Analytica Chimica Acta 905 (2016) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

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

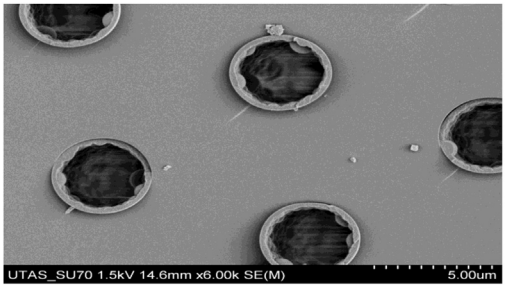
Artaches A. Kazarian [a, c], Estrella Sanz Rodriguez [a], Jeremy A. Deverell [a], James McCord [c], David C. Muddiman [c], Brett Paull [a, b, *]

[a] Australian Centre for Research on Separation Science, School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia
[b] ARC Centre of Excellence for Electromaterials Science, School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia
[c] W.M. Keck FT-ICR-MS Laboratory, Department of Chemistry, North Carolina State University, Raleigh, NC, USA

HIGHLIGHTS

- Novel PS-DVB modified photonic crystal fibres for in-capillary micro-extraction.
- New method for micro-extraction of PAHs and HPLC-FL detection at sub-ppb levels.
- Demonstration of PS-DVB modified photonic crystal fibres for capillary bioseparations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form
1 October 2015
Accepted 7 October 2015
Available online 21 October 2015

Keywords:
Polymer monolith
Porous layer open tubular column
Fused silica photonic crystal fibres
In-capillary extraction
Bioseparations

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 ± 0.1 μm and 0.26 ± 0.02 μm 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 μg L^{-1} levels of polyaromatic hydrocarbons were extracted at a flow rate of 10 μL 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 μg 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%.

* Corresponding author. Australian Centre for Research on Separation Science, School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia.
E-mail address: Brett.Paull@utas.edu.au (B. Paull).

http://dx.doi.org/10.1016/j.aca.2015.10.005
0003-2670/

1. Introduction

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

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

Theoretical considerations reported by Jorgenson and Guthrie, concluded the optimum diameter for open tubular columns for chromatographic separations should be in the order of 1–3 μm [21]. However, to-date capillary PLOT columns (more typically ~10–20 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 back pressures) 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 microbore 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 the separations of dye labelled peptides and DNA [26,28], and as multi-channel electrospray emitters in MS [22], have been reported. In addition, a recent application of wall modified FS-PCFs for extraction purposes has been described, specifically polyaromatic hydrocarbon (PAHs) extraction with subsequent separation using GC–MS [24]. However, the method reports simple adsorption of solutes into the end of the capillary only, and no in-capillary extraction or flow through extraction is explored or described. The solitary application to HPLC separations of these modified FS-PCF columns was reported by Daley et al., who investigated the chromatographic performance of capillaries following their internal wall modification using fluorosilane reagents [25]. A reasonable separation was shown for 2–3 fluorinated species, achieved using gradient elution.

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

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

2. Experimental section

2.1. 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).

2.2. 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 C_{18} column (150 × 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 Supporting information Table S2, was applied for the separation of PAHs. The C_{18} column was held at 30 °C. The sample