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COMMUNICATION

3D printed metal columns for capillary liquid chromatography.

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Coiled planar capillary chromatography columns (0.9 mm I.D. x 60 cm L) were 3D printed in stainless steel (316L), and titanium (Ti-6Al-4V) alloys (external dimensions of ~5 x 30 x 58 mm), and either slurry packed with various sized reversed-phase octadecylsilica particles, or filled with an in-situ prepared methacrylate based monolith. Coiled printed columns were coupled directly with 30 x 30 mm Peltier thermoelectric direct contact heater/cooler modules. Preliminary results show the potential of using such 3D printed columns in future portable chromatographic devices.

High-performance liquid chromatography (HPLC) is the most widely used laboratory based analytical technique for the separation of non-volatile solutes prior to their qualitative and quantitative determination. However, recently miniaturisation and portability of chromatographic systems, and analytical instrumentation in general, has drawn increased attention. Particular successes have been demonstrated in the field of gas chromatography (GC) and capillary electrophoresis (CE) [1-3]. However, the portability of HPLC systems has been somewhat hampered by miniaturisation of high-pressure pumps, columns and heating systems [1]. Ideally, the column for a portable HPLC system should perform similarly to those of standard dimensions. However, equally it should have a much reduced footprint, be of capillary scale I.D. (i.e. less than 1mm to reduce mobile phase consumption), and a length and capacity sufficient for desired chromatographic application. Obviously, a reduced column footprint facilitates further miniaturisation of associated instrumental compartments, such as column ovens/thermostats, providing good control over column temperature and facilitating increased overall performance.

Additive fabrication tools, such as 3D, printing are still considered to be within their development phase, with constant improvements in resolution and range of printable materials being reported [4-5]. However, the technology has already had a significant impact in the

area of instrumentation development and prototyping of new analytical platforms, with the first publications on 3D printing of microfluidic platforms dating back to 2002 [6]. Recent comment has placed 3D printing as one route to achieve both portability and enhanced analysis throughput, having already been applied to produce integrated microfluidic chips, "plug and play" CE system cartridges, or indeed to print actual stationary phases [3, 6-10].

One key advantage of 3D printing is the ability to fabricate solid objects with complex internal features. Intricate internal designs can be readily created, using freely available computer aided design software environments, and printed in various materials, including plastics, metals and ceramics. Techniques for printing include fused deposition modelling (FDM), stereolithography (SLA), and selective laser melting (SLM) [11]. Using this latter method for additive fabrication in metals and alloys provides the opportunity to create new column housing designs, of varying degrees of complexity, which were previously simply not possible. The materials used can also facilitate excellent heat transfer characteristics, which are important for the use of direct contact column ovens for high temperature separations, or indeed provide surface chemistry suitable for surface modification e.g. titanium, which provides for strong covalent attachment of in-situ fabricated polymer monolithic materials [12].

In the work presented here, a Realizer SLM 50 (Realizer GmbH) SLM system was used to produce prototype spiral columns with dimensions of 600 mm L x 0.9 mm I.D., in a footprint of 5 x 30 x 58 mm, in either stainless steel (SS) (Grade 316 LTLs Technik Germany) or titanium alloy (Grade Ti6Al4V – TLS Technik Germany). A schematic

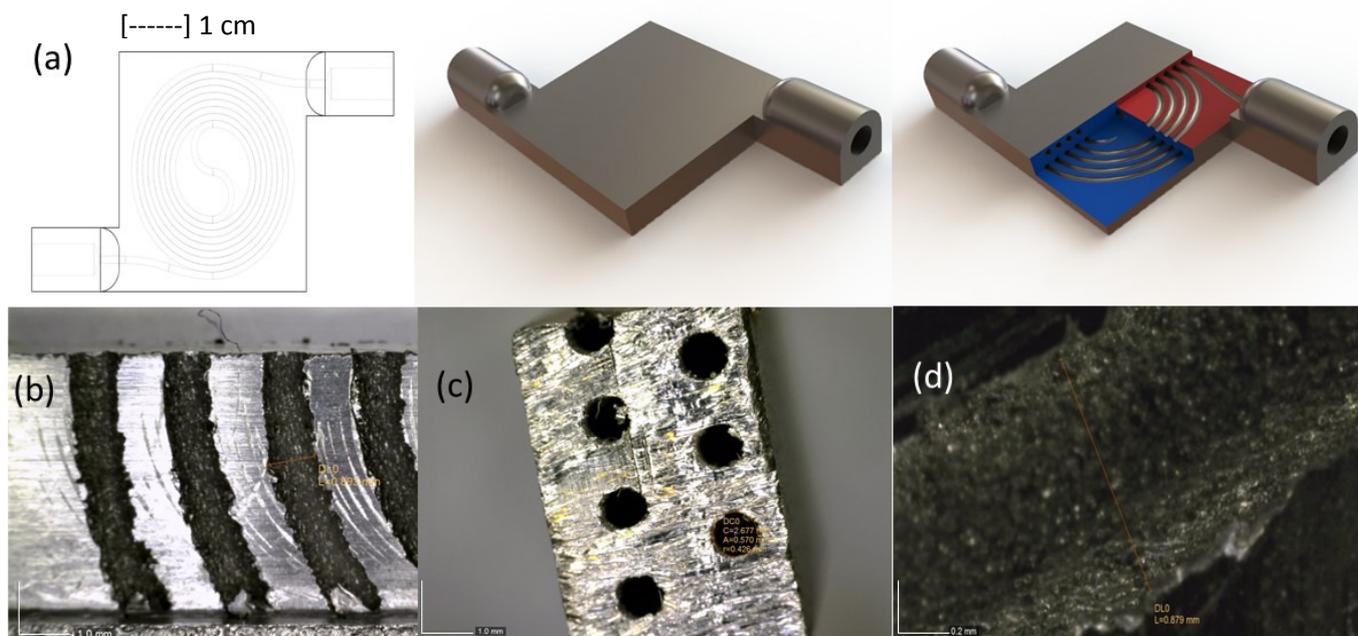


Figure 1(a) Representation of spiral columns printed in stainless steel (SS) and titanium, and (b-d) image of exposed internal channels from sectioned SS coiled columns showing surface roughness of approximately 40 μm .

representation of the column design is shown in Fig. 1(a), with the internal capillary channels of a sectioned SS spiral column shown in Fig. 1(b). The SLM 50 system can print in layers of $\sim 25 \mu\text{m}$, with a minimum feature size of $\sim 150 \mu\text{m}$. Resolution and surface roughness are defined by material properties and operating settings. The process results in loosely bound particles generally between $25 \mu\text{m}$ and $65 \mu\text{m}$ sitting on the material surface. External surfaces are typically media blasted to remove such particles. However, this is not possible for small internal features, hence here the surface roughness approximately corresponds to the largest particle size (i.e. $< 65 \mu\text{m}$). In this work this was confirmed by visual microscopy analysis of an exposed internal channel within a SS column with a surface roughness, Ra value, of $\sim 50 \mu\text{m}$ (see Fig. 1(c) and (d)).

The printed columns were designed with 10/32 standard ports [13], which were manually tapped for compatibility with standard 10/32 fittings, to connect either 1/16" peek tubing or 250 μm I.D. fused silica capillary (FSC) within a sleeve. For FSC connections, 5 mm porous monolithic styrene-divinylbenzene frits were formed in-situ (see Electronic Supplementary Information) within the capillary and attached to the outlet port. This system was tested to 4000 PSI (276 bar).

Two types of stationary phase were used to evaluate the potential of the printed columns. Firstly, SS columns were slurry particle packed with reversed-phase silica particles (octadecylsilica) ranging from 50 down to 5 μm . Packing was carried out at 4000 psi in each case (using a glycerol and isopropanol mixture (50/50 v/v) for 20, 10 and 5 μm phases, and a 70/30 v/v slurry composition for the 50 μm particles. Secondly, a methacrylate polymer monolith (butylmethacrylate-

ethylene glycol dimethacrylate, polyBuMA-co-EDMA) was prepared within the printed titanium coiled column, and bonded to the column walls following a previously reported procedure [12]. This procedure was based upon thermal oxidation of the titanium surface, followed by silanisation, and thermal polymerisation of the monolith mixture. A temperature gradient was used for polymerisation starting from room temperature up to 55 $^{\circ}\text{C}$ with the rate of 0.3 $^{\circ}\text{C}/\text{min}$ and the total polymerisation time of 570 min (more details can be found within Electronic Supplementary Information).

The planar coiled column design was produced in the first instance to facilitate direct contact heating using small 30 x 30 mm Peltier thermoelectric direct contact heater/cooler modules. Therefore, to compliment the printed columns, a small direct contact heating platform based on Peltier thermoelectric heater/coolers was developed to enable precise control of column temperature during

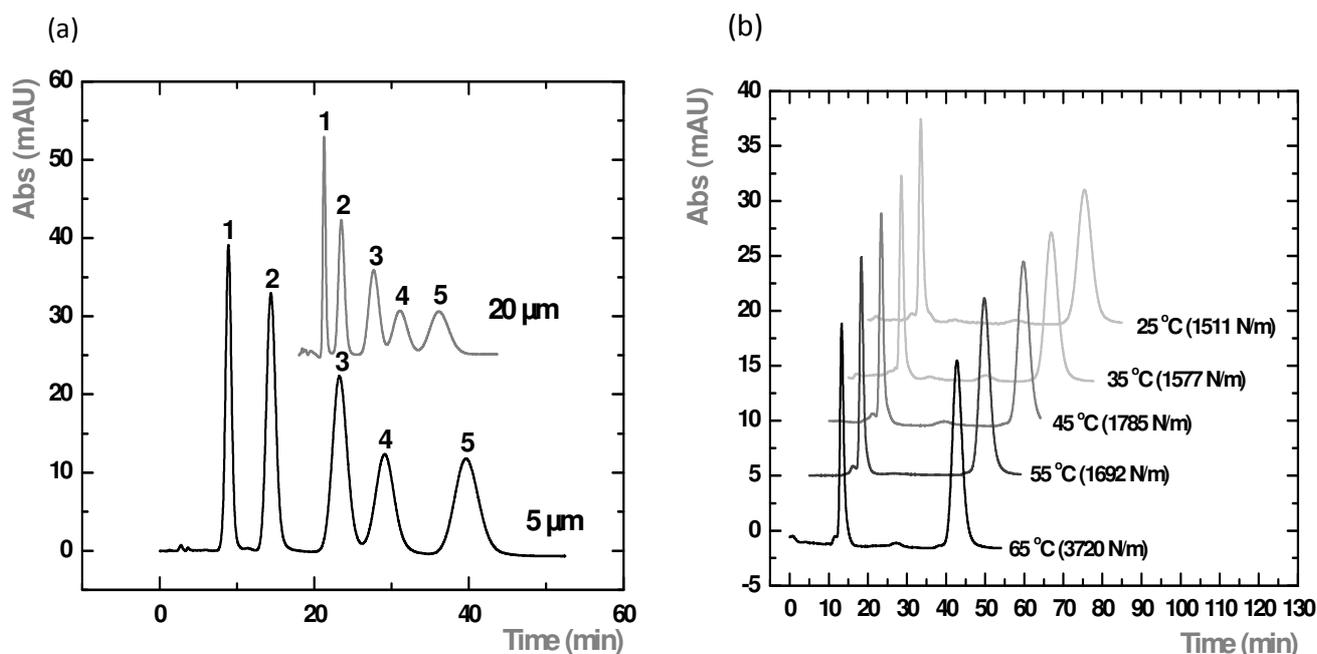


Figure 2(a) Separation of the phenone mixture, 1-benzophenone, 2-acetophenone, 3-propiophenone, 4-butyrophenone, and 5-valerophenone on ODS packed 3D printed SS columns (0.9 mm I.D. x 60 mm L). Mobile phase 40% ACN in water, flow rate 25 $\mu\text{L}/\text{min}$, UV detection at 254 nm, injected volume 1 μL . (b) The effect of temperature on retention and efficiency of uracil and acetophenone for a 5 μm packed SS column. Temperature was applied through direct contact Peltier TEC module,

chromatographic evaluation (see Electronic Supplementary Information). The system was able to control the column temperature from room temperature to 90 $^{\circ}\text{C}$, with an accuracy of 2 $^{\circ}\text{C}$. A similar system was used by Collins *et al.* for temperature control of capillary columns [14].

Although the aim of this investigation was to ascertain potential of such fabrication tools for future complex column designs, it was also important to demonstrate actual chromatographic performance, even for non-ideal column geometries (for liquid chromatography), such as coiled columns. Simple chromatographic evaluation of the packed SS printed columns was therefore carried out to examine, via the generation of van Deemter plots, the efficiency of the columns for each particle size, injection volume (for both see Electronic Supplementary Information), and column temperature.

A van Deemter curve was plotted using an uracil and acetophenone test mixture at 65 $^{\circ}\text{C}$ for flow rates ranging from 100 $\mu\text{L}/\text{min}$ down to 20 $\mu\text{L}/\text{min}$. Due to the length of the coiled column (~60 cm), a relatively low range of flow rates were applied, since increases in flow rate above 100 $\mu\text{L}/\text{min}$ led to a backpressure of more than 400 bars (although this

did demonstrate the ultra-high pressure stability of these metal printed columns). The flow rate study unsurprisingly showed the highest plate height and lowest efficiency for the large 50 μm particles, whereas the efficiencies for 20, 10 and 5 μm particle packed printed columns showed very similar trends, ranging from between 3909 plates/meter (20 μm) to 3285 plates/meter (10 μm). The failure to obtain a significant increase in efficiency with concomitant decrease in particle size, can be attributed to the significant contribution to longitudinal band broadening from the coiled column structure, and the inner surface roughness of the column, which would have a detrimental effect upon the homogeneity of the packed stationary phase, particularly for the smaller particles. The results advocate the use of stationary phase with comparable particle diameter to the surface roughness of the column in order to obtain maximum efficiency with lower backpressure.

To demonstrate the chromatographic performance of SS column packed with the various ODS stationary phases, a separation of a test mixture of five homologous phenones was performed under isocratic conditions (60/40 water/ACN). Separation of all five analytes was readily achieved on sub-20 μm particle packed columns. Typical chromatograms for the 20 μm and 5 μm packed columns are shown within Fig. 2(a). Clearly the separation was achieved in approximately half the time with the lower capacity 20 μm column, however, the separation obtained with 5 μm particles showed enhanced peak resolution, and the run time could be reduced even further through an increase in ACN content within the mobile phase (see Electronic Supplementary Information). Fig. 2(b) shows the effect of temperature on the efficiency of slurry packed SS column for the 5 μm ODS phase. A test mixture of 20 $\mu\text{g}/\text{mL}$ of uracil (void marker) and acetophenone

Table 1: Plates per meter and optimal flow rate per particle size for ODS packed 3D printed SS columns (0.9 mm I.D. x 60 mm L).

Particle Size (μm)	Optimum Flow Rate ($\mu\text{L}/\text{min}$)	Number of Plates/meter
50	45	791
20	25	3909
10	15	3285
5	25	3720

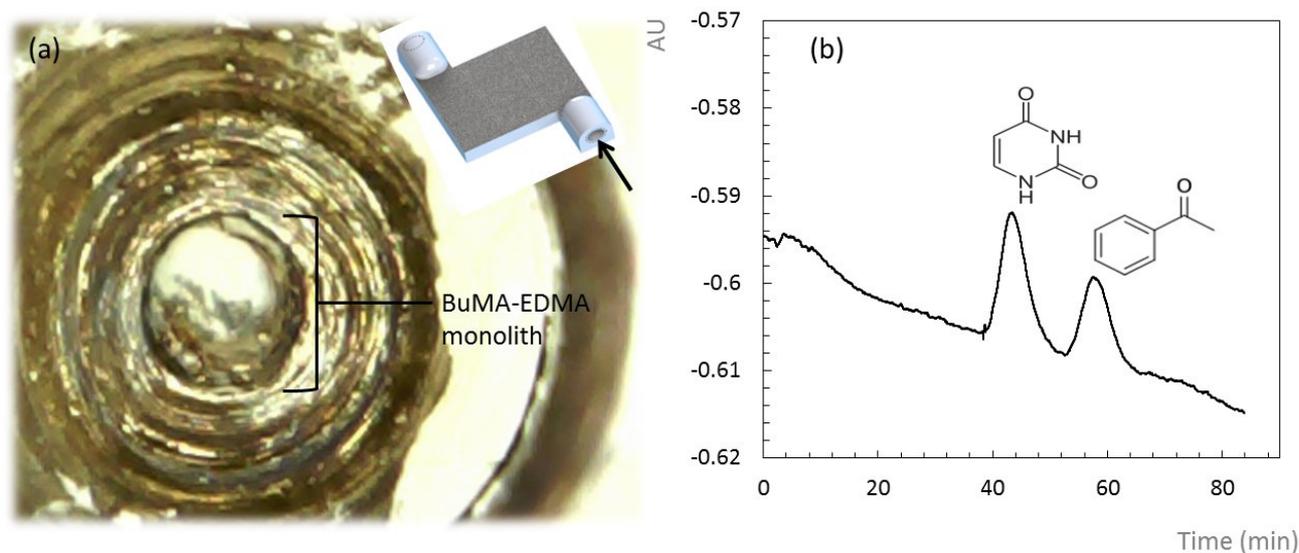


Figure 3(a) BuMA-EDMA monolith housed within 3D printed titanium coiled column (at inlet) and (b) the separation of uracil and acetophenone on the 0.8 mm x 60 mm BuMA-EDMA polymer monolith. Mobile phase 70% ACN in water, flow rate 5 $\mu\text{L}/\text{min}$, UV detection at 245 nm, column temperature 45 $^{\circ}\text{C}$, injected volume 10 μL .

(retention compound) was used, under isocratic conditions of 60:40 water:ACN (injection volume = 1 μL). Further increase in temperature beyond 65 $^{\circ}\text{C}$ did not increase the efficiency for 5 μm particles. Previous work of Nesterenko *et al.* demonstrated the successful formation and wall bonding of methacrylate based monoliths within thermally oxidised titanium tubing [12]. Here a similar procedure was applied, and resulted in a particularly dense monolith (see Fig. 3(a)), which although not optimised for liquid phase separations, did exhibit limited permeability, as evident from a pressure drop of ~ 130 bar at a flow rate of only 5 $\mu\text{L}/\text{min}$ (70% ACN). In addition, the monolith remained stable within the printed column under applied pressures in excess of 200 bar without retaining frits, confirming the wall bonding was also successful. Under the above limited flow conditions, uracil (Fig. 3(b), retention time 43 minutes) and acetophenone (Fig. 3(b), retention time 58 minutes) could be separated, albeit with an efficiency of only 1043 P/m (measured on acetophenone).

Conclusions

For the first time, 3D printing of metal alloys has been used for the creation of long capillary columns (600 mm) within small footprint designs (30 mm x 58 mm). Although these early designs are far from optimised for efficient chromatographic performance (the subject of on-going work), the potential for application to compact and portable liquid chromatography systems is already very clear. Whilst the applicability of the titanium monolithic column for HPLC was hindered by the low permeability of the prepared monolith, encouraging results were obtained using the packed SS columns. These results demonstrate the potential of the technology to produce previously unobtainable complex column designs allowing for future fundamental

and applied studies. Further work will include measures to improve packing, reduce band broadening and thus increase separation efficiency. The preparation of more porous polymer monoliths, including wall bonded open tubular structures [15], suitable for various chromatographic separations is also under investigation.

Notes and references

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Electronic Supplementary Information (ESI) available: [Details of printing process, Formation of monolithic polymer frits and phases, van Deemter curves, Design of heating system, Acknowledgements]. See DOI: 10.1039/c000000x/

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Acknowledgements

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30 B.P, P.N.N, S.S and V.G would like to acknowledge funding to take part in
31 this work from the Australian Research Council (DP130101518). BH would
32 like to acknowledge the FP7-PEOPLE-2010-IRSES Marie Curie Action:
33 'Materials and Advanced Sensor Knowledge Exchange (MASK)' for funding
34 an exchange from Dublin City University to the University of Tasmania,
35 and the Irish Research Council (IRC) under the Enterprise Partnership
36 Scheme in collaboration with Te Laboratories, as part of the International
37 SmartOcean Graduate Enterprise initiative (ISGEI) for funding of his PhD
38 research. S.B, F.T and G.G.W acknowledge the continued support of the
39 Australian Research Council and the use of additive fabrication facilities
40 within the Australian National Fabrication Facility (ANFF) Materials Node.
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