Anal Bioanal Chem (2011) 399:1845–1852
DOI 10.1007/s00216-010-4578-7

ORIGINAL PAPER

Potential of poly(styrene-*co*-divinylbenzene) monolithic columns for the LC-MS analysis of protein digests

Michiel H. M. van de Meent · Sebastiaan Eeltink · Gerhardus J. de Jong

Received: 20 July 2010 / Revised: 1 December 2010 / Accepted: 2 December 2010 / Published online: 24 December 2010

Abstract Two polystyrene-based capillary monolithic columns of different length (50 and 250 mm) were used to evaluate the effects of column length on gradient separation of protein digests. A tryptic digest of a 9-protein mixture was used as a test sample. Peak capacities were determined from selected extracted ion chromatograms, and tandem mass spectrometry data were used for database matching using the MASCOT search engine. Peak capacities and protein identification scores were higher for the long column with all gradients. Peak capacities appear to approach a plateau for longer gradient times; maximum peak capacity was estimated to be 294 for the short column and 370 for the long column. Analyses with similar gradient slope produced a ratio of the peak capacities of 3.36 for the long and the short column, which is slightly higher than the expected value of the square root of the column length ratio. The use of a longer monolith improves peptide separation, as reflected by higher peak capacity, and also increases protein identification, as observed from higher identification scores and a larger number of identified peptides. Attention has also been paid to the peak production rate (PPR, peak capacity per unit time). For short analysis times, the short column produces a higher PPR, while for analysis times longer than 40 min, the PPR of the 250-mm column is higher.

Keywords Capillary LC · Mass spectrometry · Peak capacity · Monoliths · Protein digests

Electronic supplementary material The online version of this article (doi:10.1007/s00216-010-4578-7) contains supplementary material, which is available to authorized users.

M. H. M. van de Meent (✉) · G. J. de Jong
Division of Biomedical Analysis,
Department of Pharmaceutical Sciences, Utrecht University,
P.O. Box 80082, 3508 TB Utrecht, The Netherlands
e-mail: m.h.m.vandemeent@uu.nl

S. Eeltink
Department of Chemical Engineering, Vrije Universiteit Brussel,
Pleinlaan 2,
1050 Brussels, Belgium

Introduction

Identification of proteins by mass spectrometry (MS) of proteolytic digests is an important tool in proteomics research. In order to obtain a maximum amount of data from a digest sample, a separation of the digest prior to introduction into the mass spectrometer is necessary. Due to its high resolution and ease of coupling with (nano)electrospray ionisation-MS, reversed-phase liquid chromatography (LC) is the method of choice for the separation of peptide mixtures. Columns packed with C18-bonded silica particles are most widely used for peptide separation, but organic polymer-based materials, like styrene/divinylbenzene copolymers (PS-DVB), are also employed.

Attempts to increase the throughput of protein identification from LC-MS experiments can be broadly divided into two categories. The first strategy is improving the separation efficiency of the LC column by either increasing column length [1, 2] or reducing the particle size of the column packing material [3–5]. These approaches offer high efficiency separations but suffer from high back-pressure, which necessitates either the use of low flow rates, leading to long run times, or the use of special equipment compatible with the high pressure. The second approach for increase of the throughput is using high-porosity materials which allow very fast analyses. Monoliths are a highly promising type of column materials, possessing a bimodal pore structure with large throughpores

and shallow mesopores. These unique properties result in high permeability (low column backpressure) and enhanced mass transfer (improved chromatographic efficiency) [6]. This allows the use of either high flow rates for fast separations or relatively long columns combined with conventional LC apparatus. Moreover, the high mass transfer makes monolithic columns well suited for the analysis of macromolecules [7].

Monolithic columns are available in several stationary phase chemistries. The first (polymeric) monoliths were synthesised in the early 1990s, first as methacrylate-based membranes [8], later also as methacrylate- and polystyrene-based rods [9, 10]. In 1996, a silica-based monolith was synthesised for use in HPLC [11]. Due to the absence of stagnant mesopores in the polymer backbone, these stationary phases typically offer high-efficiency peptide separations (no intra-particle mass transfer). The efficiency can be optimized further by tuning the morphology, while covalent attachment of the monolith against the fused-silica capillary wall ensures high robustness and eliminates channelling effects. While the advantages of using longer columns have been proven for silica-based monoliths [12–16], the use of polymer-based monoliths has been limited to the application of short (5–6 cm) PS-DVB-based columns for fast separations of relatively simple mixtures [7, 17, 18].

Evaluation of the performance of gradient separations is usually carried out by comparison of peak capacities (PC). Peak capacity was first defined by Giddings [19] as the maximum number of peaks that will fit within the applied elution window with a resolution of 1.0. The concept was adapted for gradient chromatography by Horváth and Lipsky [20]. Since peak width is about constant throughout a gradient separation, the experimental peak capacity can be calculated using Eq. 1:

$$\mathrm{PC} = 1 + \frac{t_\mathrm{G}}{w_\mathrm{av}} \tag{1}$$

where t_G is the gradient time and w_av is the average peak width (4σ). For large values of PC, this approaches $t_\mathrm{G}/w_\mathrm{av}$. When comparing gradient separations using columns of different length, one should also compare different gradients. A theoretical description of peak capacity is given in Eq. 2 [21]:

$$\mathrm{PC} = 1 + \frac{\sqrt{N}}{4}\,\frac{S\Delta\varphi}{S\Delta\varphi(t_0/t_\mathrm{G}) + 1} \tag{2}$$

where N is the column plate number, S is the slope of the plot of the natural logarithm of the retention factor versus solvent composition and $\Delta\varphi$ is the change in volume fraction organic modifier during the gradient. When stationary phase and gradient composition are constant, PC is only dependent on t_0/t_G and $\sqrt{N}$ (proportional to column length). Thus, the effect of column length can be evaluated when the ratio t_0/t_G is held constant, i.e. by scaling t_G to the column length. When comparing columns in this way, the peak capacity ratio should be close to the square root of the column length ratio [21].

An alternative means of evaluating the efficiency of a digest separation is by the identification of the proteins using database search engines like SEQUEST [22] or MASCOT (http://www.matrixscience.com) [23]. The number of identified peptides and the degree of protein sequence coverage are an indication of separation performance. Co-elution of peptides can lead to mutual ion suppression and the loss of sequence information when too many peptides co-elute [24].

In this paper, capillary poly(styrene-divinylbenzene) monolithic columns of two different lengths are compared for the LC-MS analysis of a tryptic digest of a 9-protein mixture. The digest contains approximately 175 peptides with a mass higher than 500 Da, including several peptides larger than 2,500 Da and a number of peptides containing known phosphorylation sites. A standard PS-DVB monolithic column of 50×0.2 mm was compared to a new 250×0.2 mm monolith using gradients between 5 and 300 min. Column performance was evaluated with regard to peak capacity, peak production rate (PPR, peaks per minute) and MASCOT identification scores.

Experimental

Materials and reagents

Cytochrome *c* (bovine), serum albumin (bov.), β-lactoglobulin A (bov.), carbonic anhydrase (bov.), lysozyme (chicken), myoglobin (horse), ribonuclease A (bov.), α-lactalbumin (bov.), trypsin (porcine, type IX-S, EC 3.4.21.4) and 1,4-dithiotreitol (DTT) were purchased from Sigma (St. Louis, MO, USA). Catalase (bov.), MS grade formic acid (FA) and acetonitrile (ACN), iodoacetamide (IAA) and ammonium hydrogen carbonate were obtained from Fluka (Buchs, Switzerland). All solutions were prepared using water from a Synergy UV water purifying system (Millipore, Bedford, MA, USA).

All reagents for the digestion of the protein mixture were prepared in 200 mM NH_4HCO_3 buffer (pH 8) unless stated otherwise. Protein stocks were prepared in water at 5.0 mg/ml for BSA and 2.0 mg/ml for all other proteins, resulting in protein concentrations as listed in Table 1 [25]. A protein mixture was prepared by mixing equal amounts of the stock solutions and adding an extra aliquot of water to achieve a tenfold dilution. The mixture was digested as follows: 100 µl of the protein solution was set to pH 8 by addition of 25 µl of a 1-M NH_4HCO_3 buffer (pH 8). After

Table 1 Composition of the protein stock solution

Protein	Concentration (μM)
Cytochrome *c*	161.8
Bovine serum albumin	75.2
β-Lactoglobulin A	108.9
Carbonic anhydrase	68.9
Catalase	34.7
Lysozyme	139.8
Myoglobin	118.0
Ribonuclease A	146.2
α-Lactalbumin	141.0

addition of 25 μl of a 10-mM DTT solution, the sample was incubated at 50 °C for 30 min to reduce disulfide bonds. After cooling to room temperature, 25 μl of a 30-mM IAA solution was added, and the sample was incubated in the dark for 60 min to alkylate the free thiols. Trypsin was dissolved in 10 μl of NH_4HCO_3 buffer to obtain a trypsin-to-protein mass ratio of 1:50 in the final solution, and the trypsin solution was added to the sample, which was then incubated overnight (16 h) at 37 °C. The digestion was stopped by the addition of 15 μl of 50% FA, and the digest was stored at −20 °C until further use. Before injection, the digest was diluted five times by addition of mobile phase A (water+0.5% FA), resulting in a 100-fold dilution of the original protein stock concentrations (0.35–1.62 nM).

Apparatus and LC columns

All analyses were performed using an Agilent 1100 nanoLC system (Waldbronn, Germany), consisting of a vacuum degasser, a binary Nano-Pump, a μ-well plate sampler and a thermostatted column compartment, which was maintained at 60 °C throughout the experiments. UV detection during gradient optimisation experiments was performed using an MU 701 UV-VIS detector (ATAS GL International, Veldhoven, The Netherlands), equipped with an external optical-fibre flow cell (6 nl, 3 mm light path)with detection at 215 nm. MS detection was performed using an Agilent LC/MSD trap XCT-ultra (Waldbronn, Germany) ion-trap mass spectrometer, equipped with an orthogonal ESI interface. PS-DVB monolithic capillary columns were obtained from Dionex Corporation (Amsterdam, The Netherlands); the short column was 50 mm×200 μm i.d., and the long column, 250 mm×200 μm i.d.

Method and data analysis

LC solvent A was water+0.5% formic acid (*v*/*v*); solvent B was water/ACN (1:1)+0.5% FA (*v*/*v*). After injection of 1.0 μl of the digest (0.35–1.62 pmol per peptide, assuming complete digestion), the sample was trapped on the top of the column, and the gradient was started. All separations were performed in duplicate in full-scan mode for peak capacity determination and a third time in MS fragmentation mode for protein identification. Gradient conditions were evaluated by a 30-min analysis of a BSA tryptic digest using the short column in the LC-UV system. The final gradient was 2–62% solvent B (1–31% ACN) with gradient times varying from 5 to 300 min at a flow rate of 3.0 μl/min, resulting in a backpressure of approximately 40 bar on the 50-mm column and 130 bar on the 250-mm column. Electrospray conditions were optimised using both a 30-min gradient separation of a reference BSA digest as well as direct infusion of the same digest dissolved in 5% solvent B at a flow rate of 5 μl/min. The quality of the signal was judged by visual assessment of the intensity of various peptide signals over an *m/z*-range of 400–2,000. An electrospray voltage of −4.00 kV was used for all experiments; MS spectra were acquired at an *m/z* range of 400–2,000. MS/MS was performed in data-dependent mode, with fragmentation of the three most intense ions and dynamic exclusion for a 0.5-min period after three scans. Fragment spectra were acquired over a 100–2,200-*m/z* range.

Peak capacities were calculated according to Eq. 1. The average peak width (4σ) was calculated using the peak width at half maximum (2.35σ) determined from extracted ion chromatograms of ten peptides from different parts of the gradient, representing seven of the nine proteins in the mixture. The peptides used are shown in Table 2, with the numbers corresponding to those in Fig. 1. The effect of separation efficiency on protein identification was evaluated using the MS/MS ions search feature of the MASCOT search engine (http://www.matrixscience.com) [23]. LC-MS/MS data files were converted to MASCOT generic format (.mgf) using the XCTs data analysis software. The peak lists were searched against the SwissProt database for tryptic peptides from all Chordata in the database, allowing two missed cleavages per peptide and containing carbamidomethyl cysteine as a fixed modification and Met-oxidation and Ser/Thr/Tyr-phosphorylation as variable modifications. Peptide mass tolerance was set to ±1.2 Da, MS/MS tolerance to ±0.9 Da.

Results and discussion

Chromatographic efficiency

Column-to-column repeatability for short monolithic column was demonstrated previously [26]. The repeatability of the 25-cm column was assessed by LC analysis of a cytochrome *c* digest. Two separate batches of seven monolithic columns

Table 2 Peptides used for peak capacity calculations

Number[a]	Peptide	*m/z*	Protein
1	EDLIAYLK	482.77 (2+)	Cytochrome *c*
2	TGQAPGFSYTDANK	728.84 (2+)	Cytochrome *c*
3	LVNELTEFAK	582.32 (2+)	BSA
4	HLVDEPQNLIK	653.36 (2+)	BSA
5	LSFNPTQLEEQCHI	858.40 (2+)	β-Lactoglobulin A
6	VLDALDSIK	487.28 (2+)	Carbonic anhydrase II
7	GTDVQAWIR	523.27 (2+)	Lysozyme
8	FESNFNTQATNR	714.83 (2+)	Lysozyme
9	VEADIAGHGQEVLIR	803.93 (2+)	Myoglobin
10	LDQWLCEK	546.26 (2+)	α-Lactalbumin

[a] Numbers correspond to numbered peaks in Fig. 1

were used for separation of the digest using a 7.5-min water-acetonitrile gradient at a flow rate of 2 μl/min. Table 3 shows the variation in retention time measured for a late eluting peptide. RSD values were below 1%, and the difference in average retention time between the two batches is not statistically significant.

Choice of the right gradient conditions is an important step in optimisation of the peak capacity. Therefore, it is necessary to gain insight in the influence of gradient slope on peak capacity for different column lengths. Retention times and peak widths for three tryptic peptides are listed in Electronic Supplementary Material Table S1. Base peak chromatograms for the 30-min gradient on both columns and the 150-min gradient on the long column are shown in Fig. 1a–c. A blow-up of the extracted ion chromatograms of peptide 5 (*m/z* 858.40) and a second peptide (*m/z* 545.40) is inserted in the figures to demonstrate the gain in resolution when using the long column. The peak capacities for these analyses are 79 and 99 for the short gradient on the short and long columns, respectively, and 266 for the long gradient. Comparison of peak capacities for the short (30 min gradient) and long (150 min gradient) columns results in a peak capacity ratio of 3.36, which is about 50% higher than the expected value of $\sqrt{5}$. This number is comparable to results obtained in the comparison of C18-silica monoliths of different length [16].

Higher peak capacities were obtained for the long column for all gradient lengths. PC increases rapidly for short gradients and appears to level off, approaching a maximum at longer gradient times (Fig. 2). This results from the observed linearity between peak width (4σ) and gradient time over the gradient range used in these experiments (Fig. 3). The smaller peak widths obtained for the long column result in higher peak capacities. The peak capacity appears to approach a plateau at long gradient times, this PC_{max} can be estimated under the assumption that w_{av} vs. t_G remains linear at very long gradient times. Where w_{av} vs. t_G is linear, the relationship is described by the following equation:

$$w_{av} = a \cdot t_G + b \tag{3}$$

where a is the slope and b represents the y-intercept. Combining this with Eq. 1 gives

$$PC = \frac{t_G}{a \cdot t_G + b} \tag{4}$$

For very long gradient times, b becomes negligible compared to $a \cdot t_G$, and thus,

$$PC_{max} \approx \frac{t_G}{a \cdot t_G} = \frac{1}{a} \tag{5}$$

Therefore, PC_{max} for a given separation system can be estimated from the slope of the w_{av} vs. t_G plot (Fig. 3). Maximum peak capacities are 294 and 370 for the short and long column, respectively.

Direct comparison of the performance of polystyrene and silica-based monoliths is difficult since column lengths usually differ; the two materials display different retention properties and are typically run under different temperatures (ambient temperature for C18-silica, 60 °C for PS-DVB). However, there does not seem to be any indication that C18-silica monoliths outperform the PS-DVB columns, as suggested in literature [27]. While the peak capacities obtained for the C18-silica monolith in [27] are similar to those we found earlier [16], the authors find much lower values for the PS-DVB column. A peak capacity of 65 was reported for a gradient of 0.25%ACN min^{-1}, whereas the peak capacity at such a gradient would be around 170 in our system. The difference in peak capacities might be the result of using different temperatures. The peak capacity in [27] was obtained at ambient temperature, while our experiments were carried out at 60 °C. A higher temperature will increase separation efficiency as it reduces the

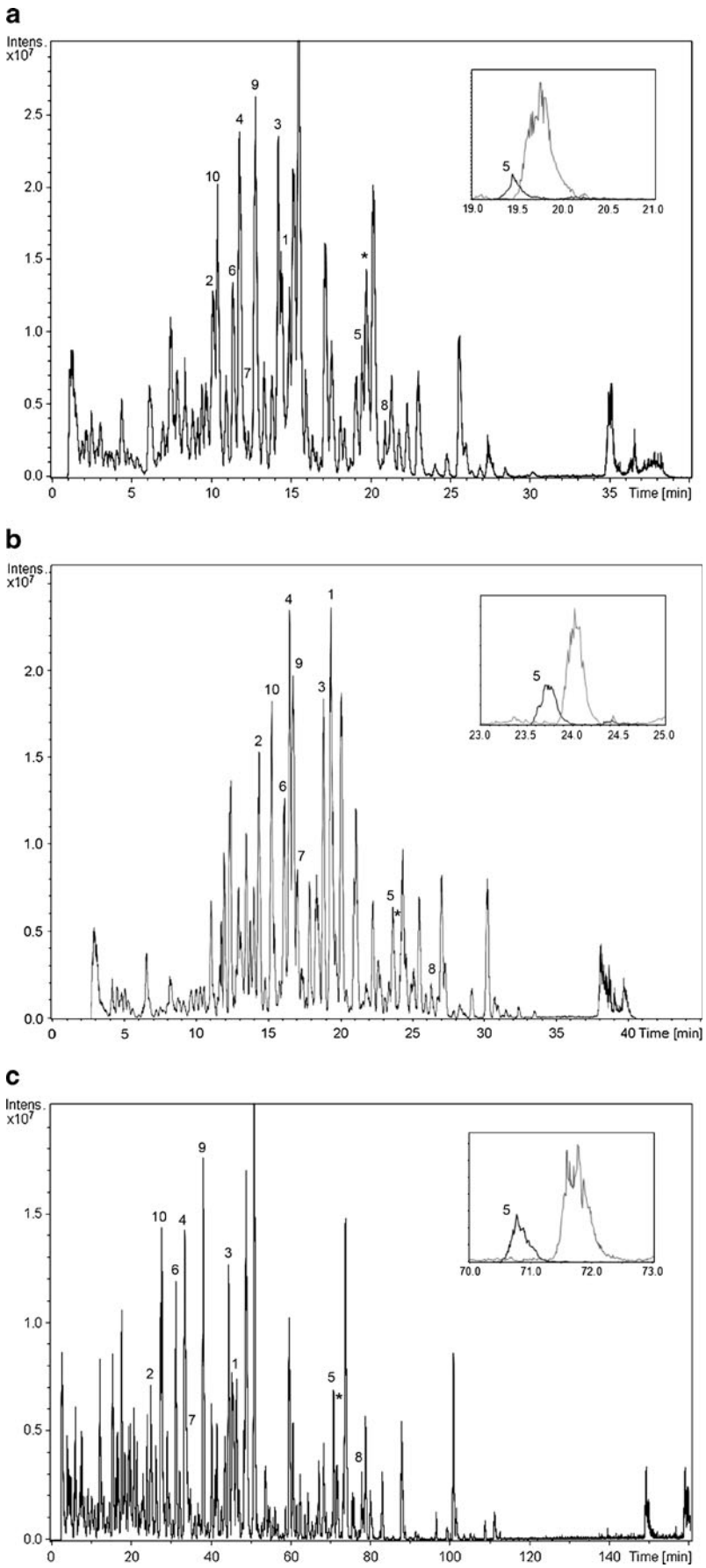

Fig. 1 Base peak chromatograms of a 9-protein digest mixture, separated on PS-DVB monolithic columns of 50× 0.2 mm i.d. (**a**) and 250×0.2 mm i.d. (**b**, **c**), using a gradient of 1–31% ACN in water (both ACN and water containing 0.5% FA). **a**, b Thirty-minute gradient; (**c**) 150-min gradient. *Numbered peaks* correspond to the peptides in Table 2. *Inserts* blow-up of extracted ion chromatograms of peptide 5 (*m*/*z* 858.40) and a peak with *m*/*z* 545.40

Table 3 Repeatability of two different batches of 25 cm×0.2 mm i.d. PS-DVB monolithic columns

	Batch 1	Batch 2
Mean t_r (min)[a]	13.75	13.86
RSD (%)[b]	0.95	0.80

[a] Late eluting peptide in a cytochrome *c* digest

[b] $n=7$

resistance to mass transfer by higher diffusivity of the analytes. This is in accordance with literature data [28, 29]. However, pilot experiments using a BSA tryptic digest and UV detection indicated that increasing the temperature from ambient to 60 °C will only yield a gain in PC of approximately 15% (data not shown). A second explanation for the observed difference may be differences in flow rate, Guryča et al. used 300 nl/min for a 100-μm ID column, while the flow for our 200 μm ID column was 3 μl/min. It is difficult to predict the exact effect of using a 2.5-fold higher linear flow rate on peak capacity as changing the column temperature typically also changes the optimum flow rate for the column.

Peak production rate

Another way to judge LC performance is by evaluating the number of peaks separated per unit of time. This is commonly called productivity [30] and is most easily calculated by dividing peak capacity by total analysis time. The performance of different columns can be judged by comparing the productivity of the columns for a fixed

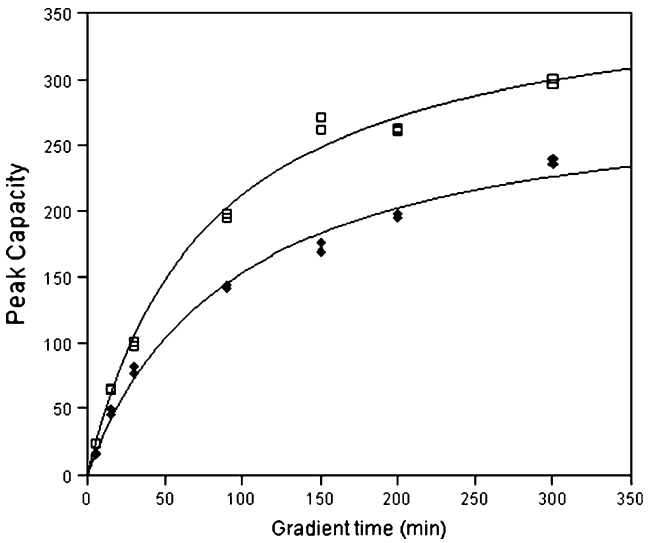

Fig. 2 Peak capacity vs. gradient time for long and short PS-DVB columns, 1–31% ACN gradient in water (both ACN and water containing 0.5% FA). *Diamonds* 50×0.2 mm i.d. column, *squares* 250×0.2 mm i.d. column

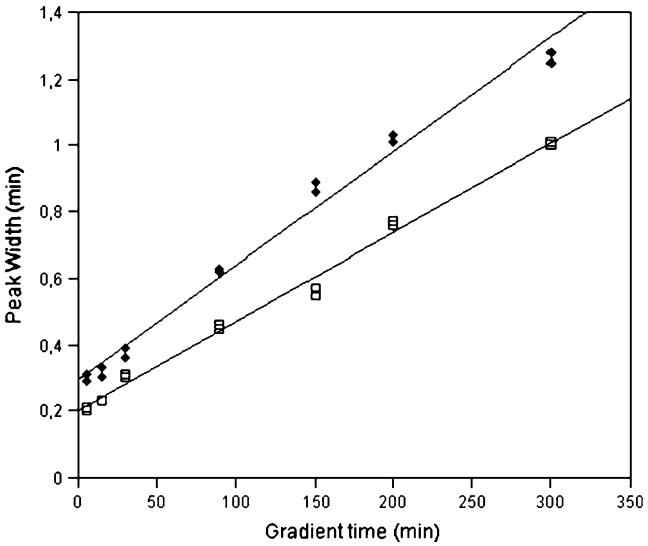

Fig. 3 Peak width (4σ) vs. gradient time for long and short PS-DVB columns, 1–31% ACN gradient in water (both ACN and water containing 0.5% FA). *Diamonds* 50×0.2 mm i.d. column, *squares* 250×0.2 mm i.d. column

analysis time, e.g. 20 min for a fast analysis and 300 min for an analysis with high peak capacity. The gradient time is easily calculated by subtracting the system dead time (t_0) and the equilibration time (t_{eq}). For longer columns, t_0 and t_{eq} will be longer and thus limit the available gradient time. As monolithic columns contain only shallow mesopores, they require less equilibration time than packed columns. Five times the column volume seems a reasonable estimate. System t_0 was determined to be 1.06 min for the short column and 2.72 min for the long column. Column dead times were 0.42 min for the short column and 2.08 min for the long column, and void volumes of 1.2 and 6.0 μl, respectively, were calculated from these values. Peak capacities can be calculated from t_G using Eq. 4, and the productivity or PPR is obtained by dividing this peak capacity by the total analysis time (Table 4). Figure 4 shows PPR (calculated from averages of measured PC values) vs. total analysis time for both columns. The PPR increases rapidly with the increasing PC for short gradients. The highest PPR is obtained for short analysis times using the short column. For longer analysis times, the relative contribution of t_0 and t_{eq} to the total analysis time is reduced, and the PPR of the long column exceeds that of the short column as a result of the higher peak capacity. This effect is observed after a total analysis time of approximately 40 min, where peak capacities for both columns are the same despite the 10-min difference in gradient time. A maximum PPR for the short column is obtained for an analysis time of about 20 min, while the long column shows a maximum at approximately 50 min. After this optimum, the PPR decreases as the PC approaches a plateau for longer gradient times. The

Table 4 Peak capacity and productivity of digest separation on PS-DVB capillary monolithic columns for different analysis times

Total analysis time (min)	L[a] (mm)	t_0[b] (min)	t_G[c] (min)	t_{eq}[d] (min)	PC[e]	PC/time (min^{-1})
20	50	1.1	16.9	2.1	48	2.4
20	250	2.7	6.9	10.4	31	1.6
100	50	1.1	96.9	2.1	155	1.5
100	250	2.7	86.9	10.4	199	2.0
300	50	1.1	296.9	2.1	227	0.8
300	250	2.7	286.9	10.4	294	1.0

L column length, t_0 system dwell time, t_G gradient time, t_{eq} column equilibration time, *PC* peak capacity

absolute difference in PPR between the two columns is lower for longer analysis times, but the PPR of the long column will always be about 25% higher because of the higher PC at long gradient times.

Mass spectrometry

Data files from the LC-MS/MS experiments were searched against the SwissProt database using the MS/MS ions search feature of the MASCOT search engine. Identification scores for both columns using various gradient times are summarised in Table 5, and scores for individual proteins are presented in Electronic Supplementary Material Table S2. Both the cumulative score and the number of identified peptides generally increase with gradient length, with a plateau at long gradient times where the peak capacity is high with respect to the number of peptides in the digest. For all gradient lengths, more peptides are identified using the long column than using the short column, which is expected since peak capacities are also higher for the long column. MASCOT scores are also higher for the long column and increase with longer gradients. The highest identification scores are obtained for BSA, which is the largest protein in the mixture. The highest sequence coverage is obtained for cytochrome *c* (60%). This can be explained by the fact that cytochrome *c* is easily digested using trypsin, and most peptides are in the 500–2,500-Da mass range.

For all gradients and both columns, at least eight proteins have been identified. Ribonuclease A was identified by only one or two peptide matches for the analyses with the long column but not identified for four gradients using the short column. The fact that ribonuclease is only identified in analyses in which separation performance is high indicates that only a few identifiable ribonuclease peptides are present in the digest. This could be the result of low digestion efficiency or the composition of the digest. A ribonuclease tryptic digest contains only a few peptides with an *m/z* between 600 and 1,200, which is the optimum range for this analysis.

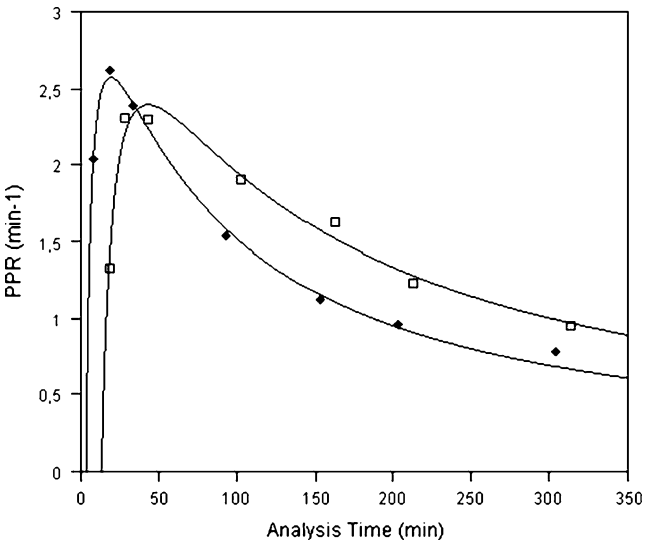

Fig. 4 Peak production rate (PPR) vs. total analysis time for long and short PS-DVB columns. *Diamonds* 50×0.2 mm i.d. column, *squares* 250×0.2 mm i.d. column. PPR is the ratio of the peak capacity and the total analysis time composed of the system dead time t_0, the gradient time t_G and the column re-equilibration time t_{eq} (see Table 3 for values of t_0 and t_{eq})

Conclusions

Our experiments have shown that monolithic columns are very well suited for the analysis of protein digest mixtures. Both chromatographic efficiency and protein identification have been compared for poly(styrene-*co*-divinylbenzene) monoliths of different lengths. As expected, the longer column produces higher peak capacities than the standard

Table 5 Protein identification data

Column	50×0.2 mm		250×0.2 mm	
t_G (min)	Score[a]	Peptides[b]	Score[a]	Peptides[b]
5	1,372	32	1,641	37
15	2,141	44	2,681	55
30	2,115	45	2,968	65
90	2,832	58	3,385	78
150	2,794	58	3,491	78

[a] Cumulative MASCOT score over all identified proteins

[b] Total number of identified peptides

(short) column, which was also observed for C18-bonded silica monoliths [15, 16]. For the relatively short PS-DVB monoliths, higher peak capacities were obtained than for the silica-based monoliths if comparable gradient times were used, but this difference can be partly attributed to difference in column temperature. Peak capacities are comparable to those obtained using 15-cm C18-silica particulate columns [31, 32], but as a result of the faster re-equilibration, the PPR of the PS-DVB monolith is higher. As was also observed for C18-bonded silica monoliths [15, 16], the gain in peak capacity for analyses on both columns with equal gradient steepness (30 min for the short column and 150 min for the long column) is higher than the expected value of $\sqrt{5}$. This may be explained by a small difference in average macropore size of the two monolithic columns. The higher peak capacities obtained with the long column are accompanied by better protein identification with higher MASCOT scores and a larger number of identified peptides for all gradients.

For short analysis times, the short PS-DVB column achieves a higher PPR than the long column. For analysis times above 40 min, the higher peak capacity of the long column compensates for the longer equilibration time, which limits the gradient time. For high throughput, it is advisable to employ fast analyses using the 50-mm column, thus exploiting the high PPR at short analysis time. For high efficiency separations, where analysis time is less important, the 250-mm column offers a high peak capacity and a higher PPR than the 50-mm column.

Acknowledgements This work was supported by The Netherlands Proteomics Center (http://www.netherlandsproteomicscenter.nl). The authors would like to thank Dionex Corporation for generously providing the PS-DVB monolithic capillary columns.

References

1. Shen Y, Zhao R, Belov ME, Conrads TP, Anderson GA, Tang K, Pasa-Tolic L, Veenstra TD, Lipton MS, Udseth HR, Smith RD (2001) Anal Chem 73:1766–1775
2. Shen Y, Zhao R, Berger SJ, Anderson GA, Rodriguez N, Smith RD (2002) Anal Chem 74:4235–4249
3. MacNair JE, Patel KD, Jorgenson JW (1999) Anal Chem 71:700–708
4. Shen Y, Smith RD, Unger KK, Kumar D, Lubda D (2005) Anal Chem 77:6692–6701
5. Tolley L, Jorgenson JW, Moseley MA (2001) Anal Chem 73:2985–2991
6. Meyers JJ, Liapis AI (1999) J Chromatogr A 852:3–23
7. Premstaller A, Oberacher H, Walcher W, Timperio AM, Zolla L, Chervet J, Cavusoglu N, Dorsselaer Av, Huber CG (2001) Anal Chem 73:2390–2396
8. Tennikova TB, Bleha M, Svec F, Almazova TV, Belenkii BG (1991) J Chromatogr A 555:97–107
9. Svec F, Frechet JMJ (1992) Anal Chem 64:820–822
10. Wang QC, Svec F, Frechet JMJ (1993) Anal Chem 65:2243–2248
11. Minakuchi H, Nakanishi K, Soga N, Ishizuka N, Tanaka N (1996) Anal Chem 68:3498–3501
12. Ikegami T, Dicks E, Kobayashi H, Morisaka H, Tokuda D, Cabrera K, Hosoya K, Tanaka N (2004) J Sep Sci 27:1292–1302
13. Luo Q, Shen Y, Hixson KK, Zhao R, Yang F, Moore RJ, Mottaz HM, Smith RD (2005) Anal Chem 77:5028–5035
14. Tolstikov VV, Lommen A, Nakanishi K, Tanaka N, Fiehn O (2003) Anal Chem 75:6737–6740
15. Van De Meent MHM, De Jong GJ (2007) Anal Bioanal Chem 388:195–200
16. Van De Meent MHM, de Jong GJ (2009) J Sep Sci 32:487–493
17. Batycka M, Inglis NF, Cook K, Adam A, Fraser-Pitt D, Smith DGE, Main L, Lubben A, Kessler BM (2006) Rapid Commun Mass Spectrom 20:2074–2080
18. Walcher W, Toll H, Ingendoh A, Huber CG (2004) J Chromatogr A 1053:107–117
19. Giddings JC (1967) Anal Chem 39:1027–1028
20. Horvath CG, Lipski SR (1967) Anal Chem 39:1893
21. Wang X, Barber WE, Carr PW (2006) J Chromatogr A 1107:139–151
22. Eng JK, McCormack AL, Yates I, John R (1994) J Am Soc Mass Spectrom 5:976–989
23. Perkins DN, Pappin DJC, Creasy DM, Cottrell JS (1999) Electrophoresis 20:3551–3567
24. Schmidt A, Karas M, Dulcks T (2003) J Am Soc Mass Spectrom 14:492–500
25. Schley C, Swart R, Huber CG (2006) J Chromatogr A 1136:210–220
26. Oberacher H, Premstaller A, Huber CG (2004) J Chromatogr A 1030:201–208
27. Guryča V, Kieffer-Jaquinod S, Garin J, Masselon CD (2008) Anal Bioanal Chem 392:1291–1297
28. Wang X, Stoll DR, Schellinger AP, Carr PW (2006) Anal Chem 78:3406–3416
29. Eeltink S, Dolman S, Swart R, Ursem M, Schoenmakers PJ (2009) J Chromatogr A 1216:7368–7374
30. Gilar M, Daly AE, Kele M, Neue UD, Gebler JC (2004) J Chromatogr A 1061:183–192
31. Toll H, Wintringer R, Schweiger-Hufnagel U, Huber CG (2005) J Sep Sci 28:1666–1674
32. Gilar M, Olivova P, Daly AE, Gebler JC (2005) Anal Chem 77:6426–6434