Wide-bore P-methylstyrene-*co*-dimethylbis(p-vinylbenzyl) silane Based-monoliths Columns for Separation of Peptides and Proteins

Said H. Lubbad*

Institute for Polymer Chemistry, Univerisät Stuttgart, Pfaffenwaldring 55, 70569 Stuttgart, Germany

* Corresponding author: E-mail: said.lubbad @ipoc.uni-stuttgart.de
Tel: +49 (0) 711-68564431; Fax: +49 (0) 711-68564396

Abstract

Wide-bore monolithic columns of p-methylstyrene-*co*-dimethylbis(p-vinylbenzyl)silane were prepared within glass columns (100 × 3 mm I.D.). Monolithic columns differing in the amount of initiator were fabricated. The pressure drop *vs.* flow-rate measurements and the relatively low swelling propensity factor indicated the good cross-linking homogeneity and good mechanically stability. The chromatographic performance of monoliths prepared was assessed by analysis of protein and peptide mixtures. Five proteins were separated in less than 2 minutes. Moreover, a mixture of 9 peptides was separated in less than 11 minutes, aside from the co-elution of vasopressin [arg8] and methionine enkephalin.

Keywords: Wide-bore monolith, dimethylbis(*p*-vinylbenzyl)silane, monolith, peptides, proteins, bioseparation.

1. Introduction

Monolithic columns are attractive for analysts applying high-performance liquid chromatography (HPLC) because they are characterized by the speed of analysis due to the ample open channel-network all over the polymer support, which allows high flow rate at low pressure drop. Moreover the resistance to mass transfer is reduced in the case of monolithic support compared to packed support due to better convection and diminished diffusion.[1–3] Monolithic chromatographic beds are presented in literature as two types differing in chemistry such as silica based-, and polymer based-supports. Silica based-monoliths established a good reputation in separating small molecules such as pharmaceuticals and environmental pollutants.[4–6] However; these types of monoliths do not endure the highly basic or acidic mobile phases; which lead to degradation of the support damaging the column performance and reproducibility. Also the sol-gel process of preparing silica monoliths is critical, tedious and time consuming, where it takes over three days to prepare such a monolith. Besides, further surface modification mainly immobilization of octyl- or octadecyl- alkyl chain is a constraining requirement. The organic based-monoliths demonstrated uniqueness mainly for the separation of large biomolecules such as peptides, proteins and nucleic acids.[7–10] These monoliths are also distinctive as they need no further surface modification to suite the task of separation; which is not the case for their counterpart silica based monoliths. So far few polymer based-monoliths that are capable of separating small molecules were recently reported.[11–16]

Organic polymers as chromatographic supports suffer from shrinkage in water and swelling in organic solvents due to the low degree of crosslinking homogeneity. The instability of the chromatographic support due to changing solvents leads to reduced column efficiency and loss of resolution.[17,18] The right solution to such a problem is to establish a highly homogenous cross linked material. A reported monomer of 1,2-bis(p-vinylphenyl)ethane (BVPE) was introduced as crosslinker in monolithic support which demonstrated homogenous polymerization as a result of the spacer between the two styrene groups.[19,20] Another cross linker of dimethylbis(p-vinylbenzyl)silane (DMBVBS) showed a high degree of homogeneous crosslinked polymer as well.[21] Yet, DMBVBS (liquid) is superior to BVPE (solid), because of its better solubility than that of the latter in various protic-organic solvent at room temperature. This allows more choices of porogens in monolith making. Furthermore, the procedure for monolith

making is way easier in case of DMBVBS compared to BVPE, as no need to preheat the tools or the monomer mixture prior to polymerization, ensuring easy handling.[19–22] The strict conditions required in preparation of BVPE-based monolith put some limitation and require high skills to save reproducibility.

The performance of monolithic column is usually optimized by systematic changes in the mass content of monomers and porogens, the polymerization temperature or in the amount of initiator as well. In this study, the recently reported material based on alkylsilane chemistry is applied for wide-bore conventional size monolithic supports, which was characterized and optimized for peptides and proteins separation. Monolithic structure and chromatographic optimization to suite separation of biomolecules is to be presented.

2. Experimental

2. 1. Chemicals & Reagents

HPLC-grade acetonitrile (ACN), and trifluoroacetic acid (TFA) were purchased from Sigma and Bakers (Germany). α,α´-azoisobutyronitrile (AIBN) was purchased from Sigma-Aldrich (Germany). Diethyl ether and petroleum ether were purchased from Riedel-de Häen (Germany). Toluene and tetrahydrofuran (THF) purchased from Sigma-Aldrich (Germany) were dried over sodium prior to use. p-Methylstyrene purchased from Aldrich was purified before use by washing with 10% sodium bicarbonate solution, dried and distilled under vacuum. Magnesium, p-vinylbenzyl chloride and dichlorodimethylsilane, purchased from Fluka (Germany), were used with no further treatment. 2-propanol (Aldrich, Germany) was used as received. Analytes such as 9-peptide standard mixture (1) bradykinin fragment 1–5; (2) vasopressin [arg8]; (3) methionine enkephalin; (4) leucine enkephalin; (5) oxytocin; (6) bradykinin; (7) LHRH; (8) bombesin; and (9) substance P, and protein standards, (1) ribonuclease, (2) A, cytochrome, C, (3) α-lactalbumin, (4) β-lactaglobulin, (5) ovalbumin, were purchased from Sigma Life Sciences (Germany). HPLC-grade tetrahydrofuran (THF) was purchased from Aldrich (Germany) and polystyrene standards for inverse size-exclusion chromatography (ISEC) were obtained from Pressure Chemicals (Pittsburgh, PA, USA) (M.Wt.: 800, 1681, 4000, 13502, 65000, 129200, 670000, 2000000 g/mol) and from Polymer Standards Service (PSS) (Mainz, Germany) (M.Wt.: 370, 271000, 1103000, 3000000, 4060000, 8090000 g/mol). Borosilicate glass columns (100 × 3 mm I.D) were purchased from CP-Analytica (Vienna, Austria).

2. 2. Instrumentation

A Transgenomic HPLC system consisted of a pump, an oven, an auto sampler, and a UV-Vis detector was used.

Wavemaker 4.1 software was used for data acquisition and processing. Peptides and proteins were detected by UV-Vis detector at wavelength of 214 and 280 nm, respectively. Nanopure infinity ultra pure water and HPLC grade acetonitrile were used in HPLC analysis.

2. 3. Synthesis of Dimethylbis(p-vinylbenzyl) Silane (DMBVBS)

DMBVBS was synthesized by Grignard coupling reaction of p-vinylbenzyl chloride and dichlorodimethylsilane (Figure. 1).[21] Magnesium (7.3 g, 0.12 mol) and 200 ml THF were placed in a schlenck tube under argon. After addition of dichlorodimethylsilane (12 ml; 0.04 mol) and p-vinylbenzyl chloride (28.2 ml; 0.08 mol) the mixture was carefully stirred to initiate the Grignard reaction. Afterward, the solution was sonicated for 2 h, and then washed consecutively with brine, saturated $NaHCO_3$, brine and finally dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the resulting crude product was purified by flash column chromatography (petroleum ether/diethyl ether 95:5) to yield DMBVBS as a viscous colorless product. The purity of the product was checked and confirmed by ^{1}H and ^{13}C NMR. ^{1}H NMR, δ ppm ($CDCl_3$, 300 MHz): 7.32, 7.29 (d, 4 H, aromatic); 6.98, 6.96 (d, 4H, aromatic); 6.75–6.65 (d × d, 2H, vinyl CH); 5.73, 5.67 (d, 2 H, vinyl CH_2, trans); 5.20, 5.17 (d, 2 H, vinyl CH_2, cis); 2.12 (s, 4H; methylene); –0.02 (s, 6H, methyl); ^{13}C NMR, δ ppm ($CDCl_3$, 300 MHz): 139.96, 133.81 (aromatic quaternary); 136.99 (vinyl CH_2); 128.57, 126.42 (CH, aromatic); 112.45 (vinyl, CH); 25.34 (methylene); –3.61 (methyl).

2. 4. Silanization of Borosilicate Glass Columns

The borosilicate glass columns were silanized first by sonicating them in a mixture of 1:1 ethanol/acetone, then etched by soaking them in 2 M KOH/ethanol solution over night at temperature of 60 °C. The following day, these columns were washed with frequent amount of water while sonication till reaching neutral effluents, then dried under high vacuum for six hours. A mixture (1:1, v:v), of 0.01% of 2,2-diphenyl-1-picrylhydrazyl hydrate in dimethyl formamide (DPPH/DMF) and 3-(trimethoxysilyl)propyl methacrylate, was filled into the glass columns, which were sealed using 2-ml Eppendorf vials, and kept to react at temperature of 100 °C for six hours. These columns were sonicated in acetone, water then ethanol for 15 minutes each, then dried under high vacuum for six hours and stored under argon for further use.

2. 5. Preparation of Monolith

Specific amounts of *p*-methyl styrene, dimethylbis(*p*-vinylbenzyl) silane, 2-propanol and toluene were

added to α,α´-azoisobutyronitrile (AIBN) as initiator (Table 1). The reaction mixture was degassed and sonicated for 15 minutes to obtain a clear homogeneous solution. Then it was introduced in a presilanized borosilicate glass column (100 × 3 mm I.D.), and sealed using eppendorf 2-ml vials. The monomers were left to polymerize at 65 °C for 24 hours. Copolymerization process is shown in Figure 1. The resulting monolith cut flat at both ends and the fitting were placed, and the whole monolith was put in the stainless steel housing then flushed with ACN for one hour to remove porogens and unreacted monomers and conditioned for further analysis.

Table 1. Recipes, porosity asset and the pressure drop of monoliths 1–3 (100 × 3 mm I.D.).

Monolith[1]	AIBN wt.-%	ε_p[2]	ε_z[3]	ε_t[4]	Pressure drop[5] (MPa)
1	0.5	0.13	0.78	0.91	3.40
2	1.0	0.16	0.72	0.88	3.60
3	2.0	0.25	0.60	0.86	3.80

[1] *p*-methylstyrene**,** dimethylbis(*p*-vinylbenzyl)silane, 2-propanol and toluene as 18.5, 18.5, 55, 8 as Vol-%. [2] ε_p: volume fraction of pores, [3] ε_z: volume fraction of inter-microglobule void volume, [4] ε_t: total volume fraction of pores, [5] Pressure drop at flow-rate of 1.5 ml/min of solvent of 15 % ACN/water.
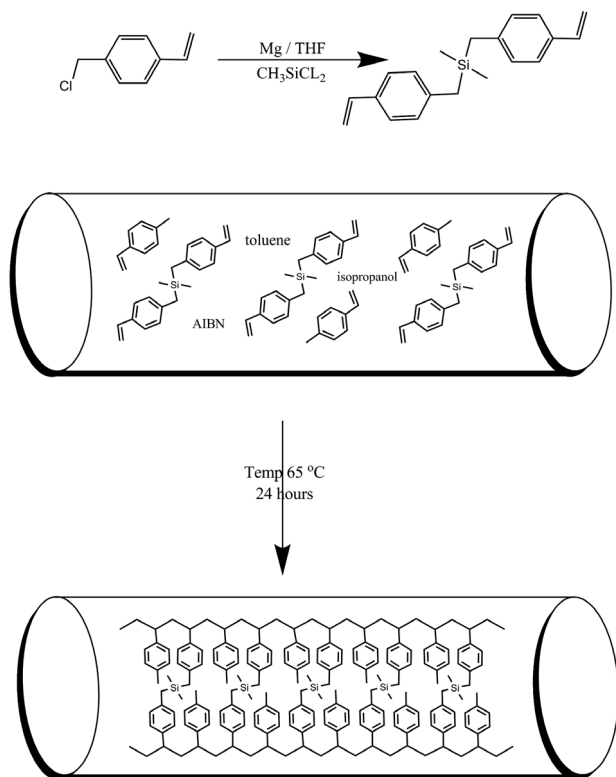

Figure 1. Crosslinker synthesis and monolith preparation

3. Results and Discussion

Three selected wide-bore monoliths (100 × 3 mm I.D.) were prepared and optimized for chromatographic separation of peptides and proteins by changing the initiator mass content (Table 1). Other monoliths of 35 and 40 mass-% monomers were prepared applying 2% initiator; however, the earlier showed poor mechanical stability. The latter was highly rigid but lacked good solvent permeability because of higher initiator content forming smaller microglobule that lead to high back pressure and consequently low solvent flow-through. In a previous study, monoliths were prepared of different recipe; however the initiator content was half amount of that for monoliths prepared in this study, which reasonably lead to different monolith properties.[21] Also these monoliths were characterized by scanning electronic microscope (SEM), and studied for swelling propensity and pressure drop *vs.* flow-rate.

3. 1. Mechanical Stability, Swelling Propensity and Monolith Permeability

The mechanical stability of monoliths 1–3 (see composition in Table 1) was studied by checking of the linearity of monolith pressure drops at different flow-rates. The linearity of this plot (Figure 2) was of (r^2 =) 0.9975, 0.9949, and 0.9882 using solvents of water, tetrahydrofuran and acetonitrile, respectively. The column backpressure for these monoliths is relatively low at high flow-rates saving the good mechanical stability (Table 1). The monolith permeability calculated for monolith 3 ($B_0 = 3.2 \times 10^{-10}$ m^2/s^2) is very good compared to reported values for other monoliths.[22]

An additional common check for the stability of a material in different solvents is the swelling propensity test.[17] The swelling propensity (SP) is a criterion for the swelling behavior of organic material which might lead to problems such as poor stability of chromatographic co-
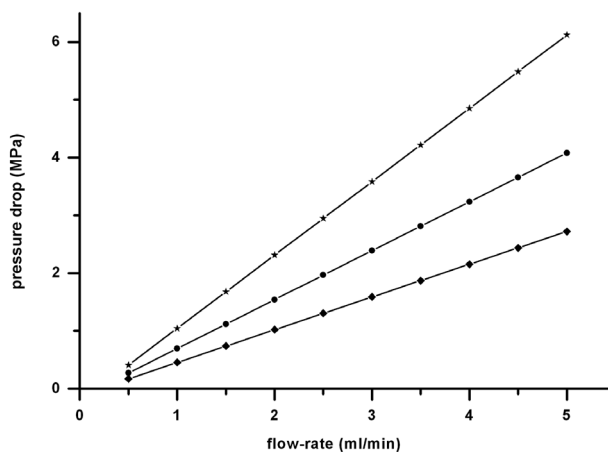

Figure 2. Plot of pressure drop vs. flow-rate for * water, ● tetrahydrofuran, and ◆ acetonitrile performed on borosilicate glass monolith 3 (100 × 3 mm I.D.).

lumns, rapidly causing reduced efficiency and loss of resolution. The reproducibility of the retention times may also be affected. Polymer-based packing materials are more or less sensitive to solvent changes. Tetrahydrofuran leads to swelling while polar eluents like methanol or acetonitrile result in irreversible shrinking of the packing bed. The SP factor is also a criterion for the shrinkage and swelling of the material in different solvents. The closer the value of the swelling propensity factor to zero, the lower the swelling propensity is, and less shrinkage problems occurs. Water was applied as the mobile phase for 10 minutes and the pressure drop was measured. The mobile phase was switched to THF and the pressure drop was measured again once the system had stabilized. The swelling propensity as determined for monoliths **1–3** applying THF was found to be around 0.55. This value swelling propensity factor is relatively low establishing rigid material; where other monolithic materials reported have SP values ranging from 0.7–1.2,[19,21,23,24] and 3.2–37 for some conventional columns packing materials.[17] The mechanical stability of wide-bore 1,2-bis(p-vinylphenyl)ethane (BVPE) based-monoliths showed stability and reproducibility problems due to low polymer support rigidity.[23] Dimethylbis(*p*-vinylbenzyl)silane based-monoliths showed better performance most probably due to the better mixing of the monomers and porogens solution at room temperature by sonication, as was not the case for BVDE, which necessitated short sonication ti-

me at high temperature in order to avoid early polymerization of the monomers before filled into the column. It is worth mentioning that this material demonstrated good stability and efficient separation for conventional sized-monoliths compared to other materials that was good only for capillary column. The mechanical stability and low swelling propensity might furnish successful up scaling of monolith size for preparation of larger monolith.

3. 2. Monolith Morphology by Scanning Electron Microscopy (SEM)

SEM photos of monoliths 1–3 (Figure 3, see composition in Table 1) indicate a decrease in the microglobule size by increasing initiator content. The increase of the initiator percentage from 0.5 to 1 and 2 w-% lead to a decrease in microglobule diameter from 5 to 2.5 and 1 µm for monoliths 1–3, respectively (Figure 3).

These results were as anticipated, as increasing the initiator content cause faster nucleation than growth, which leads to smaller size microglobule.

3. 3. Influence of Initiator Content on Monolith Porosity and Performance

Inverse size exclusion chromatography (ISEC) seems to be a practical method for checking monolith po-
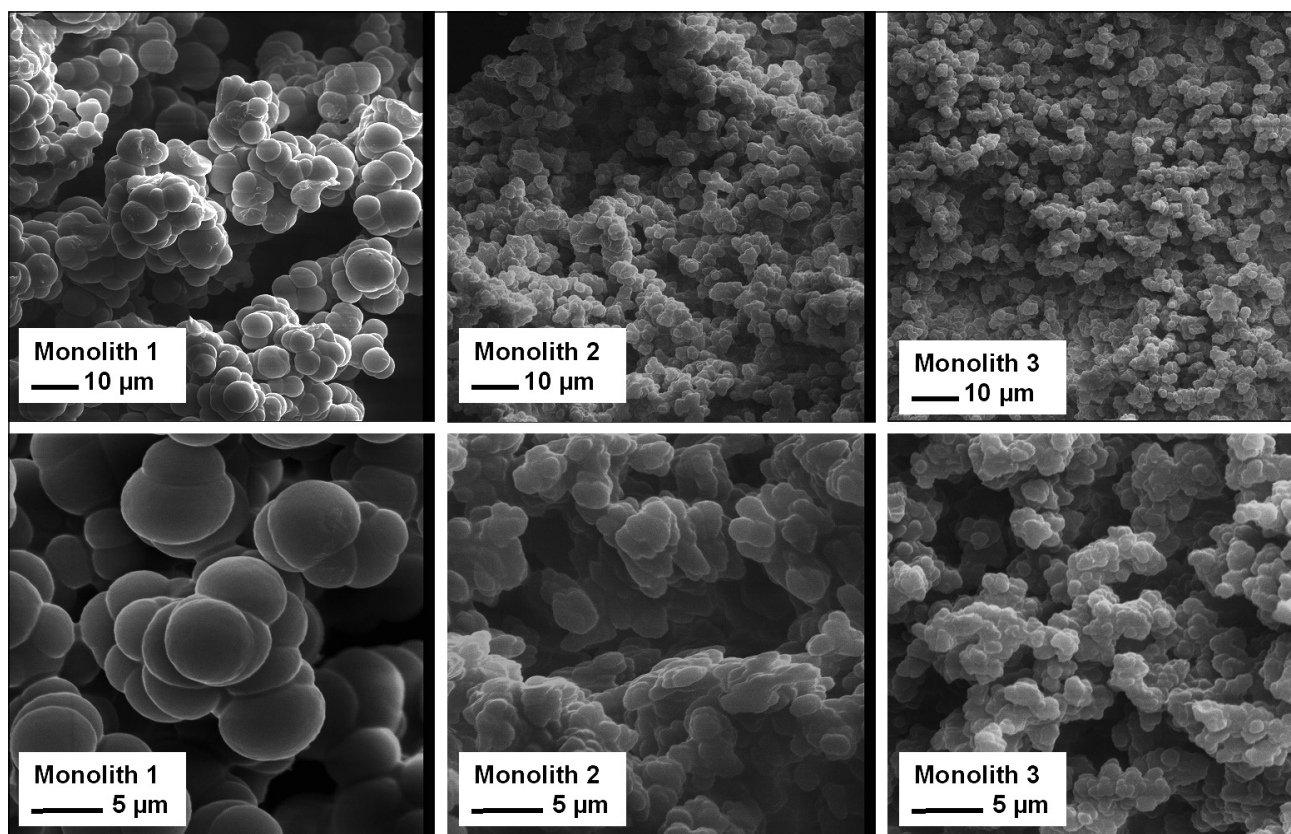

Figure 3. Scanning Electronic Microscopy (SEM) photos of three monoliths 1–3.

rosity, since it is a measure of porosity in the wet state of the monolith which is usually the case for running analysis. Moreover it can measure the whole range of pore size depending on the number and size of the polystyrene samples used.[25] To check the influence of initiator content on the monolith performance and efficiency, three monoliths of the same composition but differing in the amount of initiator were prepared as 0.5, 1.0, and 2.0 wt-% initiator, (Table 1, entries 1–3). Concerning the monolithic porosity asset, the porosity accessible to the mobile phase known as total volume fraction occupied by the mobile phase (ε_t) is classified into two main subcategories. Volume fraction of inter-microglobule void volume (ε_z) and volume fraction of pores (ε_p). The intermicroglobule void volume and volume fraction of pores are necessary for solvent flow and for analytes retention, respectively. So a good monolith performance is reached by compromising these two values to ensure good mesoporosity for analyte retention ($\Theta < 50$ nm) and good macroporosity for solvent flow ($\Theta > 50$ nm). Applying ISEC, it was found that $\varepsilon_p = 0.13$ and $1\varepsilon_z = 0.78$ for monolith 1. The low value of volume fraction of pores (ε_p) and high value of intermicroglobule void volume (ε_z) usually furnishes small surface area that impaires the monolith efficiency. For monoliths 2 and 3, it was found that $\varepsilon_p = 0.16$ and 0.25, and $\varepsilon_z = 0.72$ and 0.60, respectively (Table 1). This implies that a higher abundance of mesopores is established in monolith 2 and more in monolith 3 when the initiator content was increased by 2 and 4 folds from monolith 1 to monoliths 2 and 3, respectively.

These findings were supported by analysis of a mixture of five proteins using monoliths1–3 for comparison purposes. The shape of peaks of the 5-protein mixture analyzed on monolith-1 demonstrated poor peak symmetry with high degree of peak fronting (Figure 4a). Moreover, the peaks of the five proteins separated on monolith 1 were not as sharp as those separated on monoliths 2 and 3, applying identical chromatographic condition. Higher amount of protein sample was eluted in the injection peak incase of monolith 1 compared to monoliths 2 and 3 (Figure 4a-c). Peak fronting was reduced on monolith 2 (Figure 4b) and high symmetry was demonstrated on monolith 3 (Figure 4c). The better values of volume fraction of pores incase of monoliths 2 and 3 compared to monolith 1, resulted in better column efficiency (Figure 3a-b). Furthermore, the peak intensities of the 5 protein analytes we-
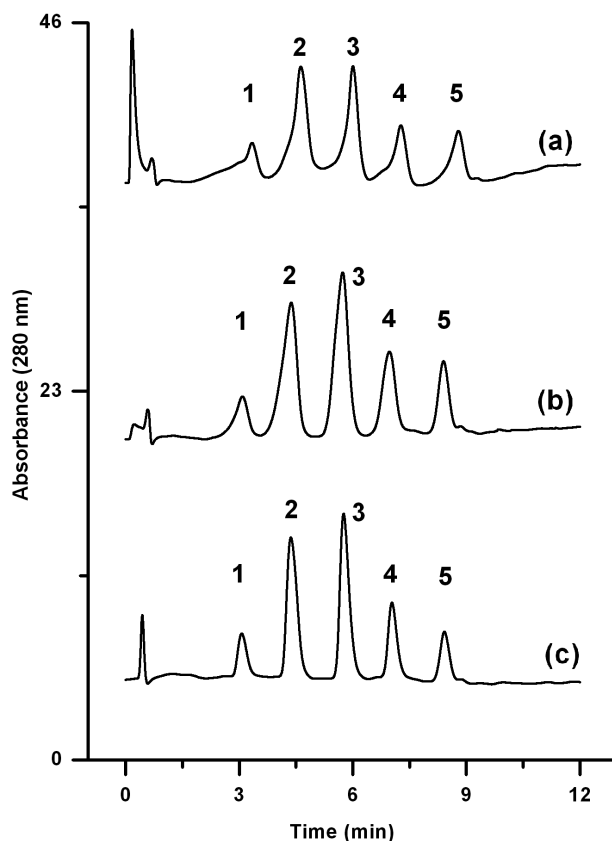

Figure 4. Influence of initiator content on monolith performance as overlay chromatogram of 5-protein mixture separated on **(a)** monolith 1, **(b)** monolith 2, and **(c)** monolith 3. Chromatographic conditions; mobile phase, A: 0.1% TFA/water, B: 0.1% TFA/ACN; gradient, 15–50 % B in 10 minutes; flow-rate, 1.5 ml/min; temperature, 25 °C; λ, **280 nm**; peak identification, (1) ribonuclease, (2) A, cytochrome, C, (3) α-lactalbumin, (4) β-lactaglobulin, (5) ovalbumin; sample, 20 μg each protein.

re sharper in monolith 3 than 2 and 1, and the injection peak was getting smaller. The total volume fraction occupied by the mobile phase (ε_t) were almost comparable for the three monoliths (Table 1) with a difference of ~ 3%, as anticipated where the total monomer content for the three monoliths were equal. Comparing the resolution and peak width at half height supported the improvement of column efficiency when increasing the initiator content (Table2). The Peak width t half height ($\omega_{0.5}$) changed from a range of 19–25 sec in monolith 1 to a range of 13–17 sec in mo-

Table 2. Comparison between retention times (t_R, min), peak half width ($\omega_{0.5}$, sec), and resolution (t_R) of five proteins separated on three borosilicate glass monoliths 1–3 (100 × 3 mm I.D.) showing the influence of initiator on monolith efficiency.

Protein	Monolith 1			Monolith 2			Monolith 3		
	t_R	$\omega_{0.5}$	R_s	t_R	$\omega_{0.5}$	R_s	t_R	$\omega_{0.5}$	R_s
ribonuclease A	3.34	19	1.84	3.09	23	1.54	3.07	13	2.53
cytochrome *c*	4.63	23	1.71	4.37	27	1.64	4.36	17	2.55
α-lactalbumin	6.00	25	1.72	5.73	23	1.77	5.76	16	2.33
β-lactaglobulin	7.27	19	2.19	6.97	19	2.47	7.04	17	2.71
ovalbumin	8.80	23	–	8.40	16	–	8.42	13	–

nolith 3. Moreover resolution increased by almost 35% while maintaining almost similar analysis time (Table 2).

3.4 Separation of Proteins and Peptides.

Five proteins as (1) ribonuclease, (2) A, cytochrome, C, (3) α-lactalbumin, (4) β-lactaglobulin, (5) ovalbumin were analyzed on monolith 3. For chromatographic optimization of protein separation, applying a single step gradient of 15 to 50 % acetonitrile in 10 minutes and a flow rate of 1.5 mL/min (Figure 5a) allowed baseline separation of 5 proteins in less than 9 minutes.
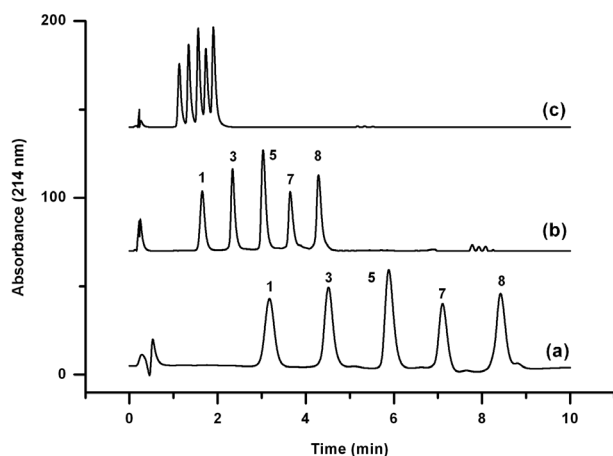

Figure 5. Optimization of chromatographic conditions for the separation of five-protein mixture on borosilicate glass monolith 3 (100x3 mm I.D.). Chromatographic conditions; mobile phase; A: 0.1% TFA/water, B: 0.1% TFA/ACN; **(a)** gradient, 15–50% B in 10 minutes; flow-rate, 1.5 ml/min; **(b)** gradient, 15–50 % B in 5 minutes; flow-rate, 3 ml/min and **(c)** gradient, 15–50% B in 2.5 minutes; flow-rate, 6 ml/min; temperature, 25 °C; λ, **214 nm**; peak identification: (1) ribonuclease A, (2) insulin, (3) cytochrome *c*, (4) lysozyme, (5) β-lactalbumin, (6) bovine serum albumin (BSA), (7) β-lactoglobulin B, (8) ovalbumin; sample, 5 μg each protein.

No loss in efficiency was observed in the separation of these proteins when bridging the gradient time within 10 and 5 minutes and doubling the flow rate to 3 ml/min, resulting in separation within 5 minutes (Figure 5b).

Moreover, by steeping the gradient of 15–50 acetonitrile in 2.5 minutes and increasing the flow-rate to 6 ml/min, good efficiency was maintained and separation time was reduced to less than 2 minutes (Figure 5c). This is supported

by comparing the values of peak width and half height ($\omega_{0.5}$) and resolution (R_s) in both cases (Table 3), where $\omega_{0.5}$ was 5.6 and 3.8 seconds, and the decrease in resolution became in the range of 3.14–3.70 and 1.36–1.73 indicating baseline separation of these proteins. Another concern was a potential sample dilution by the operating column at very high mobile phase velocity. However, the peak intensity of the five analytes in Figure 5a-c demonstrates that there was no loss in sensitivity at high mobile phase flow when the gradient slope and the solvent flowrate were altered proportionately.

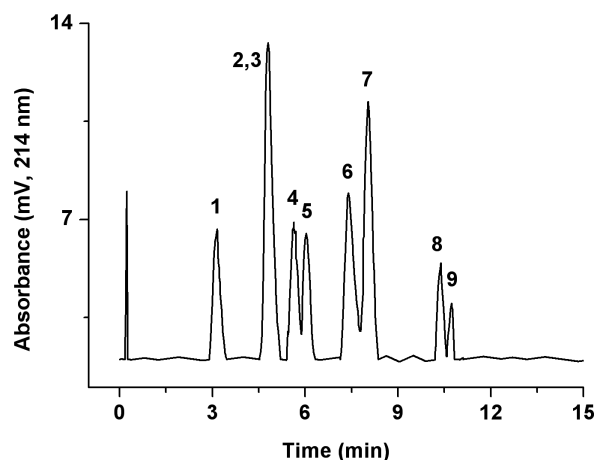Proteins are usually easy to separate but peptides separation represents a challenge. So 9-peptide mixture was

Figure 6. Separation of 9-peptide mixture on borosilicate glass monolith 3 (100 × 3 mm I.D.). Chromatographic conditions; mobile phase, A: 0.1% TFA/water, B: 0.1% TFA/ACN; gradient, 0–10 % B in 7 minutes,10–15% B in 3 minutes; flow-rate, 2 ml/min; temperature, 25 °C; λ, 214 nm; peak identification, (1) bradykinin fragment 1–5; (2) vasopressin [arg8]; (3) methionine enkephalin; (4) leucine enkephalin; (5) oxytocin; (6) bradykinin; (7) LHRH; (8) bombesin; and (9) substance P.; sample, 25 ng each.

applied as criterion for monolith performance. Peptides are considered medium-sized molecules compared to the bulky proteins. Their separation requires specific porosity. A mixture of 9 peptides (of (1) bradykinin fragment 1–5; (2) vasopressin [arg8]; (3) methionine enkephalin; (4) leucine enkephalin; (5) oxytocin; (6) bradykinin; (7) LHRH; (8) bombesin; and (9) substance P.) was separated on monolith 3 (Figure 6). These peptides were clearly separated

Table 3. Comparison of retention times (t_R, min), peak half width ($\omega_{0.5}$, sec), and resolution (t_R) of five proteins separated on borosilicate glass monoliths 3 (100 × 3 mm I.D.) under different chromatographic conditions as in Figure 5a-c.

Protein	Figure a			Figure b			Figure c		
	t_R (min)	$\omega_{0.5}$ (S)	R_s	t_R (min)	$\omega_{0.5}$ (S)	R_s	t_R (min)	$\omega_{0.5}$ (S)	R_s
ribonuclease A	3.177	15	2.86	1.65	7.5	3.14	1.130	3.8	1.69
cytochrome *c*	4.517	13	3.12	2.337	5.6	3.70	1.341	3.8	1.73
α-lactalbumin	5.881	13	3.01	3.031	5.6	3.29	1.557	3.8	1.41
β-lactoglobulin	7.103	11	3.00	3.647	5.6	3.41	1.733	3.8	1.36
ovalbumin	8.417	15	–	4.286	5.6	–	1.903	3.8	–

except for the two peptides of vasopressin [arg8] and methionine enkephalin totally coeluted (Figure 6, Table 4).

Table 4. Retention times (t_R, min), peak half width ($\omega_{0.5}$, sec), and resolution (t_R) of nine peptides separated on borosilicate glass monoliths 3 (100 × 3 mm I.D.).

Peptide	t_R (min)	$\omega_{0.5}$ (S)	R_s
bradykinin fragment 1–5	3.214	30	3.00
vasopressin [arg8]	4.812	34	1.00
methionine enkephalin	–	–	–
leucine enkephalin	5.321	27	3.49
oxytocin	6.803	24	1.35
bradykinin	7.435	32	1.88
LHRH	8.312	24	5.14
bombesin	10.452	26	0.77
substance P.	10.749	20	–

3. 5. Run-to-Run and Batch-to-Batch Reproducibility

To check the run-to-run reproducibility, 15 injections of 5-protein mixture were injected under identical conditions on monolith **3**; only very minor differences in retention times were observed. The calculated *RSD* values for the retention time (t_R) and resolution (R_s) were found to be between 1.1–1.8 and 2.7–3.9% for the five proteins, respectively. To check the long term reproducibility and time stability, monolith 3 was stored in acetonitrile for six months. Then the monolith was conditioned overnight. Another 15 injections of 5-protein mixture were performed preceded 5 blank runs. The RSD in the retention time (t_R) and resolution (R_s) were 1.42–2.34 and 3.43–4.29 for the five proteins, respectively. Furthermore, to check the reproducibility of monolith preparation, three monoliths of recipe 3 (Table 1) were prepared and tested for the separation of the 5-protein mixture. The RSD values for the retention time and resolution ranged from 2.41 to 2.53 % and from 4.12 to 4.43, respectively.

4. Conclusion

Wide-bore polymer based-monolithic support synthesized from *p*-methylstyrene-*co*-dimethylbis(*p*-vinylbenzyl)silane (MS/DMBVBS) suited reversed-phase separation of peptides and proteins. Due to the good stability and low pressure drop experienced on these monolithic supports, fast separation of proteins was accomplished where 5 proteins were separated in less than 2 minutes. These monoliths show high capacity and high performance which suites both analytical and micro-preparative scales reaching analyte doses ranging from ~25 ng for peptides to ~20 μg for proteins. The monolith performance improves by the higher initiator mass content, as a result of

optimized monolith morphology and porosity. The best resolution, peak symmetry and peak intensities were demonstrated for monoliths of initiator content of 2 wt.-percent.

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Povzetek

Na osnovi kopolimera *p*-metilstiren-dimetil-bis(*p*-vinilbenzil)silana smo v steklenih valjih (100 × 3 mm I.D.) pripravili monolitne kolone z velikimi odprtinami. Posamezni monoliti so bili pripravljeni z različnimi količinami iniciatorja. Meritve padca tlaka v odvisnosti od hitrosti pretoka in precej nizek faktor nabrekanja nakazujejo dobro homogeno zamre enje in dobro mehansko stabilnost. Kromatografsko učinkovitost pripravljenih monolitov smo ocenili na osnovi analize mešanice proteinov in peptidov. Tako je separacija petih proteinov potekla v manj kot dveh minutah, medtem ko je bila zmes devetih peptidov ločena v manj kot enajstih minutah, ob hkratni eluciji vazopresina [arg8] in metionin enkefalina.