We also studied effect of gradient steepness on the separation of proteins and loading capacity of monolithic columns varying in the inner diameter. Results of this study are available in Supporting Information.

CONCLUSION

This study demonstrates that porous polymer monolithic columns with comparable properties can be prepared in capillaries scaled down to 5 μm. Since the kinetics of polymerization play a decisive role in the formation of the desired monolithic structure, its morphology is controlled by a variety of factors including volume-to-surface ratio, polymerization temperature, diffusion of the growing polymer radicals, and density of coverage of the capillary wall with polymerizable groups. Slow polymerization at lower temperatures and/or excessive coverage of the wall with polymerizable groups lead to the formation of a nearly impermeable polymer layer attached to the wall resulting in a heterogeneous porous structure. The thickness of this layer can be minimized via (i) a decrease in vinylization time or (ii) an increase in polymerization temperature. The former enables the formation of monolithic structures comparable to those of larger diameter column monoliths prepared from a specific polymerization mixture at lower temperature and low initiation rate of the polymerization. The latter is more convenient but may require adjustments in the composition of polymerization mixture to obtain monoliths with the targeted pore size. These findings provide an important tool for the preparation of small diameter columns both in open tubular and porous monolithic formats.

Our chromatographic tests have confirmed that the use of narrow bore monolithic capillary columns decreases the peak width and improves the sensitivity in mass spectrometric detection. An additional benefit of the small diameter columns is that only a very small sample volume is sufficient for both separation and detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Support of this research by a grant of the National Institute Institutes of Health (GM48364) is gratefully acknowledged. Portions of this work were performed at the Molecular Foundry, Lawrence Berkeley National Laboratory, which is supported by the Office of Science, Office of Basic Energy Sciences, U.S. Department of Energy, under Contract No. DE-AC02-05CH11231.

Abbreviations

BuMA, butyl methacrylate; EDMA, ethylene dimethacrylate; AIBN, azobisisobutyronitrile; ACN, acetonitrile; SEM, scanning electron microscopy; I.D., internal diameter.

REFERENCES

(1). Janasek D, Franzke J, Manz A. Nature 2006;442:374–380. [PubMed: 16871204]
(2). Guiochon G. J. Chromatogr., A 2007;1168:101–168. [PubMed: 17640660]
(3). Kennedy RT, Jorgenson JW. Anal. Chem 1989;61:1128–1135.
(4). Hsieh SC, Jorgenson JW. Anal. Chem 1996;68:1212–1217. [PubMed: 8651494]
(5). Svec F, Fréchet JMJ. Anal. Chem 1992;64:820–822.
(6). Wang QC, Svec F, Fréchet JMJ. Anal. Chem 1993;65:2243–2248. [PubMed: 8238925]
(7). Deyl, Z.; Svec, F., editors. Capillary Electrochromatography. Elsevier; Amsterdam: 2001.
(8). Liao JL, Chen N, Ericson C, Hjertén S. Anal. Chem 1996;68:3468–3472.
(9). Peters EC, Petro M, Svec F, Fréchet JMJ. Anal. Chem 1997;69:3646–3649. [PubMed: 9286168]
(10). Palm A, Novotny MV. Anal. Chem 1997;69:4499–4507.
(11). Svec F, Peters EC, Sýkora D, Yu G, Fréchet JMJ. HRC-J 2000;23:3–18.

(12). Hilder EF, Svec F, Fréchet JMJ. Electrophoresis 2002;23:3934–3953. [PubMed: 12481286]
(13). Hilder EF, Svec F, Fréchet JMJ. J. Chromatogr., A 2004;1044:3–22. [PubMed: 15354426]
(14). Bedair M, El Rassi Z. Electrophoresis 2004;25:4110–4119. [PubMed: 15597411]
(15). Legido-Quigley C, Marlin ND, Melin V, Manz A, Smith NW. Electrophoresis 2003;24:917–944. [PubMed: 12658680]
(16). Wu R, Hu L, Wang F, Ye M, Zou H. J. Chromatogr., A 2008;1184:369–392. [PubMed: 17923135]
(17). Peters EC, Svec F, Fréchet JMJ. Adv. Mat 1999;11:1169–1181.
(18). Moravcova D, Jandera P, Urban J, Planeta J. J. Sep. Sci 2003;26:1005–1016.
(19). Urban J, Eeltink S, Jandera P, Schoenmakers PJ. J. Chromatogr., A 2008;1182:161–168. [PubMed: 18206896]
(20). Premstaller A, Oberacher H, Huber CG. Anal. Chem 2000;72:4386–4393. [PubMed: 11008774]
(21). Premstaller A, Oberacher H, Walcher W, Timperio AM, Zolla L, Chervet JP, Cavusoglu N, van Dorsselaer A, Huber CG. Anal. Chem 2001;73:2390–2396. [PubMed: 11403277]
(22). Schlemmer B, Gatschelhofer C, Pieber TR, Sinner FM, Buchmeiser MR. J. Chromatogr., A 2006;1132:124–131. [PubMed: 16934281]
(23). Eeltink S, Desmet G, Vivo-Truyols G, Rozing GP, Schoenmakers PJ, Kok WT. J. Chromatogr., A 2006;1104:256–262. [PubMed: 16364339]
(24). Eeltink S, Geiser L, Svec F, Fréchet JMJ. J. Sep. Sci 2007;30:2814–2820. [PubMed: 17893847]
(25). Buszewski B, Szumski M, Sus S. LC-GC Europe 2002;15:792–798.
(26). Peterson DS, Rohr T, Svec F, Fréchet JMJ. Anal. Chem 2003;75:5328–5335. [PubMed: 14710809]
(27). Sykora D, Peters EC, Svec F, Fréchet JMJ. Macromol. Mater. Eng 2000;275:42–47.
(28). Geiser L, Eeltink S, Svec F, Frchet JMJ. J. Chromatog. A 2008;1188:88–96. [PubMed: 18342870]
(29). Sinner FM, Gatschelhofer C, Mautner A, Magnes C, Buchmeiser MR, Pieber TR. J. Chromatogr., A 2008;1191:274–281. [PubMed: 18242625]
(30). Ivanov AR, Zang L, Karger BL. Anal. Chem 2003;75:5306–5316. [PubMed: 14710807]
(31). He M, Zeng Y, Sun XJ, Harrison DJ. Electrophoresis 2008;29:2980–2986. [PubMed: 18551717]
(32). Eeltink S, Svec F, Fréchet JMJ. Electrophoresis 2006;27:4249–4256. [PubMed: 17024717]
(33). Luo Q, Yue G, Valaskovic GA, Gu Y, Wu SL, Karger BL. Anal. Chem 2007;79:6174–6181. [PubMed: 17625912]
(34). Yue G, Luo Q, Zhang J, Wu SL, Karger BL. Anal. Chem 2007;79:938–946. [PubMed: 17263319]
(35). Luo Q, Gu Y, Wu SL, Rejtar T, Karger BL. Electrophoresis 2008;29:1604–1611. [PubMed: 18383016]
(36). Luo Q, Rejtar T, Wu SL, Karger BL. J. Chromatogr., A 2009;1216:1223–1231. [PubMed: 18945436]
(37). Nischang I, Svec F, Fréchet JMJ. J. Chromatogr., A 2009;1216:2355–2361. [PubMed: 19201413]
(38). Geiser L, Eeltink S, Svec F, Fréchet JMJ. J. Chromatogr., A 2007;1140:140–146. [PubMed: 17182044]
(39). Viklund C, Svec F, Fréchet JMJ, Irgum K. Chem. Mater 1996;8:744–750.
(40). Gusev I, Huang X, Horváth C. J. Chromatogr., A 1999;855:273–290. [PubMed: 10514993]
(41). Rozing G, van de Goor T, Yin HF, Killeen K, Glatz B, Kraiczek K, Lauer HH. J. Sep. Sci 2004;27:1391–1401. [PubMed: 15638148]
(42). Jung S, Ehlert S, Mora JA, Kraiczek K, Dittmann M, Rozing GP, Tallarek U. J. Chromatogr., A 2009;1216:264–273. [PubMed: 19091319]
(43). Svec F, Fréchet JMJ. Macromolecules 1995;28:7580–7582.
(44). Huang X, Horváth C. J. Chromatogr., A 1997;788:155–164. [PubMed: 9419876]
(45). Courtois J, Szumski M, Bystrom E, Iwasiewicz A, Shchukarev A, Irgum K. J. Sep. Sci 2006;29:325.
(46). Jandera P. J. Chromatogr., A 2006;1126:195–218. [PubMed: 16787650]

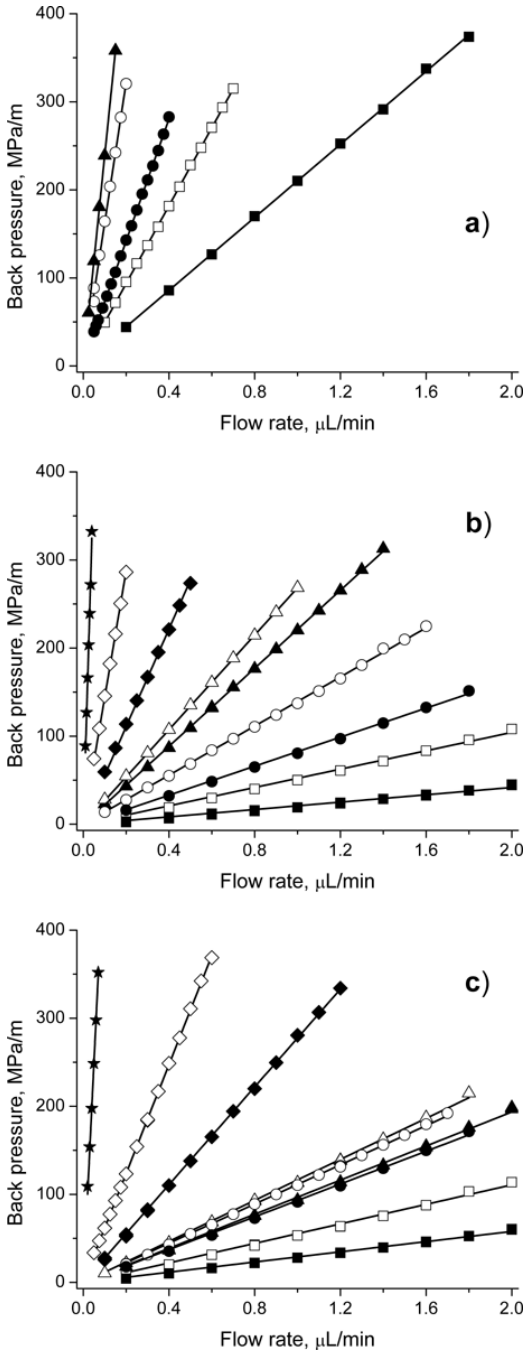

Figure 1.

Effect of flow rate on back pressure of poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths prepared from the same initial polymerization mixture in capillaries differing in inner diameter at a temperature of (a) 70 °C, (b) 60 °C, and (c) 50 °C. Capillary diameter: (■) 75 μm, (□) 50 μm, (●) 40 μm, (○) 30 μm, (▲) 25 μm, (△) 20 μm, (◆) 15 μm, (◇) 10 μm, and (★) 5 μm. Mobile phase: 50/50 % (v/v) acetonitrile-water containing 0.1 % (v/v) formic acid.

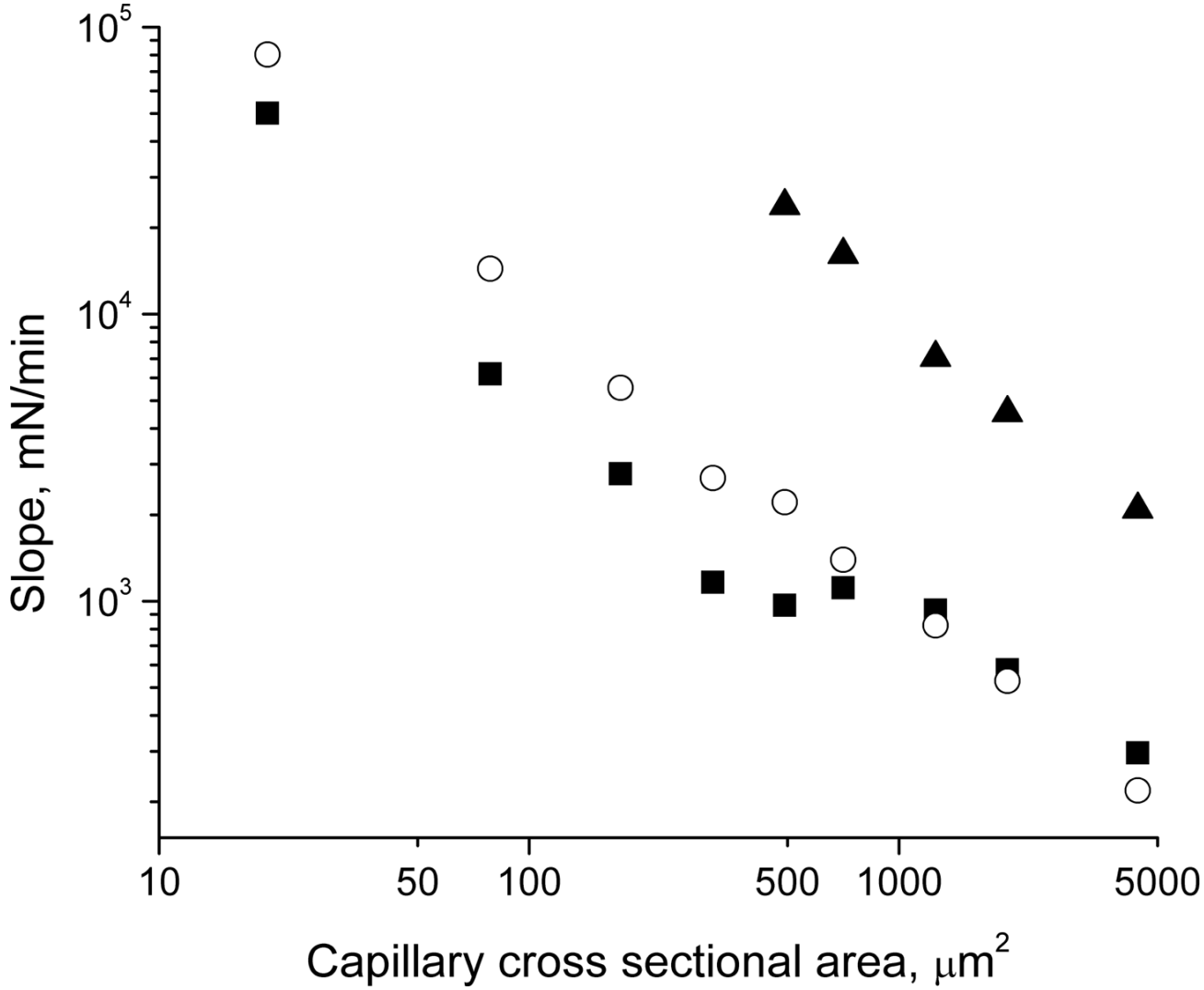

Figure 2.
Slope of plots of back pressure against flow rate shown in Fig. 1 as a function of capillary cross sectional area measured for poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths prepared from the same polymerization mixture at temperatures of (■) 50 °C, (○) 60 °C, and (▲) 70 °C.

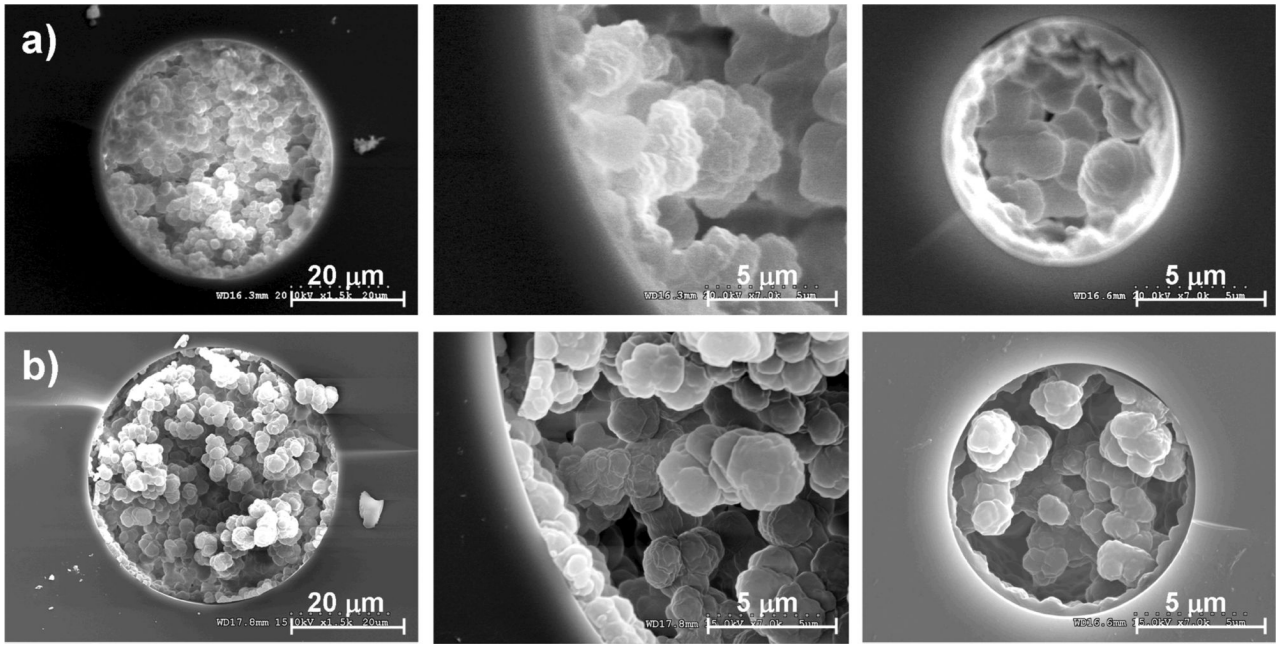

Figure 3.

SEM micrographs of the cross section of the poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths prepared at temperatures of (a) 50 °C, and (b) 60 °C in 50 µm (left) and 10 µm (right) fused silica capillaries. The center micrographs show the morphology of the monolith at the wall in 50 µm capillary magnified to the scale of the 10 µm capillary for direct comparison.

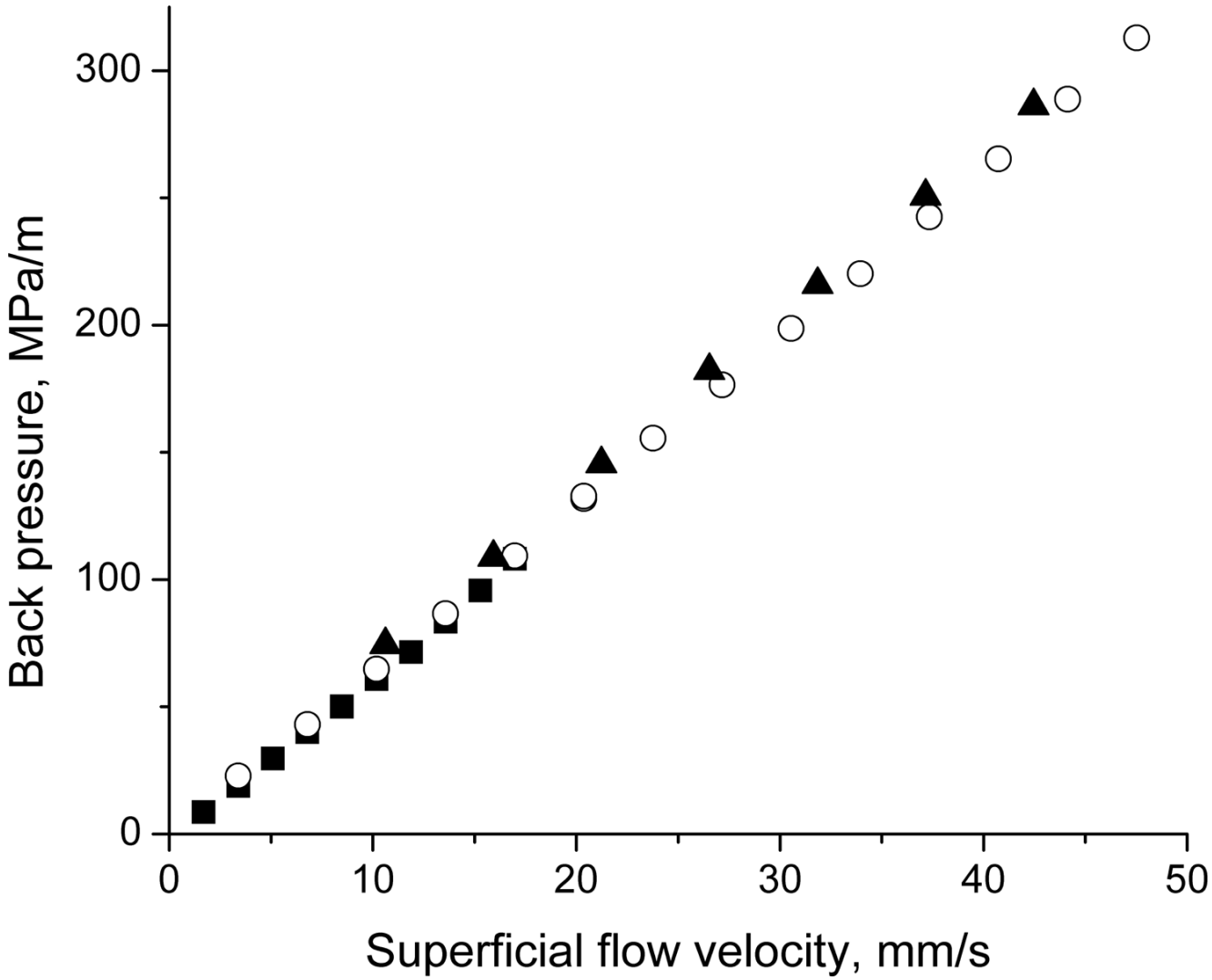

Figure 4.
Back pressure generated by poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths in (■) 50 μm, (○) 25 μm, and (▲) 10 μm I.D. capillaries prepared at 60 °C as a function of superficial flow velocity.

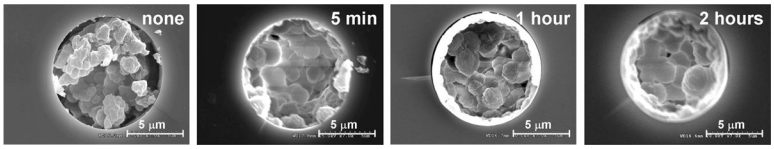

Figure 5.
SEM micrographs of the cross section of poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths in 10 μm I.D. fused silica capillaries modified with 3-(trimethoxysilyl)propyl methacrylate for different periods of time and polymerized at a temperature of 50 °C.

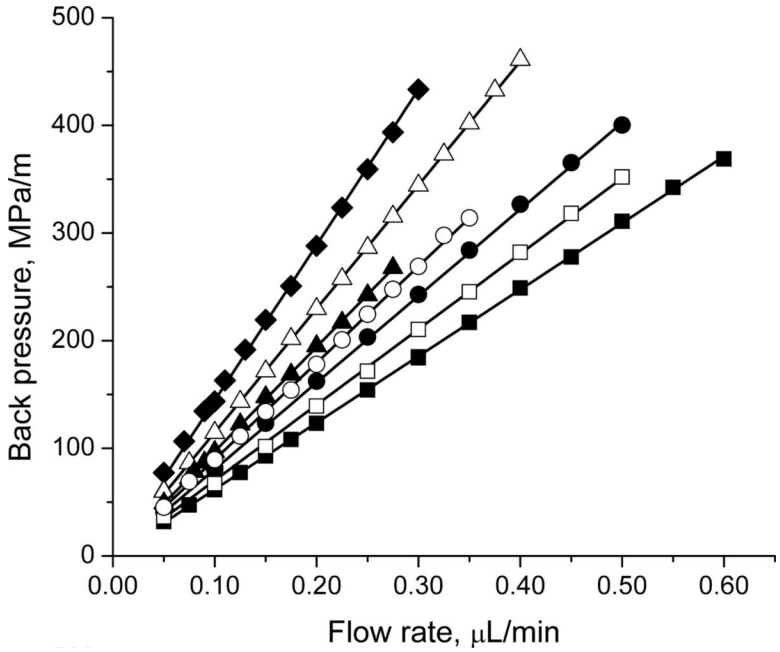

Figure 6.

Effect of duration of the surface modification with 3-(trimethoxysilyl)propyl methacrylate on back pressure generated in the 10 μm I.D. capillary containing poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths prepared at 50 °C. Surface modification time: (■) 120 min, (□) 60 min, (●) 30 min, (○) 25 min, (▲) 15 min, (△) 10 min, and (◆) 5 min.

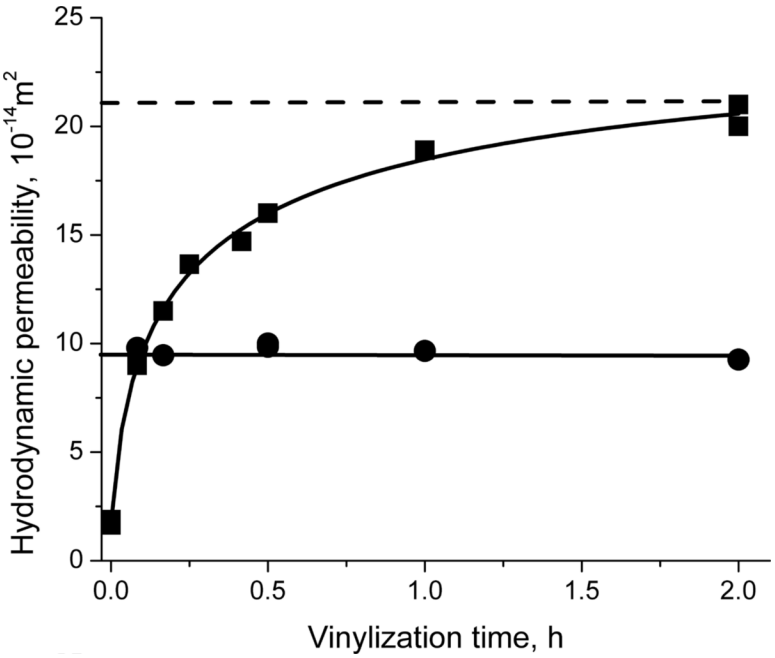

Figure 7.

Impact of time of surface vinylization with 3-(trimethoxysilyl)propyl methacrylate on the hydrodynamic permeability of porous polymer monolith prepared at 50 °C calculated using Eq. 3 for the (■) 10 μm and (●) 50 μm I.D. capillary. Mobile phase: 50/50 % (v/v) acetonitrile-water containing 0.1 % (v/v) of formic acid.

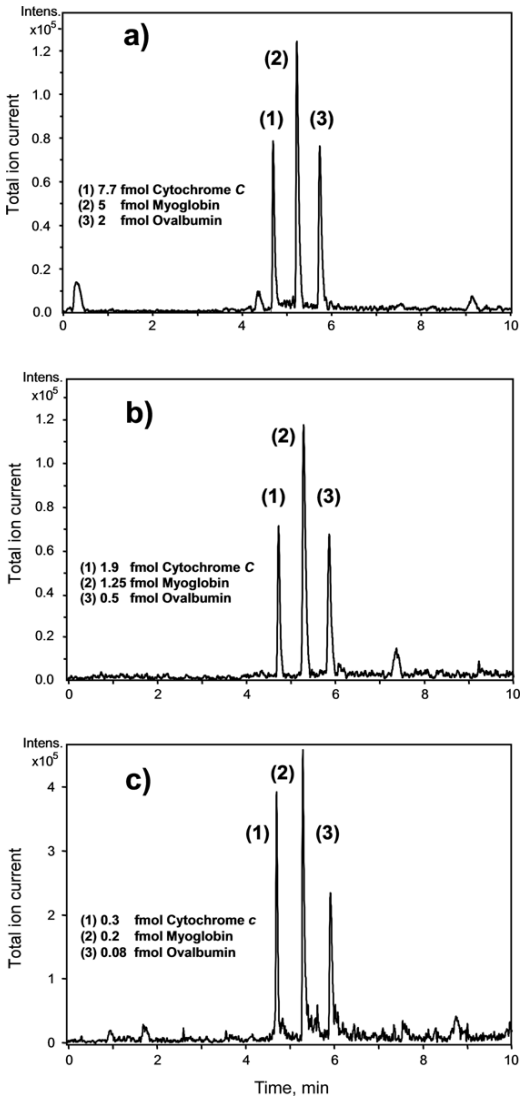

Figure 8.

Chromatographic separations of cytochrome *c*, myoglobin, and ovalbumin using gradient elution in poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolithic capillary columns varying in inner diameter prepared at 60 °C. Conditions: column length 15 cm, mobile phase A: 0.1 %v/v formic acid solution in water, B: 0.1 %v/v formic acid solution in acetonitrile, gradient 0–50 % B in A in 3.2 min, superficial flow velocity 13.6 mm/s, injection volume 50 nL. (a) Capillary 50 μm I.D.; flow rate of 1.6 μl/min, (b) capillary 25 μm I.D.; flow rate of 0.4 μl/min, (c) capillary 10 μm I.D.; flow rate of 0.064 μl/min.

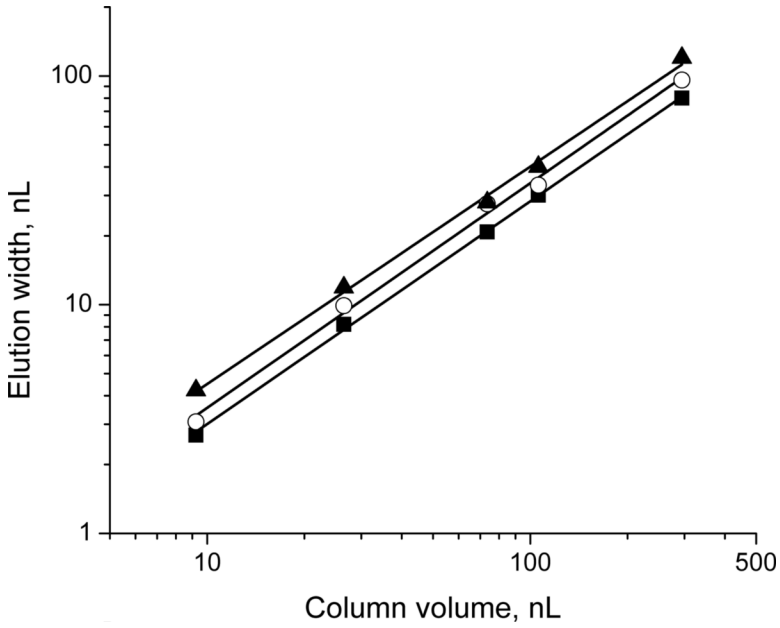

Figure 9.
Effect of column volume on the peak width at half height expressed in volume units for (■) cytochrome *c*, (○) myoglobin, and (▲) ovalbumin. For conditions see Figure 8.