Wall modified photonic crystal fibre capillaries as porous layer open tubular columns for in-capillary micro-extraction and capillary chromatography.

Artaches A. Kazarian^{1,3}, Estrella Sanz Rodriguez¹, Jeremy A. Deverell¹, James McCord³, David C. Muddiman³, Brett Paull^{1,2*}

¹Australian Centre for Research on Separation Science, and ²ARC Centre of Excellence for Electromaterials Science, School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia.

³W.M. Keck FT-ICR-MS Laboratory, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, USA.

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ABSTRACT

Wall modified photonic crystal fibre capillary columns for in-capillary micro-extraction and liquid chromatographic separations is presented. Columns contained 126 internal parallel 4 μm channels, each containing a wall bonded porous monolithic type polystyrene-divinylbenzene layer in open tubular column format (PLOT). Modification longitudinal homogeneity was monitored using scanning contactless conductivity detection and scanning electron microscopy. The multichannel open tubular capillary column showed channel diameter and polymer layer consistency of $4.2 \pm 0.1 \mu\text{m}$ and $0.26 \pm 0.02 \mu\text{m}$ respectively, and modification of 100% of the parallel channels with the monolithic polymer. The modified multi-channel capillaries were applied to the in-capillary micro-extraction of water samples. 500 μL of water samples containing single $\mu\text{g L}^{-1}$ levels of polyaromatic hydrocarbons were extracted at a flow rate of $10 \mu\text{L min}^{-1}$, and eluted in 50 μL of acetonitrile for analysis using HPLC with fluorescence detection. HPLC LODs were 0.08, 0.02 and $0.05 \mu\text{g L}^{-1}$ for acenaphthene, anthracene and pyrene, respectively, with extraction recoveries of between 77 and 103 %. The modified capillaries were also investigated briefly for direct application to liquid chromatographic separations, with the retention and elution of a standard protein (cytochrome c) under isocratic conditions demonstrated, proving chromatographic potential of the new column format, with run-to-run retention time reproducibility of below 1 %.

34 INTRODUCTION

35 Separation of intact proteins and peptides in HPLC has always presented
36 challenges [1-2], and the development of porous layer open tubular (PLOT) capillary
37 columns for such bioseparations is one approach to overcome of these challenges [3-
38 4]. These capillary PLOT columns generally have limited sample capacity, and hence
39 a small sample size, but simultaneously reduce mobile phase consumption and
40 provide the possibility of providing low pressure separations. Such columns can be
41 operated at low nanolitre flow rates and easily interfaced with ESI-MS, producing
42 smaller droplets and thus minimising ion-suppression and yielding improvements in
43 sensitivity [5]. To-date a great deal of attention has been focused upon the fabrication
44 of capillary PLOT columns of various forms and dimensions [1, 5-19]. For example,
45 Karger and coworkers have published a series of articles on the development of
46 polystyrene-divinylbenzene (PS-DVB) based PLOT supports using a simple,
47 reproducible fabrication approach, and applying the resultant micro-bore capillary
48 columns to the high-resolution separation and characterisation of peptides [5, 20].

49 Other groups have also investigated both methacrylate and PS-DVB based
50 PLOT columns, specifically focusing on layer morphology and thickness, obtained
51 using both photoinitiated and thermal methods of monolith formation. Column
52 characterisation was largely carried out using scanning electron microscopy (SEM)
53 and scanning capacitively coupled contactless conductivity (sC4D), with the
54 chromatographic performance of the columns generally evaluated with protein
55 separations [10, 12-13, 15, 17].

56 Theoretical considerations reported by Jorgenson and Guthrie, concluded the
57 optimum diameter for open tubular columns for chromatographic separations should
58 be in the order of 1-3 μm [21]. However, to-date capillary PLOT columns (more

typically ~10 to 20 μm I.D.) have tended to exhibit a limited capacity, which necessitates the need to produce longer columns (>1 m), which can be challenging in terms of uniform layer formation and increased back pressures. Additionally, in the case of microbore capillary PLOT columns (e.g. sub-10 μm I.D.), the low operational flow rates (due to high column backpressures) can also present stability issues especially for gradient elution, ultimately compromising both repeatability and reproducibility [5]. These issues of limited capacity (stemming from a low surface area) and low flow rates, have also acted to limited the use of such open tubular capillaries for in-capillary micro-extraction.

A promising alternative to the conventional PLOT capillary formats is the use of multiple parallel capillary columns, which are widely available in numerous designs and dimensions, one such source being fused silica photonic crystal fibres (FS-PCFs), also described as micro-structured fibres (MSFs). The potential advantage of these structures is related to the large number of precisely uniform and parallel micro-channels, which collectively provide increased column capacity as a consequence of higher surface area, and thus capacity for larger sample loading, providing for greater solute-surface interactions, shorter diffusion pathways (than within a single large open channel), together with low flow resistivity and thus higher operational flow rates. These capillary formats are available with channel dimensions in the single digit micron range (e.g. 4 μm) and below, and therefore offer the potential for the development of both selective micro-extraction capillaries and PLOT capillary columns for liquid chromatographic separations, similar to those obtained using conventional micro-bore PLOT capillary columns [22-29].

To-date there has been very limited work reported using such FS-PCFs in separation science. Some preliminary applications within capillary electrophoresis, for

84 the separations of dye labeled peptides and DNA [26, 28], and as multi-channel
85 electrospray emitters in MS [22], have been reported. In addition, a recent application
86 of wall modified FS-PCFs for extraction purposes has been described, specifically
87 polyaromatic hydrocarbon (PAHs) extraction with subsequent separation using GC-
88 MS [24]. However, the method reports simple adsorption of solutes into the end of the
89 capillary only, and no in-capillary extraction or flow through extraction is explored or
90 described. The solitary application to HPLC separations of these modified FS-PCF
91 columns was reported by Daley *et al.*, who investigated the chromatographic
92 performance of capillaries following their internal wall modification using fluorosilane
93 reagents [25]. A reasonable separation was shown for 2-3 fluorinated species,
94 achieved using gradient elution.

95 To develop these capillary columns further in both micro-extraction and HPLC,
96 and to increase stationary phase capacity, the bonding of porous polymer layers within
97 these FS-PCFs merits greater investigation. The production of uniform bonded porous
98 polymer layers within each of the parallel channels is technically challenging but may
99 provide the appropriate stationary phase for both application as an extraction and/or
100 separation phase. Some previous work by Fu *et al.* [23] and Bachus *et al.* [30] has
101 been reported using FS-PCFs as templates for fabricating nanotubes, wires and
102 porous monoliths [24], however none of these examples have been applied or
103 investigated with regard to liquid chromatographic performance.

104 Therefore herein are described results from a study to investigate the
105 modification of 4 μm I.D. parallel micro-channels within FS-PCFs, with a uniform
106 porous PS-DVB layer, and be the first to demonstrate this new column format within
107 in-capillary micro-extraction and HPLC. The production of a highly reproducible and
108 homogeneous PS-DVB wall bonded layer throughout all parallel channels within the

FS-PCF column was the aim of this work, and the simple demonstration of its potential in both micro-extraction and low pressure chromatographic separations.

EXPERIMENTAL SECTION

Chemicals and Materials

Deionised water was obtained using a Millipore (Bedford, MA, USA) Milli-Q water purification system. Styrene, divinylbenzene, ethanol, octanol, AIBN, sodium hydroxide and cytochrome C were obtained from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile (ACN) and trifluoroacetic acid, formic acid, disodium hydrogen orthophosphate, hydrochloric acid and acetic acid were sourced from Ajax Finechem (Sydney, Australia). Acenaphthene (purity 97%), anthracene (purity 99%) and pyrene (purity 98 %) were obtained from Aldrich (St. Louis, MO, USA). Photonic crystal fibre capillary LMA-15 was sourced from NKT Photonics (Birkerød, Denmark).

Instrumentation

A Waters 2695 HPLC with a Waters 474 scanning fluorescence detector (Milford, USA), was used for the reversed-phase HPLC quantification of PAHs extracted using the modified FS-PCFs. The fluorescence detector was applied with a wavelength program based upon individual PAH retention times to maximise sensitivity to each PAH. The fluorescence detector operating conditions along with chromatographic parameters are summarised in the Electronic Supporting Information within Table S1. The separation was carried out using a C₁₈ column (150 x 4.6 mm, 5 µm particle size) from Thermo Scientific (Waltham, MA, USA), with a gradient ACN:water mobile phase. A gradient program, as detailed in Electronic Supplementary Information Table S2, was applied for the separation of PAHs. The C₁₈ column was

held at 30 °C. The sample injection volume was 50 μ L. The HPLC method LODs were calculated as 3.3 times the standard deviation of the peak area obtained for each compound after the analysis of 10 replicates of a procedural blank divided by the calibration slope. LOQs were calculated as 10 times the standard deviation of the peak area obtained for each compound after the analysis of 10 replicates of a procedural blank divided by the calibration slope.

An UltiMate3000 RSLC nano system (Dionex, Sunnyvale, CA, USA) equipped with a NCS-3500RS capillary LC gradient pump, VWD-3400RS UV detector (45 nL flow cell) and a WPS-3000TPL RS autosampler fitted with a 200 nL sample loop was used for the capillary chromatography of protein. Mobile phase mixtures of ACN and aqueous buffers were filtered using nylon membranes (47 mm diam., 0.22 μ m pore size). Solutions were degassed using an ultrasonic bath (Soniclean). Isocratic separations were carried out using inlet lines A and B where line A was a mixture of 95% acetonitrile in water with 0.1% (v/v) TFA while line B was 100% water with 0.1% (v/v) TFA. Gradient separations were carried out as follows; 0 min (100% B), 60 min (40% A), and flow rate: 0.4 μ L min⁻¹. All HPLC experiments were carried out under ambient conditions. All system controls, data collection and processing were carried out using the Chromeleon® (Version 6.80) software.

For the protein digest separations, an Agilent pump (Santa Clara, CA, USA) 1200 system equipped with a 20 nL VICI internal sample injector 090-0151H and a Knauer HPLC UV detector containing a 45 nL flow cell were used. The UV detector was interfaced with the HPLC system using an Agilent interface 35900E. All system controls, data collection and processing were carried out using the Chemstation software while sample injection was carried out manually.

Scanning electron micrograph images were recorded using Hitachi SU-70 (Tokyo, Japan) and Verios 460L (Oregon, USA) field emission scanning electron microscopes. Monolith layer measurements were undertaken using IC3D™ metrology software for the Verios 460L.

Scanning (capacitively coupled) contactless conductivity (sC⁴D) experiments were conducted using a Tracedec Contactless Conductivity detector (Innovative Sensor Technologies, GmbH, Germany) as described previously, with minor changes to characterise the surface properties of PLOT and empty columns [31]. Specific scanning conditions can be found within Electronic Supporting Information.

Plot column preparation

Surface modification of the columns was based on the method of Hjerten with minor changes [32]. Both standard micro-bore fused silica capillary (FSC - 10 µm I.D.) and the FS-PCF capillaries were first conditioned and silanised prior to modification (for details see Electronic Supporting Information). A polymerisation mixture containing 150 µL styrene, 150 µL divinylbenzene, 700 µL ethanol and 5 mg AIBN was prepared (monomer ratios varied for optimisation experiments). The mixture was sonicated and purged under nitrogen for 5 min, and infused into the FSC or FS-PCF for 15 min. A rubber septum was used to seal the ends of the capillaries, which were then left in the water bath at 74 °C for 16 h. Following polymerisation, the various capillaries were flushed with 100 % ACN for storage.

In-capillary micro-extraction procedure

To evaluate the applicability of the modified FS-PCF capillary columns for in-capillary micro-extraction, a 5 cm long modified FS-PCF was used throughout. A blank

(deionised) water sample was prepared, and spiked with three PAHs, at concentrations of 1.36, 0.14 and 0.22 $\mu\text{g L}^{-1}$, for acenaphthene, anthracene and pyrene, respectively. 500 μL of the spiked water sample was passed through the modified FS-PCF using a syringe pump at a flow rate of 10 $\mu\text{L min}^{-1}$, for in-capillary micro-extraction, equating to an extraction time of 50 min. The water eluate was collected and 50 μL analysed using RP-HPLC to confirm PAH removal. Following extraction, 50 μL of ACN were pumped through the FS-PCF, again at 10 $\mu\text{L min}^{-1}$ (5 min) to elute the adsorbed PAHs. The ACN eluate was collected and again analysed using RP-HPLC. Between each subsequent extraction the modified FS-PCF capillary was flushed with 200 μL of ACN. Before sample loading, 50 μL of ACN were passed through the modified FS-PCF, collected and analysed to verify no sample carryover.

Three mixed standard solutions (ST1, ST2 and ST3) were prepared, adding to the initial spiked water sample 1.31, 2.64 and 3.98 $\mu\text{g L}^{-1}$ of acenaphthene; 0.15, 0.29 and 0.44 $\mu\text{g L}^{-1}$ of anthracene; and 0.22, 0.44 and 0.66 $\mu\text{g L}^{-1}$ of pyrene, to enable calculation of % recoveries based upon a standard addition calibration. Each spiked sample was subjected to the process detailed above.

A schematic diagram of the applied in-capillary extraction procedure is shown as Figure 1.

RESULTS AND DISCUSSION

Optimisation and control of polymer layer growth

The controlled preparation of polymer stationary phase layer was based on the conditions developed by Yue *et al.* [5]. Initial optimisation experiments involved the use of standard 10 μm I.D. capillaries in place of the FS-PCF capillaries. Various parameters were studied including percentage and type of porogen, and functional

and crosslinking monomer ratios. SEM images of typical resultant polymer phases formed from this optimisation process are available within Electronic Supporting Information (Figure S1). Optimising the preparation of the PS-DVB plot column involved varying the functional (PS) to crosslinking monomer (DVB) composition between 1:1, 1:3 and 3:1, while the monomer to porogen content remained at 40 % to 60 %, respectively. Under these conditions it was evident that large globules were being formed potentially impeding the flow and there was no substantial difference in morphology and layer thickness upon changes to PS/DVB ratios.

PLOT FS-PCF preparation and characterisation using SEM

Preparation of PLOT columns within the FS-PCF 4 μm parallel channels was carried out using the above optimised conditions. Figure 2(A) shows the FS-PCF structure in its unmodified form, with 126 parallel 4 μm I.D. channels. Figure 2(B) shows a typical fibre following surface modification, which demonstrates that all 126 channels were modified, confirming the absence of any individual blockages affecting the flow of the polymer solution. Multiple replicates of the modified fibre were prepared and imaged using SEM at multiple points along the length of the capillary to confirm complete channel modification. Figure 2(C) shows the PS-DVB layers achieved within the FS-PCF capillaries, during optimisation work, under varied polymerisation conditions, where layer thicknesses of ~ 100 nm, 150 nm and 200 nm were observed, respectively.

Taking into account a desire for significant stationary phase capacity for both extraction and chromatographic applications, and the desire to maintain relatively low back pressures for the modified FS-PCF, a polymerisation time of 16 h was set, producing a PS-DVB layer of ~ 300 nm. Figure 3 shows the high resolution images of

these modified 4 μm channels, illustrating the excellent uniformity of the polymer layer between channels and the successful wall bonding of the PS-DVB phase. Indeed, these results are a substantial improvement on the work published by Mugo *et al.* who commented upon their imperfect modification, with some channels fully filled with monolith and others only partially modified [24]. Herein, to fully confirm the longitudinal uniformity, a newly fabricated FS-PCF was cut into 10 individual sections, at 2 cm intervals, and each imaged using SEM. This process resulted in average channel diameter of $4.2 \pm 0.1 \mu\text{m}$ I.D., which confirmed that the channels were highly consistent throughout the length of the fibre (and was also in-line with the work of Daley *et al.*, who reported similar channel size distribution) [25]. This imaging also confirmed the polymer layer thickness for this particular FS-PCF PLOT column to be 0.26 ± 0.02 , which obviously corresponds to less than 10 % variation. 100 % of the channels in all ten sections were modified with PS-DVB monolithic type phase, successfully demonstrating that complete and uniform modification of such sized channels with a PS-DVB layer can be achieved in these new FS-PCF capillary formats.

Contactless conductivity (sC⁴D)

For non-invasive characterisation purposes, contactless conductivity, applied in a scanning mode (sC⁴D), was used [13]. Here the detector responds to the porous layer formation on the walls of the column, which by reducing the cross-sectional area within the channel (herein the collective contribution from all parallel channels), increasing resistance and enhancing the voltage drop. Figure 4 shows two sC⁴D traces recorded for a 25 cm FS-PCF, before and after formation of the inner PS-DVB layers. Data points were recorded at 5 mm intervals. The top trace provides a clear indication of the uniformity of the internal structure of the FS-PCF, whilst the bottom

trace, following modification, confirms that such modification has been successful across the length of the fibre, maintaining an average response of ~ 25 mV, without any obvious slope from start to end. There were however, clear indications of minor internal disturbances, recorded at ~ 7 -10 cm and ~ 19 -20 cm sections, perhaps pointing towards irregularities in the polymer layer at these points. To investigate such irregularities, plus ascertain batch-to-batch reproducibility of the modified FS-PCFs, further PLOT FS-PCFs were prepared and sC⁴D profiles compared. Typical data can be found within Electronic Supporting Information (Figure S2), which shows impressive, mostly identical sC⁴D traces throughout the entire length of the two columns, emphasising reliability of the monolith preparation procedure. However, interestingly one of the columns showed a very large anomaly at around 17.5-18 cm mark, where the signal spiked dramatically, in which instance the capillary was sacrificed, cut and SEM imaged at this point, only to reveal no visual irregularities, suggesting such sharp and isolated anomalies are not caused by an irregular monolith, rather perhaps a trapped air pocket. Both of the columns showed adequate and similar permeability and were later used in both extraction and chromatography, showing no detectable differences in performance.

In-capillary micro-extraction on PS-DVB modified PS-PCFs

Using a 5 cm L piece of the PS-DVB modified FS-PCF PLOT capillary column, its application to in-capillary micro-extraction was investigated using a test mixture of three PAHs at low $\mu\text{g L}^{-1}$ concentrations. Quantification of the PAHs following extraction and elution was achieved using RP-HPLC with fluorescence detection. Figure 5(a) shows the overlaid RP-HPLC chromatograms for a standard PAH solution before extraction, and the same solution collected exiting from the capillary column

during on-going extraction. As can be seen from the chromatograms shown, complete removal of the three PAHs can be seen. Figure 5(b) shows the overlaid chromatograms obtained for the original spiked water sample and subsequent standard additions ($n = 4$). Comparison of the chromatograms for standard ST3 shown within Figure 5(a) and 5(b) demonstrate clearly the improvement in signal to noise achieved following extraction from the 500 μL sample load volume to the 50 μL of ACN eluate.

For the HPLC-FL method itself, LODs and LOQs, together with peak area and retention time precision for a mixed PAH standard (containing 1.34, 0.14 and 0.22 $\mu\text{g L}^{-1}$ of acenaphthene, anthracene, and pyrene, respectively) were determined. Based on ten replicate injections, using $3.3 \times \text{SD}$ and $10 \times \text{SD}$ of the baseline noise, LODs of 0.012, 0.016 and 0.050 $\mu\text{g L}^{-1}$ were found for acenaphthene, anthracene, and pyrene, respectively, with LOQs of 0.036, 0.048 and 0.151 $\mu\text{g L}^{-1}$. For peak area and retention time precision, inter-day repeatability was found to be 2.26, 13.74 and 13.80 % for peak area, and 0.26, 0.35 and 0.40 % for peak retention time for acenaphthene, anthracene and pyrene, respectively. This parameter was obtained by injection during different days of 4 replicates of a standard solution containing a concentration of 1.36, 0.14 and 0.22 $\mu\text{g L}^{-1}$ for acenaphthene, anthracene and pyrene, respectively.

Quantitative recovery data from the above in-capillary extraction experiments are summarised in Table1. Quantification of the original spiked water sample (containing 1.34, 0.14 and 0.22 $\mu\text{g L}^{-1}$ of acenaphthene, anthracene, and pyrene, respectively) was achieved using the standard addition calibration method, showing excellent agreement between added and determined values for acenaphthene ($<3\%$) at single $\mu\text{g L}^{-1}$ concentrations. For anthracene and pyrene, present at low to mid ng L^{-1} concentrations, the agreement was $>70\%$, which is acceptable given the

concentration and operation at close to the RP-HPLC LODs for these two solutes. For the standard addition calibration curve the regression coefficients were $R^2 = 0.999$, 0.995 and 0.999 for acenaphthene, anthracene and pyrene, respectively. Standard addition calibration graphs can be seen within Electronic Supplementary Information (Figure S3). Recoveries were certainly acceptable given the ultra-low concentrations of the standards, ranging from between 77 % for pyrene and 103 % for acenaphthene.

To evaluate the concentration factor (CF), both the spiked water sample and mixed standard solutions were analysed using RP-HPLC before extraction. The areas obtained for each compound were then compared with the areas obtained in the analysis of the ACN eluate collected following extraction. The CF was defined as: $CF = A_{ACN\ eluate} / A_{Water\ loaded}$, where $A_{ACN\ eluate}$ is the integrated area obtained from the analysis of the collected ACN eluate and $A_{Water\ loaded}$ is the integrated area obtained from spiked water sample or standard solutions. Table 2 shows the average CF obtained for each PAH over the whole concentration range ($n = 4$). Once again the best results were recorded for acenaphthene, $CF = 10.3$, based upon the 500:50, load:eluate ratio. The CFs factor for anthracene and pyrene were unfortunately lower, approximately ~4.4 and 2.6, respectively, although reasonably repeatable, given the very low concentrations investigated. The reasons for the losses were not immediately apparent and a further 50 μ L of ACN eluate only contained small traces of each PAH.

Capillary liquid chromatography using PS-DVB modified PS-PCFs

To evaluate the chromatographic suitability of the developed PS-DVB modified FS-PCF capillary PLOT column, a simple protein standard of cytochrome C was chromatographed under simple isocratic conditions. A 20 cm length of the PS-DVB modified FS-PCF capillary was used, and a 200 nL injection volume of the protein

sample used. With a mobile phase flow rate of $0.4 \mu\text{L min}^{-1}$ (~30 % ACN mobile phase), the capillary back-pressure was measured <50 bar. It should be noted that this injection volume was ~50 times greater than that which be typically used with a single 10 μm I.D. PLOT column [34], although here the aim was to simply demonstrate retention and adequate peak shape for a protein standard under such isocratic conditions.

Figure 6 demonstrates the chromatographic retention of cytochrome C (peak 1), carried out in triplicate, and with the three separate chromatograms overlaid. Conventional approaches to such protein separations on PLOT capillaries would apply a gradient elution program. However, here under isocratic conditions cytochrome C provided a retention factor of 0.6, peak efficiency of $\sim 5,000$ plates m^{-1} and excellent run-to-run reproducibility of below 1% (retention time). Encouragingly the protein peak itself showed no obvious signs of column overloading, although it was also clear the new capillary columns exhibited relatively low retention overall for the protein, but this is as expected for a relatively short length for a PLOT column. The peak shape observed for the protein standard under isocratic conditions was indeed as good as can be expected, without any sign of splitting or significant asymmetry, suggesting once more uniformity of the monolith layer throughout the 126 channels and the absence of pseudo eddy diffusion observed in FS-PCFs reported by Daley *et al.* [25].

To briefly evaluate performance of the PS-DVB modified FS-PCF PLOT column under applied gradient conditions, an *E. Coli* lysate sample (Biorad, Gladesville, Australia) was also chromatographed, in this instance using a 30 cm L modified fibre. The resultant chromatogram can be found within Electronic Supporting Information (Figure S4), which although showing little retention of the bulk of proteinaceous

material, also shows a number of well retained (presumably peptide) peaks, which on close inspection display excellent peak shapes, without any sign of peak splitting.

CONCLUSIONS

Preparation of FS-PCF based PLOT capillary columns using PS-DVB as a porous layer is described. The modified PLOT FS-PCF capillaries contained 126×4 μm channels. Polymer layer characterisation was carried out using sC⁴D and SEM demonstrating complete surface coverage and uniform porous layer thickness in 100 % of the channels. The developed FS-PCF based PLOT capillary columns were applied in novel in-capillary micro-extraction of a test solution of trace levels of PAHs with very encouraging results. Further work is now underway to optimise these new extraction capillaries for further application, including the impact of extraction rate, time, temperature etc., on selectivity and recovery of a wider range of organic analytes. The capillaries were also briefly applied to actual chromatographic separations, specifically the isocratic liquid chromatographic of a protein sample, revealing promising chromatographic potential for future such applications.

Electronic Supporting Information

Further instrumental and experimental details. sC⁴D characterisation studies showing signal traces for one empty (*) and two monolith-modified MSFs (★, ●). Standard addition calibration curves. Separation of *E. coli* lysate sample (Biorad) using a PS-DVB modified FS-PCF.

AUTHOR INFORMATION

* Corresponding Author:
Tel: +61-3-6226 6680, Fax: +61-3-6226 2858
Email: Brett.Paull@utas.edu.au

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Figure 1. A schematic diagram of the applied in-capillary micro-extraction workflow.

Figure 2. Preparation of PLOT columns within a 4 μm channel I.D. FS-PCFs. (A) FS-PCF structure in its unmodified format. (B) Typical PS-PCF following surface modification, (C) Three images displaying individual FS-PCF channels under non-optimised polymerisation conditions.

Figure 3. High resolution images of FS-PCF channels, illustrating uniformity of the polymer layer between the channels and the wall bonding of the PS-DVB phase.

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527 Figure 4. (a) RP-HPLC-FL chromatograms of ST3 mixed PAH solution before and
528 after extraction using the PS-DVB modified FS-PCF. (b) Overlaid chromatograms of
529 50 mL ACN eluates from PS-DVB modified FS-PCF capillary following concentration
530 of 500 μ L of each sample.

Figure 5. Two sC⁴D traces recorded for a 25 cm L FS-PCF, before (*) and after (●) formation of the inner PS-DVB layers.

Figure 6. Overlaid chromatograms showing the retention of cytochrome C (1) carried out in triplicate. Experimental conditions: mobile phase (isocratic): 32 % A, 68 % B, flow rate: 0.4 $\mu\text{L min}^{-1}$, injection volume: 200 nL, temperature: ambient, detector: UV at 220 nm.

Table 1. Quantification of PAHs in spiked water sample.

PAH	Added ($\mu\text{g L}^{-1}$)	Detected* ($\mu\text{g L}^{-1}$)	Recovery (%)
Acenaphthene	1.34	1.38	102.98
Anthracene	0.14	0.11	78.57
Pyrene	0.22	0.17	77.27

*based upon standard addition calibration ($n=4$).

553 Table 2. Concentration factors for each PAH obtained by in-capillary micro-extraction on PS-
 554 DVB modified FS-PCF.

PAH	CFs ¹	
	Average ²	SD ³
Acenaphthene	10.25	1.24
Anthracene	4.35	0.51
Pyrene	2.61	0.64

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556 ¹CF = Concentration factor calculated as $A_{ACN\ eluate}/A_{Water\ loaded}$, where $A_{ACN\ eluate}$ is the
 557 integrated area obtained from the analysis of the ACN eluate collected and $A_{Water\ loaded}$ is the
 558 integrated area obtained from spiked water sample and standard solutions.

559 ²Average value for CF obtained for spiked samples and standard solutions ($n=4$).

560 ³SD= Standard deviation.

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Graphical Abstract