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# Towards high peak capacity separations in normal pressure nanoflow liquid chromatography using meter long packed capillary columns

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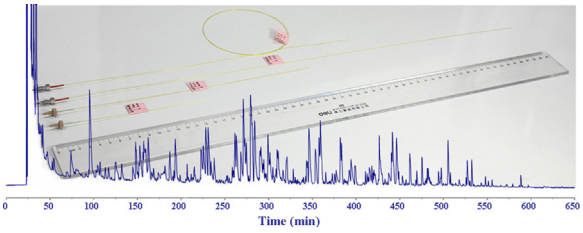
## HIGHLIGHTS

- Meter long packed capillary columns were fabricated through a facile approach.
- 100,000 plates and 800 peak capacity were generated on the meter long columns.
- The long columns can be operated within 40 MPa on normal pressure nanoLC systems.
- Consistently high peak capacity at a column-to-column level was observed.
- The columns can be used at least for 100 injections in a time span of 6 months.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Single shot proteomics is a promising approach to high throughput proteomics analysis. In this strategy, long capillary columns are needed to perform long and shallow gradients to achieve high peak capacity and good peak width for informative mass spectrometric detection. Herein, we report that meter long capillary columns, packed with 5 μm particulate material, can be facilely fabricated based on single particle fritting technology. The long columns could reliably generate high peak capacities of 800 in 10 h long gradients for protein digest separations. The operation was within the pressure range (40 MPa) of the most widely used normal pressure nanoLC systems. Due to the excellent life time (>100 injections) and inter-column performance consistency, the meter long capillary columns reported here should be of practical usefulness in single shot proteomics without the need for ultra-high pressure instrumentation.



## 1. Introduction

Single shot proteomics is an emerging strategy for high throughput proteomics analysis [1–13]. It uses long capillary columns running in long and shallow gradients to achieve high peak capacity in a single dimensional separation. In combination with fast-scan mass spectrometers (MS), the long column-based

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single shot strategy has realized identifications of thousands of proteins within several days for both prokaryotic [3,12] and eukaryotic biosystems [1,2,4–7,10]. Compared with the relatively complicated instrumentation and tedious operation of 2D LC-based approaches [14], the simplicity and effectiveness of single shot strategy greatly pushed forward its widespread use in proteomics. The advantages of single shot proteomics include: simplified sample preparation and therefore shortened analysis time, lower amount of starting material for analysis, large dynamic range and high sensitivity of down to sub-femtomole for protein identifications. Recent reports have revealed that single shot strategy can support deep-coverage analysis of proteomes of, e.g., yeast [1,2], human [2,4–6] and other mammalian cell lines [7]. Another recent work also investigated its effectiveness in targeted proteomics analysis [8], where the single shot strategy was combined with the selected reaction monitor MS method to realize quantification of targeted proteins from undepleted human serum. Although at this moment in time, the coverage of single shot proteomics can not rival that of multidimensional LC–MS based strategies, Thakur et al. predicted that, "*single-run analysis has the potential to radically simplify proteomics studies while maintaining a system-wide view of the proteome*" [1].

The best advantage of long column-based single dimensional LC is its high peak capacity (e.g., 100 s–1000) for separations of complex mixtures of peptides [15]. It significantly reduced peptide coelution, allowing acquisition of MS/MS spectra of precursor ions from highly complex samples. More importantly, the wide peak width resulting from long and shallow gradients can greatly enhance the multiplexing capacity for MS scanning and lead to improved protein identification and proteome coverage. Recent studies have revealed that repeated short gradients (as conventionally used) are less efficient than a single long gradient in terms of peptide/protein identification numbers [6].

There are a handful of choices for the appropriate material for long chromatographic columns. Capillary silica monolith, due to its low backpressure, can be used at long lengths, e.g., 1–12 m [16–20], and has been demonstrated to be an excellent material for single shot proteomic separations [3–5,13]. Although 15 and 30 cm long capillary silica monolithic columns are marketed by several providers, single-piece long capillary silica monolithic columns have not become commercially available yet. In contrast, microparticulate chromatographic material has been well commercialized with good quality and easily accessible from vendors worldwide [15,21–31].

Recently, long capillary columns (40–200 cm) packed with 3 to sub-2 μm (reversed phase chromatography) particles have been broadly reported for their use in proteomic separations running under ultra-high pressures (10–20 kpsi) [1,6,8,32]. For 3 to sub-2 μm particulate materials, however, the capillary packing itself is a major technical challenge [33]. The difficulties include: column fritting, the tendency of capillary tube blockage, and the need for ultra-high pressure packing pump, which is not always available in proteomics laboratories. After packing, there is the need for special microfittings and unions for deadvolume-free connections under ultra-high pressure [34]. Finally, there are concerns about the columns' durability and column-to-column reproducibility. These are all critical issues in practice.

We believe that, for laboratories whose focus is on proteomics analysis rather than separation science or column technology, robust and easy-to-produce capillary columns are of utmost importance. Nowadays, most proteomics laboratories are equipped with normal pressure (<40 MPa) nanoLC systems, rather than ultra-high pressure ones, coupled to MS for routine proteomics analyses. To push forward the widespread use of single shot proteomics strategy, the aim of the present work was to introduce facilely fabricated long capillary columns which can provide high peak capacities on commonly used normal pressure nanoLC systems. The practical issues we tried to solve in this study include: (1) fabrication of meter long capillary columns with excellent column-to-column reproducibility; (2) facile preparation of such long columns; (3) usability of meter long packed columns in commonly equipped normal pressure nanoLC systems (and of course ultra high pressure nanoLC) and therefore, the transferability of the column technology to routine proteomics laboratories.

## 2. Experimental

### 2.1. Materials and apparatus

Polyimide-coated fused silica capillaries were purchased from Yongnian Reafine Chromatography (Hebei, China). The porous silica particles ~110 μm in diameter with large throughpores of about 1 μm, to be used as prefabricated single particle frits, were provided by X-tec (Bromborough, UK). The packing material Ultimate XB-C18 (5 μm, 300 Å) was obtained from Welch Materials Inc. (Shanghai, China). Thiourea, $NH_4HCO_3$, methyl-, ethyl-, propyl-, and butylbenzenes of analytical grade, dithiothreitol (DTT), iodoacetamide (IAA), trifluoroacetic acid (TFA), trypsin of sequencing grade, standard protein cytochrome C and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile and acetone of HPLC grade were provided by Merck (Darmstadt, Germany). An Elite P230 high pressure pump from Dalian Elite Analytical Instruments (Dalian, China) was used for column packing. A packed capillary column, Acclaim PepMap100, 75 μm × 15 cm, C18, 5 μm, 100 Å was purchased from Thermo-Dionex (Amsterdam, The Netherlands) for performance comparison with the self-packed columns. For this direct performance comparison experiment, the self-packed capillary column was prepared with the packing material obtained from a disassembled Acclaim HPLC column (Acclaim 120, 4.6 mm × 25 cm, C18, 5 μm, 120 Å), which was also purchased from Thermo-Dionex (Amsterdam, The Netherlands).

### 2.2. Protein digestion

Protein digest samples were prepared by tryptic digestion of standard proteins in solution. Generally, proteins were solubilized in 8 M urea, 50 mM $NH_4HCO_3$. Then, the sample was reduced by DTT and alkylated by IAA. Finally, trypsin was added at a protein-to-enzyme ratio of 50:1, the digestion was incubated at 37 °C over night.

### 2.3. Nanoflow liquid chromatography

NanoLC experiments were carried out on an Ultimate 3000 nanoLC system (Thermo-Dionex, Amsterdam, The Netherlands), equipped with an autosampler and a variable wavelength UV–vis detector with a 3 nL flow cell. A 4 nL Valco nanovolume injector (VICI AG, Schenkon, Switzerland) was used for column performance evaluation under isocratic condition. For large volume injections under gradient elution, the autosampler with a 1 μL loop was adopted.

### 2.4. Single particle fritting and column packing

We have reported fabrication of short nanoLC columns (15 cm long) [31] based on single particle fritting technology [35–37]. In contrast to other fritting methods, the single particle fritting approach uses large perfusive silica beads as prefabricated frits. The frit's wide through-pores provide permeability for solvent flow going through itself during the process of column packing and subsequent runs. As shown in Fig. 1, this excellent feature also

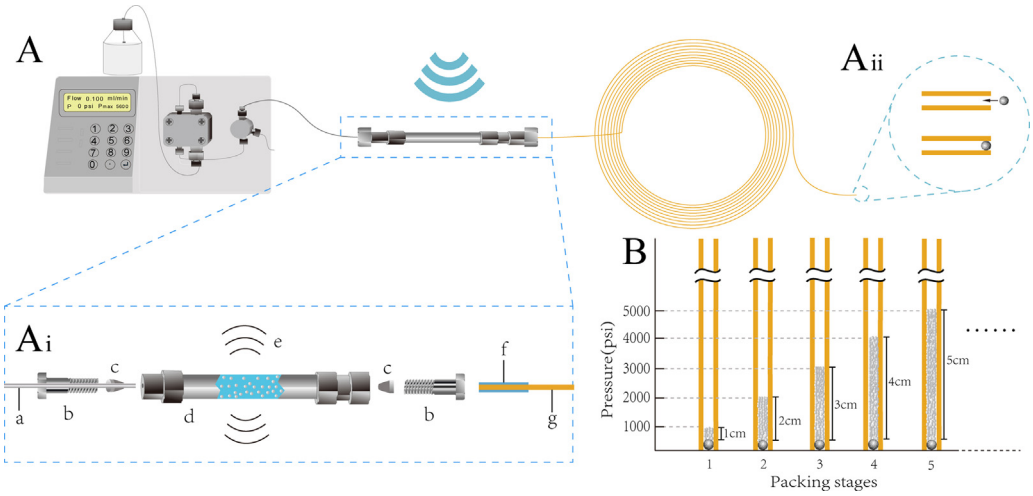


**Fig. 1.** Packing meter long capillary columns based on single particle fritting technology. A: the complete setup for capillary packing; $A_i$: interface between packing pump and capillary tube: a: 1/16″ stainless steel tube; b: 1/16″ stainless steel nut; c: 1/16″ stainless steel ferrule; d: slurry reservoir (stainless steel HPLC tube, 4.6 mm i.d., 250 mm long); e: sonication bath; f: 1/16″ PEEK tube; g: fused silica capillary; $A_{ii}$: single particle fritting, a perfusive large single particle, ~110 μm, was forced into the capillary tip and used as an end frit; B: packing procedures, the pressure was gradually increased as the packed bed was building up, after the first few centimeters, the pressure was increased to 5000–6000 psi and maintained for the remaining packing process.

enabled facile fabrication of long capillary columns: in less than 5 h, a meter long packed column could be fabricated.

First, a 100 μm i.d., 365 μm o.d. fused silica capillary was chosen as the column tubing. One end of the capillary was tapped into a micro centrifuge tube, in which a small number of ~110 μm perfusive silica beads were deposited. A single perfusive silica bead was captured, and then pushed into the capillary by pressing the end of the capillary against a plane surface [31]. This single silica bead served as the outlet frit of the column. The capillary was slurry-packed under high pressure using acetone as the packing solvent. The packing material was suspended in acetone at a concentration of 2 mg $mL^{-1}$ and ultrasonicated for 15 min. The slurry was loaded into a reservoir (4.6 mm i.d., 25 cm long) attached to a high pressure pump. The one end fritted capillary tube was connected to the reservoir via the open end. Pressure was increased gradually to 6000 psi and maintained until the column was packed. In detail, when packing the first centimeter of the column bed, the pump pressure was increased to 1000 psi; during the packing of the second centimeter, the pressure was increased to 2000 psi. In this way, when the column bed was packed to 5 cm, the pump pressure was increased to 5000–6000 psi and this high pressure was maintained for the remaining packing process. After packing, the column was cut to a desired length when the packing system was fully depressurized. Finally, another single perfusive silica bead was forced into the cut end serving as the inlet frit of the column. Before use, the column was mounted onto the nanoLC system and fully equilibrated with the mobile phase under high pressure.

For this study, columns of 15, 30, 60 and 100 cm long, respectively (three for each length, totally 12 columns), packed with Ultimate XB-C18, 5 μm, 300 Å, were fabricated for performance investigations.

## 3. Results and discussion

### 3.1. Optimization of capillary column fabrication

First of all, when <**cutting a capillary tube, the capillary end should be cut as flat as possible. For a good quality standard, a professional capillary cutter (commercially available from many vendors, e.g., GL Sciences, Agilent and Upchurch) was used rather than a ceramic tile. The quality of the cut could be checked under an optical microscope. A poor cut may cause blockage of the column and even the (nanoliter) injection valve during use. It may also give rise to dead volumes between the separation column and the connecting capillary to the post-column detector.

During packing, as shown in Fig. 1, a large slurry reservoir (e.g., 4.6 mm i.d., 250 mm long stainless steel tube) was adopted to ensure that the long capillary could be packed in one fill (of the reservoir). At the beginning of the packing, it was important to increase the packing pressure gradually (Fig. 1), in order to build up a good quality bed at the column end area. Although the slurry concentration is packing material-dependent, our experience was that relatively high concentration of slurry performed better in terms of packing throughput and column bed quality. In the choice of slurry solvent, according to our experience, acetone usually gave satisfactory packing quality for most reversed phase materials.

Although we suggest the use of sonication during packing to keep the slurry suspended, we also succeeded in packing good quality columns simply by tapping the reservoir tube from time to time during packing. The suspension tended to precipitate at the outlet end area of the reservoir, so vibration at this area could effectively help the slurry re-suspend.

After packing, the column was pressurized (5000–6000 psi) using acetone in order to solidify the column bed. When mounted on the nanoLC system, the column was fully equilibrated under high pressure before use.

### 3.2. Isocratic elution and column performance

In our previous report [31], single particle fritted, short capillary columns (15 and 20 cm long), packed with 5 μm particulate material, have realized an excellent plate height of 10.90 μm, which is equivalent to 92,000 plates per meter. In moving towards long capillary columns to pursue high peak capacities, the first question was whether the good quality still stands as the packed bed increased from the normal length (e.g., 10–20 cm) to a meter long column. As shown in Fig. 2, using a retained analyte (butylbenzene) as the standard, plate height as a function of flow rate was investigated. At the flow rate of 250 nL $min^{-1}$ (linear velocity 0.67 mm $s^{-1}$), a plate height of 10.30 μm was observed. When taking methyl- and ethylbenzenes as the standards, plate heights of 9.90 and 9.98 μm were observed, equivalent to 101,000 and 100,000 plates per column, respectively, as shown

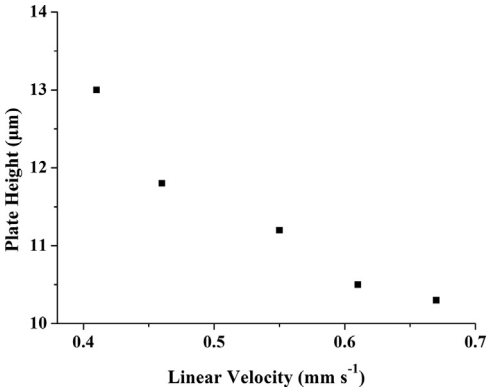


**Fig. 2.** Plate height as a function of flow rate on a meter long packed capillary column. Capillary column: ultimate XB-C18, 5 μm, 300 Å, 1,000 mm × 100 μm i.d.; mobile phase: 60% ACN; injection volume: 4 nL; UV detection: 214 nm. A retained neutral analyte, butylbenzene, was used as the standard.

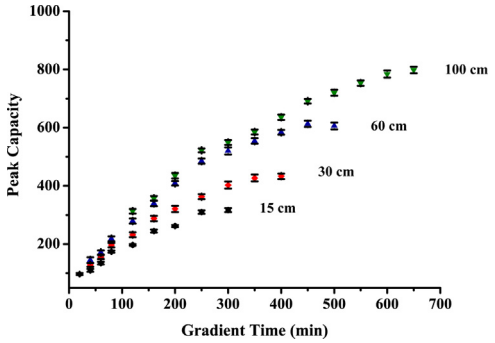


**Fig. 3.** Peak capacity as a function of gradient time on columns of different lengths. From bottom to top: columns of 15, 30, 60 and 100 cm long, 100 μm i.d., all packed with Ultimate XB-C18, 5 μm, 300 Å. Gradient separations of tryptic digest of cytochrome C were used for peak capacity evaluation. Gradient conditions: mobile phase A, $H_2O$ + 0.05% TFA, B, ACN + 0.05% TFA, 5–50% B in various gradient time; injection volume: 0.2 μL; flow rate: 400 nL $min^{-1}$ for 15, 30 and 60 cm long columns, and 250 nL $min^{-1}$ for 100 cm long columns; UV detection: 214 nm. For each column length, three columns were prepared and used for evaluation. Each data point is the average peak capacity obtained on the three columns of the same length.

in Fig. S1 in Supplementary data. The result indicates that the meter long column has an excellent packing quality. The even better separation efficiency obtained on a meter long column than on a 20 cm long column [31] should be attributed to the relatively smaller influence of extracolumn effect.

Obviously, only five data points were recorded in Fig. 2, corresponding to the volumetric flow rate range of 150–250 nL $min^{-1}$. This is due to the pressure limitation of normal pressure nanoLC instrumentation (e.g., 40 MPa for the Dionex 3000 system used in this study) and the high backpressure of meter long packed columns. For instance, at the flow rate of 250 nL $min^{-1}$ in ambient (25 °C), the backpressure of the meter long column has reached 30 MPa.

### 3.3. Gradient elution and peak capacity

Packed columns of different lengths (15, 30, 60 and 100 cm) were prepared for performance investigation in gradient separations of protein digests. Based on the van Deemter curve studied before (Fig. S2 in Supplementary data) using the same packing material [31], 400 nL $min^{-1}$ (corresponding to a linear velocity of 1.25 mm $s^{-1}$) was chosen as a relatively optimum flow rate for 15, 30 and 60 cm long columns. While for 100 cm long columns, although the pressure limit of the normal pressure nanoLC instrument is 40 MPa, the flow rate of 250 nL $min^{-1}$ (at the backpressure of 30 MPa) was chosen. This is because that, in gradient elution mode, the composition change of mobile phase may lead to fluctuations of column backpressure. Therefore, to maintain the long columns' stable operation, 250 nL $min^{-1}$ was chosen as the flow rate upper limit in the investigations.

As shown in Fig. 3, peak capacity as a function of gradient time was investigated using different column lengths. According to the previous reports [4,31], peak capacities were calculated based on the average 4$\sigma$ peak width using tryptic digest of cytochrome C as the probe. For all the four column lengths investigated, peak capacities increased as gradient times were extended, and reached a relatively constant level at certain stages. The trend coordinates well with gradient elution theories and previous work on packed and monolithic capillaries [3,6,38,39]. Practically, double the column length resulted in ~30–50% increase in the upper limit of peak capacities. Fig. 3 clearly shows that, longer columns generated higher peak capacity for a given gradient time; while for a targeted peak capacity, longer columns required shorter gradient times to realize. This is a crucial endorsement for the widespread use of long packed columns in proteomics separations targeting at highly complex mixtures (e.g., digested peptides). The data series presented in Fig. 3 also provide a practical guideline for achievable peak capacities in normal pressure nanoLC of protein digests. For required peak capacities within 800, 600, 400, or 300, columns of 100, 60, 30 and 15 cm long, respectively, should be enough for the targeted performance. Example chromatograms of BSA digest obtained on columns of different lengths are presented in Fig. 4.

In MS-based proteomics, peptide/protein identification numbers are directly dependent on peak capacities of the separations prior to MS [6]. Previously, researchers have reported high peak capacities of 500–1500 using 3, 2 and sub-2 μm particle-packed long capillary columns (40–200 cm). Due to their very high backpressure, however, the columns need to be operated on ultra-high pressure nanoLC systems (UHPLC) [1,6,8,32] under 10–20 kpsi (equivalent to ~100 MPa). This is certainly a big challenge for separation instrumentations for most laboratories.

Silica monolithic columns are well known for their excellent permeability and therefore have good potential in pursuing high resolving power using extended column length. <*****Tanaka and co-workers [16,17,20] have reported series pioneering work in high resolution capillary LC separations based on long silica monolithic columns. For example, they prepared 0.9–12.4 m long, 100 μm i.d. monolithic columns and achieved 100,000–1,000,000 theoretical plates for isocratic separation of neutral compounds, and also realized high resolution gradient separation of proteins digests using 3 m long monolithic capillaries on a non-UHPLC system [17]. In a later study, taking 20 MPa as the pump pressure limit, they have theoretically proved that 3 m long silica monolith can generate peak capacities over 1000, running in 10–40 h shallow gradients for separations of protein digests [20]. Such a peak capacity level has a clear edge over that (8 0 0) of the 1 m long packed capillary reported in the present work. In the practical term, however, Miyamoto et al. stated that, "*It was difficult to prepare a long capillary column with a homogeneous monolithic silica structure along its entire length, while shorter columns can be obtained with higher efficiency by selecting portions prepared homogeneously*" [17]. This probably can explain the fact that in the market, only capillary silica monolithic columns of short length (e.g., 15 cm) are commercially available. Nevertheless, <*****Ishihama and co-workers [3–5] recently further exploited the excellent separation power of long capillary silica monolithic columns and demonstrated their usefulness in single shot proteomics of *Escherichia coli* as well as human stem cells. It can be expected that once the key issues of fabrication reproducibility

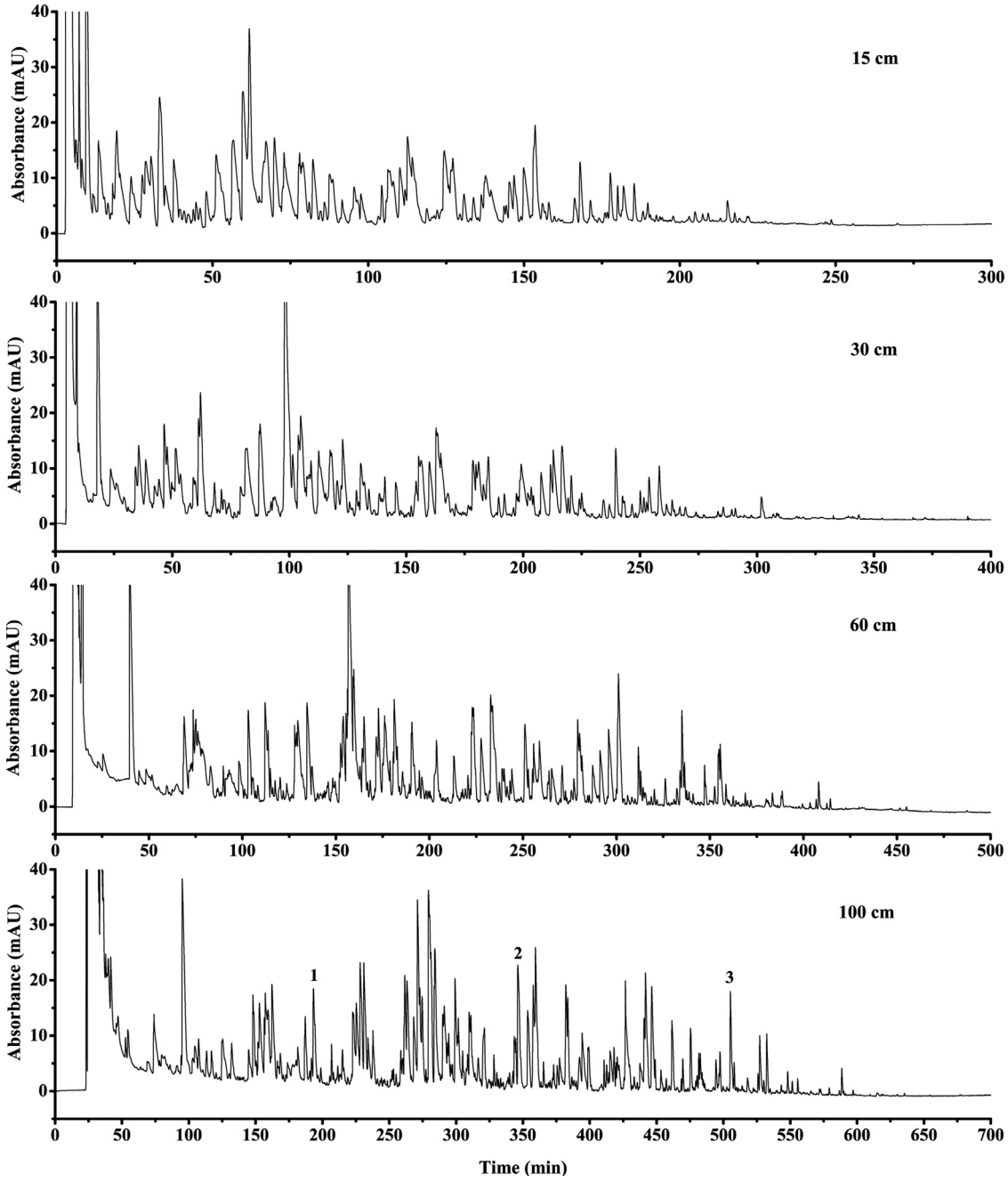


**Fig. 4.** Long gradient separations of BSA digest to reach the maximum peak capacity on columns of different lengths, as marked in each frame. Gradient conditions: 5–50% B in 300, 400, 500 and 650 min on 15, 30, 60 and 100 cm long columns, respectively. Other conditions as in Fig. 3.

and robustness are solved, the long capillary silica monolithic columns may become a working-horse in practical proteomics laboratories.

Recently, Zhou et al. reported the use of 25 μm i.d., 1 m long capillary columns, packed with 5 μm particles, in single shot analysis based on normal pressure nanoLC [7]. An excellent peak capacity of 750 was achieved in a 600 min gradient separation of a complex mammalian proteomic sample. The peak capacity compared very well with the present work using 100 μm i.d. packed columns. Apart from its limited loadability (0.2–2 μg protein digests), the 25 μm i.d. column presented significantly improved electrospray ionization efficiency due to its extremely low flow rate ($<10$ nL min$^{-1}$). The work provided another choice to push forward the performance of nanoLC–MS for microscale bioanalysis. On the other hand, it also raised a new challenge for separation scientists to fabricate good quality microcolumns with such a low aspect ratio (column diameter: particle size $\sim$5). Among others, Fanali et al. have reported the difficulty in packing such columns [40].

In contrast, the high peak capacities achievable on the single particle fritted, easy-to-produce 100 μm i.d. capillary columns should be an important advancement from the viewpoint of practical usefulness in proteomics laboratories, where ultra-high pressure packing devices as well as ultra-high pressure nanoLC

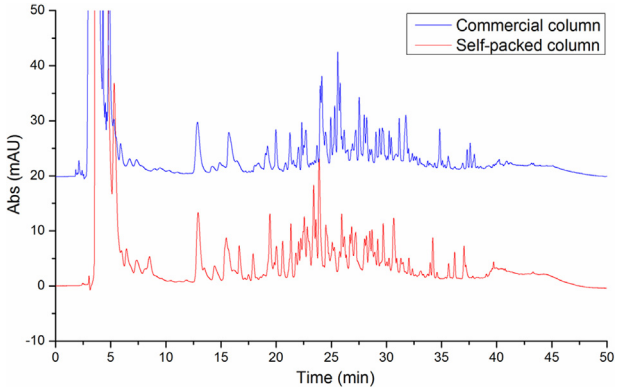


**Fig. 5.** Comparison of nanoLC separations of BSA digest using a Dionex Acclaim PepMap100 column, 75 μm × 15 cm, C18, 5 μm, 100 Å (upper graph) and a self-packed single particle fritted column (bottom graph), 100 μm × 15 cm, packed with the same Acclaim series of packing material obtained from a disassembled Dionex Acclaim HPLC column (acclaim 120, 4.6 mm × 25 cm, C18, 5 μm, 120 Å). Gradient time: 30 min, flow rates: 300 nL min$^{-1}$ for Dionex column; 350 nL min$^{-1}$ for self-packed column. Other conditions as in Fig. 3.

systems may not be available. More importantly, the high peak capacity of 800 has been among the high-end peak capacities (500–1500) one can achieve in a single dimensional nanoLC separation.

To locate a benchmark for this development, we also compared single particle fritted nanoLC columns with commercially available Acclaim PepMap columns from Dionex. The columns have shown highly comparable performance, as shown in Fig. 5. To eliminate the difference in reversed phase chemistry, we adopted the packing material from a disassembled Dionex Acclaim HPLC column for the capillary packing. The consistent results (Fig. 5) clearly demonstrated the excellent applicability and transferability of the single particle fritting technology as well as the reliable quality of the capillary columns thus fabricated. The two chromatograms shown in Fig. 5 do not have a completely matched profile. This may be due to the slightly different pore sizes (100 versus 120 Å) of the packing material, although both of them belong to the same Dionex Acclaim stationary phase series.

### 3.4. Column consistency and life time

Focusing on the practical usefulness of the long packed columns, we have also investigated their performance stability at a column-to-column level. For this purpose, three columns of each length were fabricated and investigated for peak capacity reproducibility. As shown in Fig. 3 and Table 1, consistent peak capacities were observed on each set of three columns of the same length. For instance, relative standard deviation (RSD) of peak capacity between 1.1 and 2.5% ($n = 6$) was observed on the three 100 cm long columns, indicating the long columns' reliable separation power for complex mixtures.

The life time of microcolumns, especially for these long length ones, is one of the most important parameters for their widespread use in routine proteomics laboratories. A key technical aspect that needs to be pointed out is that, the long columns reported here do not have an integrated spray tip as required in nanoLC–ESI–MS applications [41]. This should, on the contrary, enhance the long columns' flexibility in practical use. The taper-ended columns are prone to break or block at the tip area. Once this happens, the whole column can not be used even if the packed bed is still effective for chromatography. To this end, connecting a separate spray tip (e.g., commercial ones such as PicoTip [42] or custom-made ones [41]) to the analytical column should be advantageous over the integrated setups, although some band-broadening effect may inevitably take place. In terms of life time, the meter long columns could be used, at least, for 100 injections of various protein digest samples over a time span of 6 months. During the course, for short term storage (1–3 days), the column was filled with 80% ACN and left on the nanoLC system. For a longer term storage (>3 days or to be removed from the instrument), the column was thoroughly washed with highly aqueous solvent (e.g., ACN/water, 5:95) followed by highly organic solvent (e.g., ACN/water, 80:20). When dismounted, the column was sealed with ethanol/water (10/90) at the two ends using microcentrifuge tubes or end-to-end joined together via a PTFE tube. For "dirty" samples (e.g., high concentration protein digests), thorough sample preparation (e.g., centrifugation and desalting) and the use of guard/trap columns were necessary and after use, the separation column was washed with highly aqueous and highly organic solvents sequentially. Using BSA digest as the standard, RSDs of retention times of three marker peaks (as marked in Fig. 4) were recorded to be 1.0% (peak 1, retention time 193.2 min), 0.7% (peak 2, retention time 346.2 min) and 0.6% (peak 3, retention time 505.2 min), respectively, in 30 long gradient (10 h) runs. The long columns' excellent separation power and long term stability suggest their effectiveness as a separation tool in routine single-shot proteomics analysis as well as an enabling device in building up a multi-lane microseparation platform.

**Table 1**
Peak capacity reproducibility between three columns of the same length in gradient separations of protein digests.

| Column length/cm | 15 | 30 | 60 | 100 |
|---|---|---|---|---|
| Gradient time/min | 20–300 | 40–400 | 40–500 | 120–650 |
| RSD of peak capacity ($n = 6$) | 1.3–4.5% | 2.1–5.0% | 1.4–6.5% | 1.1–2.5% |

Peak capacity data were based on separations performed in gradient times between 20–650 min, as specified in Fig. 3, for each column length respectively. For each column length, three columns were used for performance evaluation, and the test was repeated twice on each column.

## 4. Conclusions

From a separation science point of view, the advancement of single shot proteomics largely depends on the achievable extreme peak capacity of single dimensional separations. In this study, high quality meter long capillary columns with excellent column-to-column reproducibility, packed with 5 μm particles, were facilely fabricated based on single particle fritting technology. The long columns enabled high resolution separations of complex mixtures of digested peptides and realized an excellent peak capacity of 800 on a normal pressure nanoLC instrument. Taking into account column fabrication, reproducibility, durability and operability (back pressure, nanoflow precision, tubing connections etc.), the facilely fabricated long capillary columns reported here should be of practical and extensive usefulness in route proteomics analyses as well as in studies of other complex mixtures requiring high peak capacity single dimensional separations.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.09.006.

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