The Utilisation of Stereolithography Rapid Prototyping, to drive the design and testing of a new Chromatographic technique, Dynamic Field Gradient Focusing (DFGF).

Thomas Wray, Peter Myers.*
Department of Chemistry, University of Liverpool, Crown Street, Liverpool, UK. L69 7ZD.
**Corresponding Author*

In developing new analytical instruments and in particular chromatography instrumentation there is a need to modify or manufacture components to perform experiments and initiate the innovation. Usually this means anything from ordering bespoke parts to fabricating components and devices in house. At the University of Liverpool the development of a new technique in chromatography, Dynamic Gradient Field Focusing (DFGF), is moving forward with the aid of some of the latest advances in Stereolithography Rapid Prototyping. With this method of design improvement the testing of various designs and configurations has led to a system coming to fruition in a reduced time frame and at a very low cost. Utilising this approach has catalysed the emergence of a new instrumentation platform.

Experimental

Rapid Prototyping

Rapid prototyping (RP) is a process developed in the 1980's as a means of producing prototypes or models[1]. As the technology has progressed RP has become the link between designs and the real world. A design drawn using Computer Aided Design (CAD) software can be interpreted into a series of layers which are then grown or sculpted to give a product, the prototype. The first rapid prototypes were simple free form cutting machines programmed by basic CAD software. There are many different types of RP devices which have been devised since then. One of these more advanced techniques is stereolithography which has been utilised for this work.

Stereolithography Rapid Prototyping

Stereolithography, from the Greek 'to write with light', is a process where a material is polymerised typically by UV light[2]. Shapes are generated by a series light manipulating devices to project a two dimensional image onto a bed of liquid, causing polymerisation. As these 2D images polymerise the new solid is lifted from the bed, these layers build up the three dimensional structure which emerges from the liquid, Figure 1.

The development of a high resolution stereolithography RP device has been undertaken by Jetinder Singh[3] at the

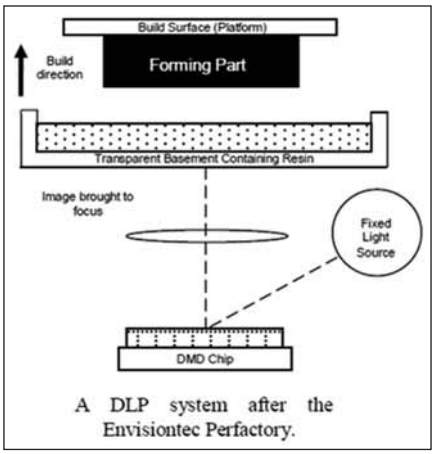

Figure 1. Schematic displaying the setup of stereolithography apparatus.

University Of Liverpool Department of Engineering. This system is capable of producing components with features accurate down to just 6 µm. This is achieved by using state of the art software, laser control and stepper motor control. The refined stereolithography RP machine provides the capability of forming complex microfluidic devices in a fraction of the time it would have normally taken going through a classical machining route with a significant reduction in cost.

Dynamic Field Gradient Focusing

Dynamic Field Gradient Focusing (DFGF) is a new innovative separation technique[4]. As the name suggests DFGF uses a dynamically adjustable electric field gradient to separate a mixture of components from a matrix. The focusing part is attained by hydrodynamic flow acting in the opposite direction to the field, resulting in analytes coming to a unique focal point according to their differences in electrophoretic mobility and hydrodynamic radius[5]. The beauty of this technique over other electrophoretic separation methods is that it allows ultimate control over both the hydrodynamic flow and the electric field, enabling the user to alter and control the movement of analytes in the separation column. Not only does this provide means to control a given separation, it is also possible to hold back concentrated bands of analytes before eluting them from the device. With the ability to separate and concentrate samples in a single step, DFGF is not only a precise analytical tool, but also has the potential to be used as a front end for further characterisation or analytical technologies, i.e. a Mass Spectrometer.

Early work with DFGF saw attempts to work towards a microfluidic DFGF system with research carried out by Peter Myers[6] at Leeds University where it was demonstrated such a device could be used to separate a mixture of proteins. The main problems with this were; the use of membranes, joule heating, monolith break down, and issues with detection. All problems which have now been be improved upon and resolved with the latest innovation.

Results

Current research focuses on the latest DFGF device (Figure 2). The design of the new instrument hinges around an open channel quartz separation column, no packing materials or monolith are required. This miniaturised system gives a larger electrode density, producing a higher electric field gradient over a smaller area to provide more control over separations. Moreover separations can now be performed using much lower voltages so allowing the miniaturisation of the power supplies driving this device. Having the ability to reduce the size of the overall instrument is favoured as many modern microfluidic devices may themselves be miniature but the equipment required to drive them often has a large laboratory footprint. Since the new DFGF device features a quartz separation column UV detection along the total length of the separation channel can now be implemented. This is achieved by housing a Photo Diode Array (PDA) detector along the face of the column and illuminating the column, from the back of the chip, with 254 nm UV light. Using this configuration a separation can be observed in real time and adjustments to the positions of analytes can be made.

Figure 3. Microfluidic DFGF device demonstrating an open channel separation of Amaranth and Bromophenol Blue. Left: Original exposure. Right: Enlarged area where the bands of analyte are focused.

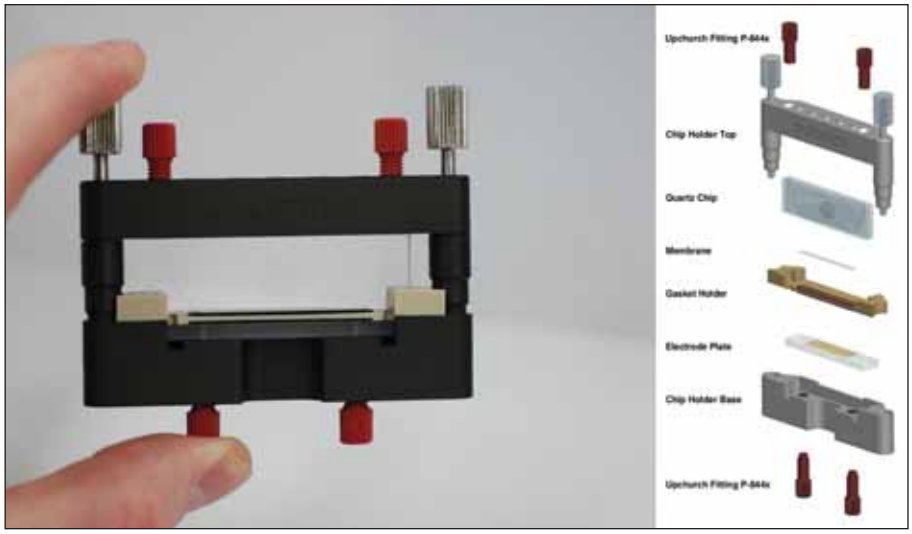

Figure 2. Microfluidic DFGF and components.

Preliminary experiments with the new DFGF system saw the open channel separation of a simple two component test mixture. A mixture of Amaranth and Bromophenol Blue was loaded onto the device which was then separated at a flow rate of 1 µL/min and a field of 100 V/cm^2. This separation was achieved in 20 minutes and exposures of the reflectance detection method are shown in Figure 3. A graphical representation of the separation is shown in Figure 4.

The separation data, shown in Figure 4, was collected by taking exposures of the whole column length and then subtracting this from an initial background exposure. Exposures where taken once a minute and plotted to give a graphical representation of the separation. The sample mixture was introduced and brought into focus and over time the single peak resolved into the two components of the test mixture. The images in Figure 3 corresponds to the final trace at 20 minutes in Figure 4. This experiment validated the functionality of the device and also highlighted some key issues.

Stereolithography RP has moved DFGF forward by accelerating the manufacture of new and modified components for testing with the system. As with any new instrumentation, it is rare that a new design will work perfectly from the outset! The new microfluidic DFGF was no exception with issues of sample loss from the separation channel and electrode degradation being the main problems encountered. Solutions came from stereolithography in the form of masks for Physical Vapour Deposition (PVD) of platinum electrodes through to complete replacement parts with significant design improvements.

The mask for the PVD went from design to being completely formed in just 12 hours,

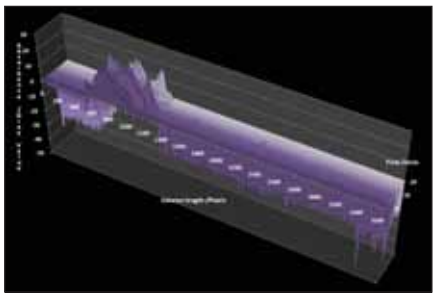

Figure 4. Reflectance data displaying the separation of Amaranth and Bromophenol Blue. With time on the z-axis it is observed that the single band advances slighty on the x-axis, column length, and resolves into two peaks corresponding to the two components of the mixture.

shown in Figure 5. This was then seated on a fused silica chip ready for the platinum electrodes to be sputtered onto the surface of the chip, Figure 6. The electrodes were made precisely and in just a few weeks from discussion over the new design. Having these to test showed us that unfortunately electrodes made in this way were definitely unsuitable for applications in DFGF as the platinum coating did not have a strong affinity to fused silica and the electrodes degraded. The solution was found to be electrodes made from platinum foil recessed into a chip. Current design work is incorporating a Printed Circuit Board (PCB) for the electrodes. In addition the RP process is allowing the testing of future electrode designs which ultimately will shape the electric field to the dimensions of the separation channel.

A simple solution to the sample leaking from the column was to redesign the 'purge channel' of the DFGF (this component is part of the 'Gasket holder', Figure 2). Part of the DFGF setup involves having the electrodes in a separate channel. The purpose of the purge channel is to keep the sample in a separate channel away from the electrodes as this eradicates any pH changes and gas evolution from destroying the analytes. The defect with the purge channel was that when originally manufactured the channel width was far too large. Using stereolithography RP an array of new purge channels with varying channel widths were constructed (Figure 7).

Figure 5. PMMA Sputtering mask for Platinum PVD.

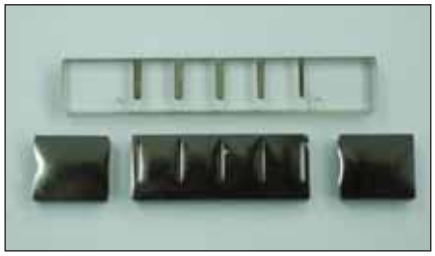

Figure 6. PMMA mask post platinum PVD, platinum electrodes formed on glass chip.

Once one of these parts had been drawn in the CAD software, it could be formed multiple times with small alterations made easily, in this case the channel width. Having tested 500, 200 and 100 micron channels it was experimentally determined that a channel width comparable to the separation channel, at 100 microns, was optimal.

Further work utilising stereolithography RP for DFGF will see a completely new design for the lower half of the device. It is common for a device to be simplified and refined with the aid of RP. An interesting example of this is where RP has led to the refinement of a low cost miniaturised Quadropole Mass Filter[7]. The number of components was reduced from around thirty to only thirteen.

Using RP a new design for the DFGF will see a group of six complex components beneath the separation channel evolve to just a single unit with a PCB insert. Once this has been demonstrated on the RP platform it is readily scalable up to a commercial manufacturing line.

Figure 7. Replacement component manufactured using stereolithography RP. Channel widths ranged from 1 mm down to 100 µm.

Conclusion

Collaborating with engineers using RP is a sure way to accelerate the development of new instrumentation and the refinement of current systems in chromatography. The advantages of receiving components within days of designing them and having the facility to produce multiple copies at minimal extra cost really drives this kind of research and development forward, as other methods of sourcing new specialist components come at a high cost with long waits. Finally the ease of manufacture gives the research a large degree of freedom for modifying what would be static design.

Acknowledgements

Thanks to; Dolomite for the silica microfabrication, Thermo Fisher Scientific for the PhD support to Thomas Wray, Pfizer Global Research & Development for supporting the project, and the University of Liverpool for a DTA award to Thomas Wray.

References

[1] C.W. Hull, (1984) U.S. Patent 4575330

[2] F.P.W. Melchels, J. Feijen, D.W. Grijpma, Biomaterials 31 (2010) 6121-6130

[3] J. Singh, js1ngh@liverpool.ac.uk , Department of Engineering, University of Liverpool. UK

[4] N.I. Tracy, C.F. Ivory, Electrophoresis 29 (2008) 2820-2827

[5] Z. Huang, Ph.D. Thesis, Washington State University (2001)

[6] P. Myers K.D. Bartle, J. Chromatogr. A. 1044 (2004) 253-258

[7] B. Brkic, N. France, A.T. Clare, C.J. Sutcliffe, P.R. Chalker, S. Taylor, Am. Soc. Mass

'Total Solution' approach that combines Automation Technologies with Gas Chromatography Systems

Under an agreement signed in November 2010*, Presearch will distribute Bruker Gas Chromatography (GC) instrumentation in the UK with the added value of complementary technologies and solutions. By combining automation solutions from CTC Analytics, and solid phase extraction from United Chemicals, (UCT, inc.), 'whole solutions' will be built around Bruker's routine single Channel 430 or flexible multi-channel 450 GC systems.

The new Bruker-Presearch partnership will provide customers with a comprehensive choice of extended technologies that will deliver reduced analysis times and improved precision. Sensitivity can be increased through MEPS, ITEX, SPME, Headspace and liquid sample handling, and by designing pre-separations using UCT SPE. Customers will also benefit from the knowledge exchange and associated experience the agreement will create, which will in turn help to simplify and speed up results for the Environmental, Food, Energy or Clinical laboratory.

Mark Dunham, Sales Manager at Presearch said, "The new partnership gives us, for the first time, the ability to offer our customers total GC solutions for their analytical needs or for their process control problems."

Richard Robinson, Sales Team Leader at Bruker, was quick to point out the benefits for customers in choosing Bruker GC from Presearch, "'Presearch's expertise and extended technologies add a further dimension in optimised end-solutions for our customers who choose this additional channel to acquire their GC solution."

For more information visit www.bruker.com www.presearch.co.uk