EXPLORING THE USE OF MICROSTRUCTURED FIBRES AS A STATIONARY PHASE SUPPORT FOR OPEN TUBULAR LIQUID CHROMATOGRAPHY

by

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Abstract:

With the rise of capillary HPLC systems, open tubular liquid chromatography (OTLC) has been garnering more attention due to the possible fundamental advantages of open tubular systems over conventional packed or monolithic systems. Performance has yet to reach its potential due in part to a variety of technical challenges, resulting in the need for very small injection volumes and sensitive detection. In this work, we have shown that with modern HPLC sample introduction and detection systems, along with careful fabrication of polymer stationary phases, that reverse phase open tubular liquid chromatography may be within reach. We have shown that, with small diameter (i.d. 30μm) open tubular columns, complex multi-component mixtures (EPA 610, in-house drug mixture) can be separated. We have also shown that these columns are robust and can function over a wide range of flow rates (200-1000 nl/min), and may be useful for general reverse phase separation in the future. However, currently, more stationary phase development and procedure refinement is needed.

Microstructured fibres (MSFs), a relatively new class of optical fibre which confine light within fibres through a refractive index change caused by the use of parallel air channels running throughout the length of the fibre, are explored as a new support material for open tubular liquid chromatography. The fine channel structures of MSFs enable reasonable sample volumes to be used compared to conventional open tubular systems, while offering a similar plug-like flow profile through the fibre. With current sample introduction and flow technologies, we have shown that the potential advantages of MSF columns is great even when simple C18 stationary phases are used; this was able to separate a four PAH mixture. However, a distribution in channel sizes caused by current manufacturing standards and a limited ability to evenly deposit polymer stationary phases in the fibres has kept MSF columns from reaching their full potential.

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List of Acronyms

Acen	Acenapthene
ACN	Acentonitrile
ACY	Acenaphthylene
AIBN	Azobisisobutyronitrile
Anth	Anthracene
ASA	acetylsalicylic acid
BaA	Benzo[a]anthracene
BAP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BDDA	Butanedioldiacrylate
B[ghi]P	Benzo[g,h,i]perylene
BkF	Benzo[k]fluoranthene
CE	capillary electrophoresis
CEC	capillary electrochromatography
Chry	Chrysene
CTMS	Chlorotrimethylsilane
DahA	Dibenz[a,h]anthracene
DVB	Divinylbenzene
ESI	electrospray ionization
FLU	Fluorene
Fluo	Fluoranthrene
GC	gas chromatography
HPLC	high performance liquid chromatography
Ind	Indeno[1,2,3-c,d]pyene
i.d.	inner diameter
LC	liquid chromatography
LMA	Laurylmethacrylate
MSF	microstructured fibre
NAP	Naphthalene
OTLC	open tubular liquid chromatography
PAH	polycyclic aromatic hydrocarbons
Phen	Phenanthrene
PLOT	porous layer open tubular
PS	Polystyrene
Pyr	Pyrene
SEM	scanning electron microscope

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Chapter 1: Introduction

1.1.0 Introduction to HPLC Separations.

1.1.1 Introduction to Chromatography

Chromatography is a powerful laboratory technique used to physically separate components of a mixture[1, 2]. The general working mechanism of chromatography involves a mixture being passed through or over a fixed "stationary phase", which in most cases is chemically bound to a solid support, such as small silica beads. As the mixture passes through, the components of the mixture interact with the stationary phase based on their chemical properties[1, 2]. Some components interact with the stationary phase more strongly than others, while some do not interact with the stationary phase at all. The stronger the interaction between the stationary phase and the particular component of the mixture, the longer the time the latter spends on the stationary phase. As a result, one analyte lags behind the other components of the mixture, effectively causing the components to be separated over time[1, 2].

There are many different chromatographic techniques. The two most important classes of chromatography are liquid chromatography (LC), where liquid samples are used and gas chromatography (GC), where samples with a high vapour pressure can be analysed[1, 2]. These classes of chromatography can then be further subdivided based on the type of interaction between components of a mixture and the stationary phase. Adsorption, partitioning, size exclusion and affinity are a few examples of commonly employed interaction types, although, adsorption and partitioning are the most widely encountered methods used in modern LC[1, 2].

Liquid chromatography has numerous modes of operation as well. The two most commonly encountered are "normal phase" LC and "reverse phase" LC. Normal phase LC is carried out using a polar stationary phase and a non-polar (organic) solvent as the mobile phase

whereas reverse phase LC is done using a non-polar stationary phase and polar solvent for the mobile phase[1, 2]. Reverse phase LC is increasingly more popular than normal phase LC as many potential analytes possess at least some non-polar character that can be exploited for a separation, allowing for reverse phase LC columns to be more versatile than their normal phase counterparts[1, 2]. Another increasingly popular feature of reverse phase LC is the use of more environmentally friendly solvents for mobile phases, such as water or alcohols, over normal phase methods which have a heavy use of organic solvents such as hexane.

Two important measures of performance in chromatography are the resolution and efficiency of separation. Resolution, which is generally regarded as how well peaks are

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} \tag{1}$$

separated relative to each other, is described by equation (1): [where Δt_r is the difference in retention times between the two peaks and w_{av} is the average width of the peaks measured at the baseline.] The resolution of peaks can be affected a great deal by the properties of the stationary phase, mobile phase and analyte[1, 2]. For instance, if you have a very polar group of analytes and are using a polar mobile phase with a nonpolar stationary phase, resolution may be improved by using a less polar mobile phase or by switching to a more polar stationary phase. Unfortunately, there is a practical upper limit to increasing resolution, as the separation will eventually take too much time to carry out and peaks will likely suffer from problems related to diffusion [1, 2]. A value of 1.5 for resolution is considered baseline resolution of two peaks.

Another important measure of performance in chromatography is efficiency of separation. Efficiency is related to the relative width of the chromatographic band and is often

measured in terms of the plate height[1, 2]. Plate height, H, is described by the van Deemter equation, equation (2), and this describes the magnitude of band broadening through three main

$$H = A + \frac{B}{u_x} + C\,u_x \qquad (2)$$

mechanisms[1, 2]. The A term represents band broadening by multiple flow paths. In packed columns for example, the fluid travelling through the column does not all take the same path, as the small particles used in the column can create resistance to flow, and as such, some of the analyte will move though the columns in a shorter time (path); while other parts of the analyte band will take a longer path, leading to an expansion of the chromatographic band[1, 2]. The B term represents band broadening through longitudinal diffusion along the column. This is caused by the analyte band simply diffusing along the mobile phase as a result of a high concentration of analyte in one place within the solution[1, 2]. This effect, however, can be limited to a degree through control of the mobile phase speed (u_x) through the column[1, 2]. Lastly the C term, called the mass transfer term, describes band broadening through the equilibration of analyte between mobile and stationary phases in the column. A slow equilibration time negatively affects the size of the chromatographic band. This is because the interaction between the stationary phase and analyte is more favourable than the analyte floating freely in solution, this in turn, causes some of the analyte to "stick" to the stationary phase while the bulk of the solution continues to travel down the column[1, 2]. This effectively spreads the analyte out resulting in wider peaks. The C term can be reduced by using thinner stationary phase layers and/or smaller columns (for open tubular columns) or smaller particles (for packed columns) which allows for faster equilibration between analyte, stationary and mobile phases[1, 2]. Plate height is an important characteristic of chromatography columns because if we are able to reduce any of the terms in the equation, we can achieve smaller plate heights, resulting in more efficient (better) separations[1, 2].

1.1.2 Miniaturisation of Liquid Chromatography

Miniaturisation of liquid chromatography techniques has been, and still is, a major area of research that has been spurred on by more and more reliable manufacturing of small glass capillaries[3-8]. There are some major differences between conventional HPLC and miniaturised (microcolumn) HPLC. Most noticeable is the column size; most microcolumn LC systems use small (10-100 μm i.d.) glass capillaries as columns, compared to the normal 4.6 mm i.d columns of conventional LC systems. Another major difference between conventional LC and microcolumn LC is the flow rates that are employed[3-8]. Conventional LC normally uses flow rates in the mL/min range, whereas microcolumn LC typically uses μL/min or lower. Using miniaturised LC systems offers a number of advantages over conventional HPLC, most notably a reduction in sample and solvent use[3-8]. Other benefits of miniaturised LC systems include: greater sensitivity, resolving power, as well as better compatibility with modern detection techniques such as mass spectrometry (nanoESI, for example) and lab-on-a-chip devices[3-8]. Miniaturized LC is not without its challenges, though, as column fabrication can be tricky at times and the introduction and detection of such small sample volumes (often in the low and sub nanolitre range) can still pose some difficulties to analysis[3-8].

1.1.3 HPLC Columns Types

The most widely encountered general classes of liquid chromatographic columns are packed columns, monolithic columns and open tubular columns[1, 2, 9, 10]. Packed columns are the most common column type and consist of a packed bed of small spherical silica particles, usually on the order of 3-5 μm in diameter. Packed columns normally use a covalently bound stationary

phase (e.g. C8, C18, or phenyl groups) for reverse phase type separations. Packed columns owe much of their success to the high surface area of small silica particles as they can allow for many fast interactions between stationary phase and analytes[1, 2, 9, 10]. Newer ultra high pressure (UPLC) columns can use particles as small as 1-2 μm in diameter[2]. Using smaller particles can increase column efficiency by reducing the A term (by allowing for more uniform packing of particles and flow in the column) and C term (by allowing for more contact/ faster interactions between the stationary phase and mobile phase) in the van Demeter equation (2). However, using smaller (1-2 μm) particles leads to substantial increases in flow induced backpressure, requiring strong and sophisticated pumping systems and more robust fittings[9, 10].

Columns made of monolithic materials have been gaining much popularity over the last few years. Monolithic chromatography columns can be made out of a variety of materials; however, most are now highly cross-linked networks of polymers, such as polystyrene/ divinylbenzene or a variety of acrylates or methacrylates[2, 9, 11]. Silica monoliths can also be used and functionalised in similar ways to silica particles. Monoliths are distinguished from packed columns in a several ways; one major difference is that unlike packed columns made of distinct particles, a monolithic column is a very porous single polymeric or silica structure. This provides them with a few advantages. One such advantage is that monolithic stationary phases are less prone to shifting and compression over time from constant high applied pressures[9]. The porous structure of monolithic columns allows for very high surface areas to be achieved. A high surface area allows for increased contact with analytes and leads to good separations[2, 9, 11]. Monolithic columns are also very versatile; they can be easily synthesised with a variety of pore sizes and functionalities and in different column/ channel architectures, where the packing of silica spheres may be difficult or impossible to do reproducibly[9, 11]. Monolithic columns also enjoy a high column to column fabrication reproducibility, with RSD values for retention time

and back pressure variation ranging between 0.5 -1.5% and 4-12% respectively, numbers which are close to those of packed columns [9,11]. Despite the many advantages of monolithic columns, it has been reported that monolithic columns can have a lower total surface area than packed particle columns, which may limit their sample capacity, and thus require more sensitive detectors. However it is thought that the higher flow rates and reduced analysis times compared to packed columns make up for this with gains in productivity, allowing for a shorter run cycle[9, 11].

Open tubular columns are most often encountered in gas chromatography; however there is a growing amount of work on using open tubular columns for liquid chromatography, now that reliable injection methods and highly sensitive detection methods are available[2, 9, 12-17]. Open tubular columns differ greatly from both packed and monolithic columns. The most obvious difference is in their construction. While packed columns and monolithic columns contain a mostly space filling stationary phase, open tubular columns have stationary phase bound just to the wall or to support particles (usually silica spheres) attached to the wall[2 ,9, 12-17]. This all but eliminates the A term in the van Deemter equation, as without particles or solid materials (monoliths) in the way, impedance to flow is reduced, as the column effectively becomes an open pipe. This in turn allows for reduced values of H to be achieved compared to monolithic or well packed columns[2]. Although there are clear advantages of open tubular columns, they do possess limitations such as high back pressure (when columns with very small i.d. are used) and require small sample volumes, which can make open tubular columns difficult to use.

1.1.4 Open Tubular Liquid Chromatography

Open tubular columns have been around for many years and have received significant attention for gas chromatography due to their inherent advantages over pack columns[2].

However, they have only received sporadic attention for liquid chromatography[12-17]. There are a few reasons as to why open tubular columns have not enjoyed the same success in liquid chromatography; most notably is the small amount of stationary phase in open tubular columns[8, 9, 11]. Open tubular columns only have stationary phase on the inside walls of the column, or on support particles attached to the column walls, resulting in a much reduced total surface area (up to 350 times less stationary phase when compared to a regular packed column with 3-5 μm particles)[10, 17]. Other issues include the small volume of sample injected onto a column, which can require sophisticated flow splitting assemblies and highly sensitive detectors[10].

In the early days of practical open tubular liquid chromatography, Jorgensen and Guthrie showed that there is an optimal size for efficient OTLC using established chromatographic theories [6, 12]. They concluded that the optimal diameter for an open tubular column should be on the order of 1-3 μm[12]. At this size, theoretical plate height is optimal over a range of operating conditions, such as column diameter and flow rate (operating pressure) [12]. Using capillaries this small however, produces significant technical challenges such as considerable backpressure and extremely small sample volumes, which have been persistent problems.

Previous OTLC work has focused on the use of thick stationary phases in order to increase the amount of sample that can be loaded onto a column. The reasoning behind the use of a thick stationary phase layer in very small capillaries was to compensate for the lower sensitivity in detectors of the past[8]. However, using thick stationary phases makes the C term of the van Deemter equation (2) larger, thus, increasing plate height (H) resulting in poorer separations. Because of this, analytes need to have a very high diffusivity in the stationary phase, so as not to reduce column performance when using a thick stationary phase[8]. This has not been as big an issue in classic open tubular systems, such as GC, because the movement of molecules (kinetics) in the bulk of the mixture as well as inside the stationary phase is

comparatively very fast for gases. It is, however, a very important consideration for open tubular liquid chromatography where the movement of analytes both in solution and in the stationary phase is much slower [2]. Thin layer stationary phases, have advantages in LC systems as they allow for faster mass transfer between the stationary phase and the bulk of the solution (i.e. smaller C term) eliminating the need for high analyte mobility in the stationary phase[8, 13, 16]. However, this is offset by the need for higher detector sensitivity and smaller sample loading[12].

There have been many different stationary phases employed for OTLC over the years, ranging from simple monolayers to complex polymer formulations such as various polysiloxanes, acrylates and even porous silica based materials[8, 17]. Monolayers of C18 bonded to the wall of a glass capillary are the simplest of the stationary phases that have been tried. Historically, monolayers have not seen as much success for OTLC uses, as they are generally considered to not provide enough stationary phase for adequate separation and detection (i.e. not enough sample capacity). When they have been used, they tend to require prohibitively small capillaries in order to achieve a high enough surface area to volume ratio to provide adequate separations[8, 10, 12]. Polymer based stationary phases have received much more attention over the years for OTLC and have gained popularity for many reasons. One positive feature of polymer layers is that most of the common polymers used (polysiloxanes, acrylates and styrene) are easier to fabricate within a column than some of the older methods using monolayers or functionalised porous silica networks[8, 10, 14-16, 18]. Polymer stationary phases also offer a relatively high degree of control over the thickness of the stationary phase layer through reaction time, which is an important factor for open tubular columns[8, 14-17].

Polysiloxanes were one of the first polymer stationary phases to be used for OTLC due to their extensive study and success with GC equipment. However, they were limited by a few aspects that made them difficult to use[8]. These limitations included the high viscosity of

polysiloxane solutions, the inherent instability of thin films of polysiloxanes in cylindrical tubes during column fabrication and the solubility of many polysiloxanes in organic solvents[8]. In more recent times, polyacrylate and polystyrene based stationary phases have been popular choices for OTLC. Polyacrylate and polystyrene can provide a wide array of functionalities by simply changing the side chain on the vinyl/styrene or acrylate backbone and by changing the amount of monomer that is used. These polymers have received a lot of attention mostly because of ease of use and customizability, as well as facile and fast fabrication[8, 14, 15, 18, 19]. The speed of fabrication is especially noticeable when photo-initiation is used, allowing for complete column fabrication in a matter of hours. Although these polymers are very versatile they suffer from low sample capacity (like all other open tubular columns) as well as some reproducibility problems[8, 14, 15].

1.1.5 Multichannel HPLC

There are many technical problems to consider when carrying out open tubular liquid chromatography, such as small sample volumes and column fabrication challenges. With these challenges in mind, it was suggested by Janik in 1976, building on the ideas of Golay, that sample volumes in gas chromatography could be increased by bundling large numbers of solid rods (wires) together and using the interstitial spaces in between them. This would provide high capacity columns, while maintaining the inherent potential advantages of open tubular columns[20, 21, 23]. It was also suggested that this could be accomplished by fusing a large number of plates with grooves in them together. These first generation columns were met with some criticism stating that the non circular cross section and sharp corners of the spaces in between the wires used to create the interstitial spaces would likely lead to poor performance from differences in flow between the channels and at the sharp edges[21, 23]. A third method of fabricating

multichannel columns was then suggested by Pierce which involved bundling together hollow glass tubes inside an outer glass casing and drawing it as one would a regular capillary, in a manner similar to preforms used today in the photonics industry for microstructured fibre manufacturing[22-25]. Technical and interfacing problems, again, led to only limited testing of these early columns.

Advancements in the fibre optic industry in the early 1980's allowed for smaller, more reliably pulled glass rods and capillaries to be manufactured. Furthermore, advancements in low volume detectors and reliable flow splitting systems have renewed interest in multicapillary column chromatography[23]. Meyer *et al.* used columns fabricated in a similar way to the one described by Janik, using the interstitial space between bundled solid optical fibres; although the optic fibres used by Meyer *et al.* were much more uniform in size, as a result of improved manufacturing processes[21,23] . Their work also outlined many of the theoretical concepts for multicapillary columns[23]. Meyer *et al.* stated that one of the most likely problems with multicapillary systems would be uneven flow distribution between the channels as a result of different channel sizes and would result in broader peaks and/or poor performance[23]. This would shown up as an 'A term' like effect in the van Deemter equation, as unlike packed columns, multichannel columns do not have multiple random flow paths to average out inconsistencies along the column, and as such, a very high manufacturing tolerance is required[23]. In fact, Meyer *et al*. concluded that inter capillary variation in diameter should be no more than one half percent (1 % in flow variation between columns) in order to surpass packed columns. These conclusions

$$\Delta P = \frac{8\mu L Q}{\pi r^4} \tag{3}$$

come from considerations such as the Hagen-Poiseuille equation (3), where μ is the dynamic viscosity, L is the length of pipe, Q is the volumetric flow rate, r is the radius of the pipe and ΔP

is the pressure drop across the pipe. The equation shows that small changes in channel diameter can have significant impacts on flow rate with the same applied pressure[23, 26]. Other more recent work using similarly made multicapillary columns for gas chromatography, also states the need for a very high tolerance between channel sizes[27, 28].

While the potential separation performance and advantages of open tubular multichannel columns over other (packed) column types are in theory excellent, even modern multichannel columns are plagued with relatively large plate heights and low efficiencies. This is likely because of technological limits on the manufacturing of multichannel capillaries, leading to uneven flow distributions as noted above, as well as persistent open tubular column issues[23, 27-29].

1.2.0 Photonic Crystal Fibres.

1.2.1 Introduction to Photonic Crystal Fibre

Photonic crystal fibres (PCF), which are also known as microstructured fibres (MSF) or "holey" fibres, are a type of optical fibre that was primarily designed for the photonics and telecommunication industry, based upon the properties of photonic crystals[30, 31]. Photonic crystals are small crystalline structures, usually on the nanometre/wavelength scale that interact very strongly with certain wavelengths of light. Photonic crystals can be thought of as the light analogue of how a semiconductor can manipulate electrons, where band gaps in energy within the material can allow or prevent movement of energy through the material[30, 31]. In general, photonic crystals are comprised of structures that have a periodic change in refractive index, in one, two, or three dimensions. The amount of periodicity in the crystals grants the different types of crystals different optical properties. For instance, one dimensional photonic crystals can

be made into thin films allowing for surfaces to be transformed into extremely reflective mirrors for specific wavelengths, while two dimensional crystals can be used as waveguides[30].

Microstructured fibres (MSFs), are simply a two dimensional photonic crystal, built/drawn down the length of a fibre. Most types of photonic crystal fibre are based on solid silica with air holes built into them[24, 25, 30, 31]. MSFs can be made from a variety of materials ranging from fused silica to a variety of polymers (PMMA, polycarbonate, Teflon™ etc.), the most common material being fused silica for its durability and established methods for manufacturing standard glass optical fibre [24]. MSFs are available in a variety of patterns and sizes, usually tailored to exploit some specific property of light. Examples of some MSFs can be seen in Figure 1. MSFs can be further sub-divided into solid core and hollow core fibres. This however, has implications for the optical properties of the fibre, such as how light is coupled into and guided along the fibre[25, 30].

There are a few ways to make MSFs, The most common method of manufacturing MSFs is to make a prefrom (or macroscopic version of the fibre) of the desired pattern, which in turn is drawn like a standard fibre optic or glass capillary[24, 25]. There are many potential applications for MSFs; they can be used as waveguides or in telecommunications[30]. MSFs also have the potential for use in many other areas other than photonics. Some other potential applications include being used for low threshold Raman scattering work, atom and particle guidance along a fibre and even in high harmonic generation, which could open many other applications in the biomedical fields[24]. Although the unique properties of MSFs and photonic crystals are interesting, most of the optical properties are rather complex and beyond the scope of the discussion here. The book by Joannopolos *et al.* and a review by Russell are good starting points for anyone who is further interested in the optical properties of MSFs[25, 30].

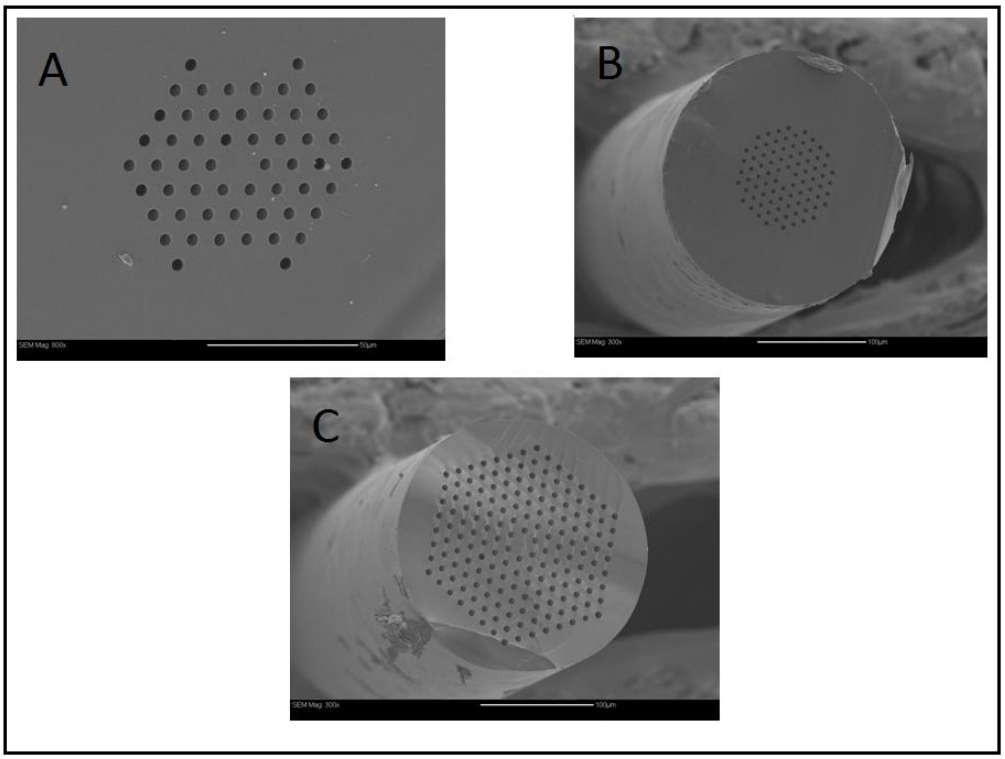


Figure 1: Variety in MSF construction. A) The holey region of a 54 holed fibre (LMA-PM-15). B) 84 holed fibre (LMA-15). C) Shows a 168 holed fibre (LMA-20). Images were taken on a JEOL JSM 840 SEM, scale bars are 50 μm in A, and 100 μm in B and C.

1.2.2 MSFs as a Multi-Capillary Column.

Multicapillary systems have a number of potential advantages over conventional single column systems such as lower back pressure and higher surface area. While MSFs may seem like an odd fit for chromatography, many of the physical aspects of MSFs make them possible candidates for use as multicapillary columns. One such aspect is the fact that in many MSFs the periodic breaks in the fibre are simply air gaps, while the rest of the structure of the fibre is made of fused silica. We can therefore treat the channels in a MSF as if it were a bundle of individual 5 μm capillary columns, allowing for facile functionalization of the surface through well known silane chemistry used in making silica particles and other types of chromatography columns[2]. The size of some MSFs (360 μm. o.d.) is also on the order of regular capillaries (365 μm o.d.), making them easy to use with existing equipment designed to fit fused silica capillary columns[32]. A third and important aspect of MSFs that make them suitable for use as multichannel chromatography columns is that the holes can be on the order of 3-5 μm inner diameter, around the size that has been suggested as the optimal for open tubular columns by Jorgenson[12, 23].

1.3.0 Introduction to Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons, also known as PAHs or polyarenes, constitute a large class of hydrocarbon molecules containing two or more fused aromatic rings as a distinctive feature[34-36]. Other common features of PAHs are their low water solubility, relatively low reactivity, environmental persistence, and a tendency to be carcinogenic. PAHs are ubiquitous in the environment and have many sources, some naturally occurring, such as forest fires and volcanoes, while others are related to human activity, for instance, motor vehicle and factory emissions[34-40]. Some of properties and issues surrounding PAHs are discussed below.

1.3.1 Properties of PAHs

The properties of PAHs vary widely with the chemical structure of the PAHs; generally, PAHs are solid, crystalline compounds, of varying colour, usually, whitish or yellow. Unsubstituted PAHs (e.g. those with just hydrogen attached to the carbon backbone) tend to have very low solubility in water and low vapour pressures, properties which make them very persistent in environmental samples[1, 34-39]. However, methylated and hyroxylated versions of the same PAHs can be substantially more soluble in water and thus much more of a health risk, as they can enter the food chain more readily[1, 34-39]. A few unsubstituted PAHs can been seen in Figure 2, along with some of their properties listed in Table 1.

1.3.2 Sources and Interests in PAHs

PAHs are formed from the incomplete combustion of organic (carbon containing) material and the vast majority of them in the environment come from anthropogenic sources[1, 34-

[39]. These include: industrial processes such as, power generation by burning of fossil fuels (coal and petroleum), petroleum processing (cracking, asphalt production etc.) and metal processing, such as aluminum and steel production. Other important human related sources are oil spills, emissions from motor vehicles, municipal/agricultural burning of waste, residential burning of wood and gas and even food production contributes to PAHs through grilling and smoking of meat products[34-40]. Although human related activities are the most prevalent and important

Table 1: Properties of various PAHs (EPA 610 mixture), for structural information (images) see Figure 2. Shown below is the PAH's shorthand abbreviation, molecular weight (MW), melting (Mp) and boiling point (Bp) in degrees Celsius, and octanol/water partition coefficient ($\log K_{ow}$) [1, 33]

Compound	Abbreviation	MW	Mp	Bp	Log Kow
		g/mol	oC	oC	at 25 oC
Naphthalene	NAP	128	81	218	3.37
Acenaphthene	Acen	152	96	278	3.92
Acenaphthylene	ACY	154	92	265	4.00
Fluorene	FLU	166	116	295	4.18
Anthrancene	Anth	178	216	340	4.54
Phenanthrene	Phen	178	101	339	4.57
Fluoranthene	Fluo	202	111	375	5.22
Pyrene	PYR	202	156	360	5.18
Benz[a]Anthracene	BaA	228	160	435	5.91
Chrysene	Chry	228	255	448	5.86
Benzo[b]Fluoranthene	BbF	252	168	481	5.80
Benzo[K]Fluoranthene	BkF	252	217	481	6.00
Benzo[a]Pyrene	BAP	252	175	495	6.04
Benzo [ghi]perylene	B[ghi]P	276	277	N/A	6.50
Indeno[1,2,3-cd] Pyrene	Ind	276	164	536	6.58
Dibenz[ah]Anthracene	DahA	278	267	524	6.75

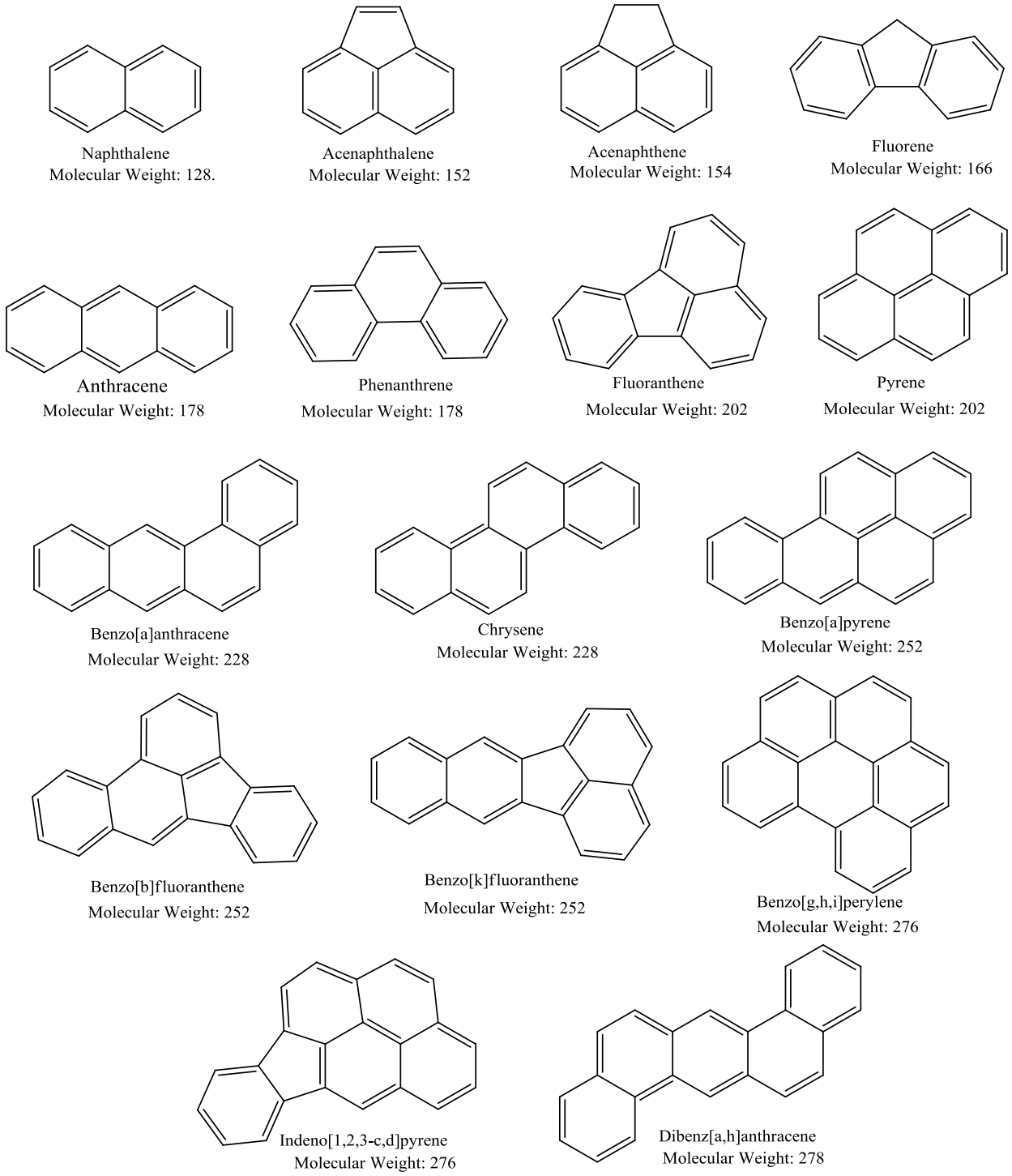
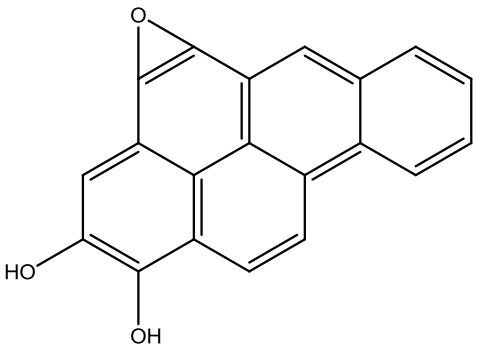


Figure 2: PAHs possess diverse properties. Shown here are the components of the EPA 610 mixture, highlighting some of the diversity

sources of PAHs, there are numerous naturally occurring sources of PAHs, such as forest fires, and volcanic eruptions[1, 34, 35].

Concentrations of PAHs in the environment are highly variable, and are dependent on meteorological conditions, seasons, type of environment, and properties of the PAHs themselves. For example, many bodies of water have lower overall concentration of PAHs due to the low solubility and photolytic degradation of the PAHs. In soils, however, PAH concentration may be many times higher (upwards of a 1000 times higher), partly due to their low solubility in water which makes them hard to remove, as well as shielding from sunlight or microorganisms that could degrade them[1, 36, 38].

There has been an increased interest in PAHs, over the last few years as it is becoming clearer that many PAHs contribute to cancer and mutagenesis in mammals, and since many PAHs are lipophilic, this leads to the risk of bioaccumulation[35-39]. Interestingly, unsubstituted PAHs have a fairly low toxicity overall, but many of their metabolites, and degradation products are very potent carcinogens and mutagens. For example, benzo[a]pyrene, which is regarded as one of the worst offenders in terms of carcinogenicity, when metabolised by cytochrome P450, can form an epoxide diol (shown in figure 3) that can bind to DNA causing DNA adducts[1, 36, 38].



Benzo[a]pyrenedihydrodiolepoxide

Figure 3: Structure of benzo[a]pyrenedihydrodiolepoxide, a benzo[a]pyrene metabolite

Other reactions can take place in air with hydroxyl and nitrate radicals, as well as ozone to produce their respective hydroxyl, oxy and nitro-PAH compounds. Although these substituted PAHs are present in significantly lower concentrations, they contribute much more to toxicity in air pollution than their parent unsubstituted PAHs[1].

1.3.3 Current Detection Methods

Currently there are a variety of ways in which PAHs can be analyzed, and the choice is somewhat determined by the sample and how much, and what kind of sample cleanup is required [1, 35, 36]. The most popular methods for the analysis of PAHs are gas chromatography (GC) and high performance liquid chromatography (HPLC). Among these popular methods, capillary electrochromatography (CEC), capillary electrophoresis (CE) (for PAHs with polar side groups or using MEKC for uncharged PAHs) and supercritical fluid chromatography (SFC), have been gaining popularity in recent years[1, 35, 36].

1.3.3.1 Gas Chromatography,

Gas chromatography (GC) is by far the most popular method of detecting PAHs, as it is best suited for detecting lower molecular weight PAHs as they tend to be more volatile. Gas chromatography is generally carried out using non-polar polysiloxane stationary phases in long (30 m), wide bore (around 300 μm inner diameter) capillary[1, 37]. There are many GC detection methods for analysing PAHs, Most use either a flame ionization detector (FID) or a mass spectrometer (MS) detector. MS is the detector of choice when using GC for analysis because of higher sensitivity and lower detection limits (typically in the low and sub part per billion range) compared to FIDs. However, for screening purposes FIDs are useful, allowing for ppb level of

detection but at much less cost[1]. Although the detection levels and efficiency of GC are better than for liquid chromatographic systems, liquid based analysis methods offer some advantages, such as the ability to analyse high boiling(>320^{o}C) and non-volatile (high mass) PAHs which can be difficult using GC[1, 37].

1.3.3.2 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is another commonly encountered method of analysis for PAHs. Although the efficiency and detection levels are not quite as good as those in gas chromatography (GC), there are several advantages to using a liquid based detection method. The first being easier sample preparation and sample introduction, as many samples are already in the liquid phase after extraction[1]. Secondly, it allows for the analysis of high molecular weight PAHs that are not volatile enough to be analysed by GC. HPLC analysis of PAHs is generally done using packed columns of either 5 μm or 3 μm octadecylsilane (ODS) treated silica particles. Common detectors used for HPLC analysis of PAHs are ultraviolet absorbance (UV), fluorescence detection (FLD) and mass spectrometry (MS) [1].

UV and FLD are the most widely encountered detection methods when using HPLC for PAH analysis; however both have advantages and disadvantages. UV detection although a simple method of detection with fairly low cost, suffers from the fact that most UV detection systems are only set up to monitor a single wavelength of light at a time; in the case of PAHs, 254 nm is most often used. However, not all PAHs absorb light efficiently at this wavelength, and can lead to sensitivity problems for some compounds[1, 40]. UV analysis by itself also makes PAH identification difficult, as many absorb in the same wavelength ranges. Some of these issues can be alleviated through wavelength programming of the detector or using diode array detectors, which can sample multiple wavelengths of the UV spectrum simultaneously, thus

allowing some compounds to be identified using their UV absorbance spectrum, along with their respective retention times[1, 40].

Fluorescence detection, while also of reasonably low cost, offers much higher sensitivity for PAH analysis than UV detection. It can also in some cases lead to direct analysis of PAHs in complex samples, as not all PAHs are fluorescent. There are however downsides to using fluorescence detection as well. One important limitation is that the excitation and emission wavelengths for PAHs are highly variable (some examples are shown in Table 2), which can make it difficult to analyse samples where more than a few PAHs are present, especially considering, that some PAHs are not fluorescent at all[1].

Lastly, a note on MS detection, MS detection provides low detection limits (ng-pg levels), but the limit that can be achieved is highly dependent on the ionization method. LC-MS detection is challenging with analysis of PAHs as it is generally hard to ionize neutral molecules such as PAHs, and requires more complex interfacing between the HPLC and MS to achieve this[1]. MS detection also runs into problems distinguishing between structural isomers of the same mass with some ionization methods, particularly softer ionization methods that result in minimal fragmentation of the PAH, such as ESI MS[1].

Table 2: Excitation and emission wavelengths for various PAHs[1].

Compound		λ Ex	λ Em
Naphthalene	NAP	280	340
Acenaphthene	Acen	289	321
Acenaphthylene	ACY	298	321
Fluorene	FLU	289	321
Phenanthrene	Phen	249	362
Anthrancene	Anth	250	400
Fluoranthene	Fluo	285	450
Pyrene	PYR	333	390
Benz[a]Anthracene	BaA	285	385
Chrysene	Chry	260	381
Benzo[b]Fluoranthene	BbF	295	420
Benzo[K]Fluoranthene	BkF	296	405
Benzo[a]Pyrene	BAP	296	405
Benzo [ghi]perylene	B[ghi]P	380	405
Indeno[1,2,3-cd] Pyrene	Ind	300	500
Dibenz[ah]Anthracene	DahA	296	405

1.4.0 Objectives

1.4.1 Single Channel Open Tubular Liquid Chromatography

Open tubular chromatography columns possess advantages over conventional packed or monolithic columns. However, a major road block to the success of open tubular columns has been a multitude of technological challenges. These technological challenges include the need for small diameter capillary, reliable introduction of very small sample volumes and detection of those small volume samples. In our experiments described here, we aim to further explore the prospects of open tubular liquid chromatography. Our main focus will be on the development and characterisation of stationary phase materials as well as an investigation of the effects of capillary size on chromatography with the aid of modern nanoLC equipment.

1.4.2 Multi-Channel Open Tubular Liquid Chromatography

Lower surface area and hence lower amounts of stationary phase, as well as high flow induced backpressures have been some of the most persistent challenges in open tubular liquid chromatography. It was suggested previously that one way in which these challenges could be overcome would be to bundle many columns together. We explore the use of microstructured fibre, originally designed for use in the photonics industry, as a support for OTLC. We investigate the behaviour of liquid flowing through an MSF, as well as how the distribution of features within an MSF and stationary phase deposition method affect chromatography.

Chapter 2: Single Channel Open Tubular Liquid Chromatography

2.1 Summary

Open tubular columns have been around for many years and have received significant attention for gas chromatography due to their inherent advantages over packed columns[2]. However, they have only received sporadic attention for liquid chromatography due to numerous technical challenges[1, 13-18]. In this chapter, the development and characterisation of stationary phase materials for capillary open tubular liquid chromatography, as well as the effect of solvent composition on the formation of the stationary phase is discussed. With a suitable stationary phase selected, additional testing and optimisation of LC conditions allowed for challenging multi-component mixtures (EPA 610, in house drug mixture) to be to be analysed through reverse phase OTLC. The effect of capillary diameter on separation performance is also explored.

2.2.0 Experimental

2.2.1 Chemicals and Materials

Hydrochloric acid, acetic acid, styrene, divinylbenzene(DVB), lauryl methacrylate (LMA), butanedioldiacrylate (BDDA), 3-(trimethoxysilyl)propylmethacrylate (γ-MPS), chlorotrimethylsilane, monochlorodimethyloctadecylsilane, acenaphthene, acenaphthalene, fluoranthene, fluorene, napthalene, phenanthrene, anthracene, pyrene, benzo[a]pyrene and pre mixed 1mL ampules of chrysene, dibenz[a,h]anthracene, benzo[k]fluoranthene, benzo[b]fluroanthene, benzo[a]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene and EPA mixture 610 were all purchased from Sigma-Aldrich (Oakville, Canada, St. Louis, USA).

Sodium hydroxide, toluene and HPLC grade acetonitrile were purchased from Fisher Scientific (Toronto, Canada, Pittsburgh, USA). Capillary was obtained from Polymicro Inc. (Phoenix, USA). Deionized water was acquired through an in house milliQ water purification system from Millipore (Billerica, USA). Ethanol was purchased from Commercial Alcohols (Toronto, Canada). Azobisisobutyronitrile (AIBN) was obtained from the Cunningham lab in the Department of Chemical Engineering at Queen`s University.

2.2.2 Open Tubular Polystyrene/Divinylbenzene Porous Layer Open Tubular Columns

Synthesis of the polystyrene/divinylbenzene (PS/DVB) porous layer open tubular (PLOT) columns were as follows. A length of capillary (10 cm, 50 cm, 2 m or 5 m) with inner diameter (i.d.) 30 μm or 10 μm was cut and pre-treated. Pre- treatment encompassed flushing the capillary for 1 hour with 0.1 M sodium hydroxide (NaOH), deionized water, 0.1 M hydrochloric acid (HCl), and again with deionised water using a Harvard Apparatus 11 plus syringe pump at 0.5 μL/min. The water washing steps were sometimes allowed to go for longer than 1 hour, if the solution coming out the end was not close to neutral when tested with pH paper. Following pre-treatment, a solution containing 50% deionised water, 20% γ-MPS, also known as Z6030, and 30% glacial acetic acid was pumped through the length of capillary for at least 1 hour to ensure the capillary was completely filled with solution, pH paper was used to check for a mildly acidic solution eluting from the capillary. The capillary was disconnected from the syringe pump and stored in the same solution (in microcentrifuge tubes) overnight. The capillaries were then flushed with a 95% acetonitrile/water solution using a Waters model 590 HPLC pump at 20 μL / min for 20-30 minutes. Once complete, a solution of 600 μL ethanol (EtOH), 200 μL styrene, 200 μL divinylbenzene (DVB) and 5 mg of azoisobuturylnitrile (AIBN)

was pumped through the capillary using a syringe pump, until the entire capillary was filled, (approximately 1 hour). The ends of the capillary were then sealed using GC septa and placed in an oven (Fisher Scientific Isotemp model 281A) for 1 hour at 80 ^{o}C . The column was removed from the oven and flushed with 95% ACN/H_2O using the Waters model 590 pump for 30 min, after which the column was ready for use. No special storage conditions were used.

2.2.3 Open Tubular LMA/BDDA PLOT Columns

A length of capillary (10 cm and 50 cm) with i.d. 30 μm or 10 μm was cut and pre-treated. Similar to above, these columns were made by pre-treating the capillaries for 1 hour with 0.1 M NaOH, deionised water, 0.1 M HCl and again with deionised water, and as before sometimes letting the water washing steps take longer if the effluent of the capillary was not near neutral after one hour. The capillaries were then flushed with a solution of 50% deionised water, 20% γ-MPS, 30% glacial acetic acid for 1 hour to ensure that the capillaries were filled with solution. They were then stored overnight in solution. After which, the capillaries were then flushed with 95% ACN/H_2O for 20-30 min. Once the silane treatment was complete, the capillaries were then filled with a solution of 700 μL EtOH, 150 μL laurylmethacrylate (LMA), 150 μL Butanedioldiacrylate (BDDA) and 5mg of AIBN. The columns were sealed with GC septa and placed in an oven for 1 hour at 80 ^{o}C and were then flushed with 95% ACN/ H_2O for 20-30 min. No special storage conditions were used.

2.2.4 Open Tubular C18 Columns.

A length of capillary (10 cm, 50 cm) with i.d. 30 μm or 10 μm was cut and pre-treated. Similarly to above these columns were made by pre-treating the capillaries with 1 hour with 0.1 M NaOH, deionised water, 0.1 M HCl and again with deionised water. A 10% v/v solution of chlorodimethyloctadecylsilane in toluene was prepared and pumped through the capillary overnight using a Harvard Apparatus 11 plus syringe pump at 0.3 μL/ min overnight. The column was then flushed with 95% ACN/H_2O.

2.2.5 Capillary Wall Etching for Stationary Phase Thickness Assessment

Short columns (25 cm) were made using the PS/DVB procedure above. They were cut into sections, at regular intervals. Following, a few of the short sections were dipped into an ammonium bifluoride solution and left to etch for twelve minutes. After etching, the sections were placed into a water bath and left for 25 minutes in order to dilute and remove the ammonium bifluoride. The sections of capillary were then allowed to dry and were trimmed down to about 0.5 cm in height. The prepared capillary samples were then gold coated (Anatech Hummer VI-1 Sputtering System) and examined using scanning electron microscopy (JEOL JSM-840).

2.3.0 Results and Discussion

2.3.1 Stationary Phase Development

Open tubular chromatography has garnered much interest over the years due to many inherent advantages over packed columns. For liquid systems though, open tubular chromatography has been somewhat of an elusive goal due to a variety of technical challenges.

Among the most prevalent are sample volume and the amount of stationary phase available in the column[8, 9, 11]. Unfortunately, for open tubular liquid chromatography (OTLC), stationary phase materials and preparation schemes are sparse in the literature [10, 15, 41-46]. Some procedures originally developed for capillary electrophoresis (CE) and electrochromatography (CEC) were selected as good starting points for stationary phase development; since many of these methods, particularly the CE and CEC methods also require thin stationary phases. Thin layers of stationary phase are needed for OTLC as they allow for faster mass transport between the stationary phase and the bulk of the solution (i.e. smaller C term in the van Deemter equation) as well as limit band broadening from slow analyte diffusion through the stationary phase. This eliminates the need for high analyte mobility in the stationary phase[8, 13, 16]. A simple surface treatment of C18 and two polymer based stationary phases [3-glycidoxypropyl-trimethoxysilane (GPTMS) and 3-(trimethoxysilyl)propylmethacrylate (γ-MPS)] differing in the amount of branching possible, were chosen as a starting point. As can be seen in Figure 4, polymer formation with both polymer methodologies was far from optimal. The resulting polymer stationary phases were spotty and inconsistently formed down the length of the column. Chromatographic performance of these columns was also disappointing with broad peaks, spanning 2-3 minutes and very low retention of test analytes (and hence almost no resolution). A representative chromatogram is shown in Figure 5.

After the disappointing results with the initial polymer methodologies, both in terms of polymer formation and chromatographic performance, focus then shifted to the simpler C18 surface treatments. Initially a triethoxyoctadecylsilane was used and again chromatographic results were poor, with next to no resolution and broad peaks, again spanning 2 -3 minutes. A

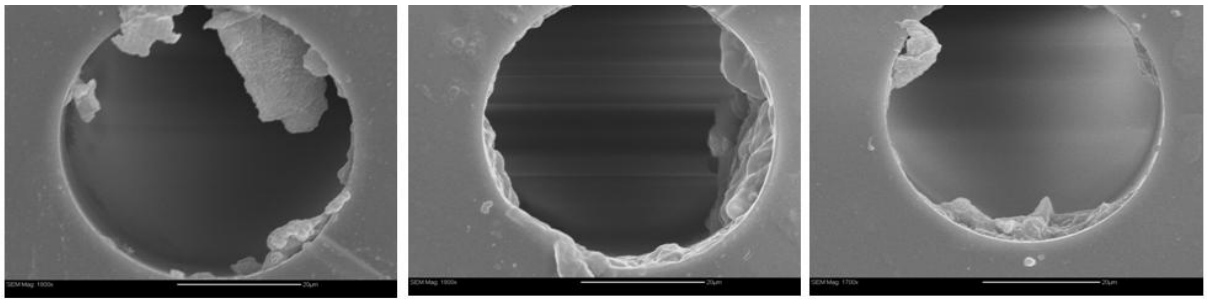


Figure 4: SEM images of polymer formation in 3 sections of i.d. 50 μm capillary, scale bars are 20 μm

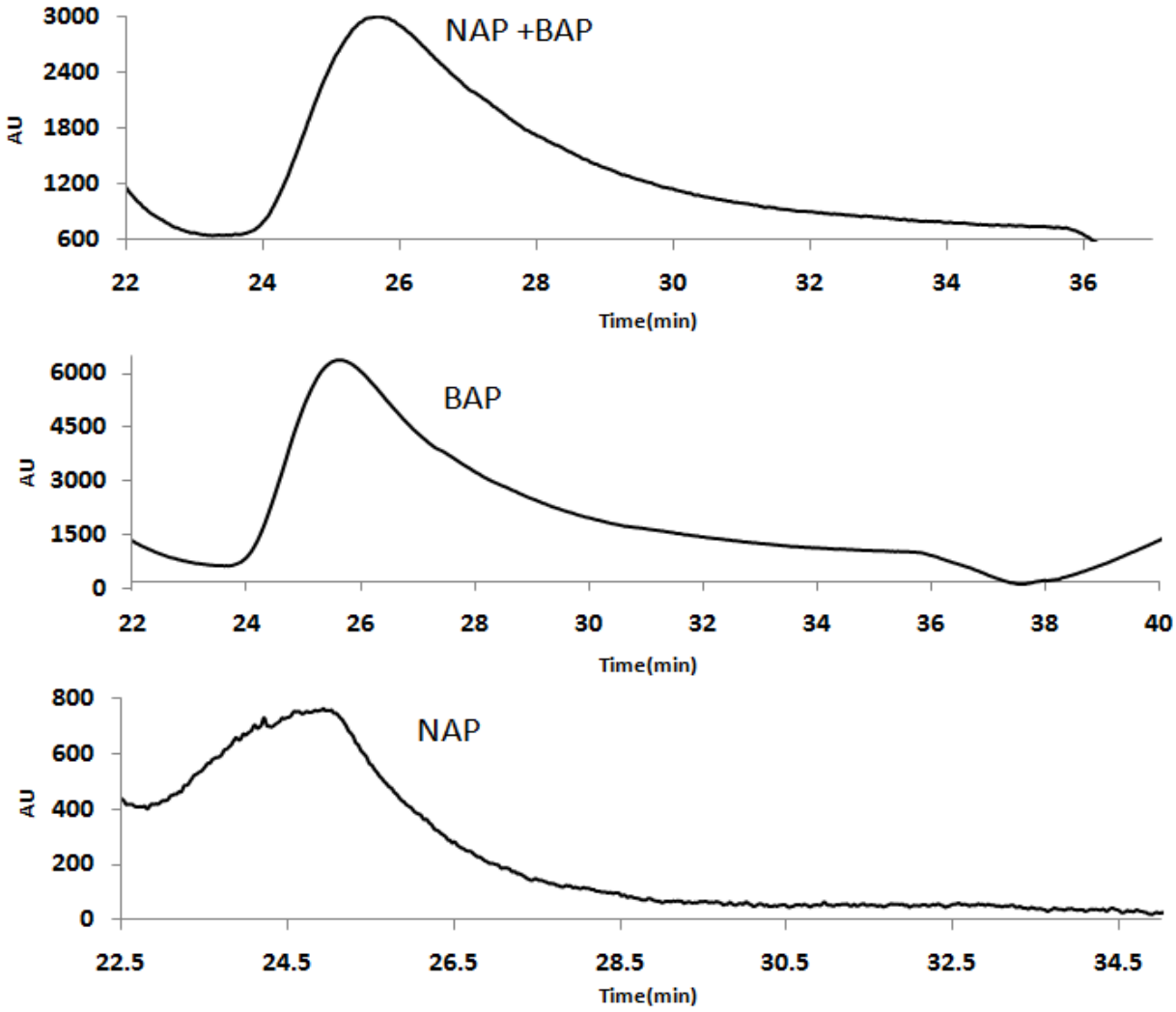


Figure 5: A representative chromatogram of early polymer layers (GPTMS and γ-MPS based) results. Note the broad peaks and very poor resolution. Analytes are naphthalene(NAP) and benzo[a]pyrene(BAP) at 300 μM and 50 μM respectively, run at a 10-99% ACN/H_2O at 400 nL/min over 15 min

switch to monochlorinatedoctadecylsilanes was then pursued based on the ease with which these types of silanes have been used in the past in the treatment of silica particles and glass surfaces[2, 47-50]. At first, results were again discouraging, however, with some slight procedural changes, results improved and a separation, albeit a rather poor one, of a two component mixture was observed as can be seen in Figure 6. The efficiency of the separation was again quite poor, as can be seen from the very wide peaks spanning almost 4 minutes. The low performance level was expected and seemed to be in line with literature stating that surface treatments (i.e. monolayer type) stationary phases have too small a phase ratio and do not provide enough retention to be of much use compared to more extended polymer stationary phases[8].

While C18 layers were providing some small successes, continued searching for potential stationary phases that could be used for OTLC applications continued. Upon which one type of procedure, focusing on the use of a polystyrene/ divinylbenzene copolymer was found[19, 20, 42]. This polymer which was referred to as a porous layer open tubular (PLOT) column appeared to be an ideal candidate as a stationary phase material, because it could be uniformly formed throughout the length of a column. Initial investigation into this type of stationary phase, using MSFs (see Ch. 3, pg 64) produced discouraging results. Polymer formation down the length of the column was good; it was consistent and seemed to have a high surface coverage, as observed through SEM imaging. However, chromatographic results were poor, resulting in minimal resolution and extremely broad ‘peaks’ 4 -5 minutes in width.

Further investigation via SEM into the PS/DVB copolymer showed that polymer formation does indeed proceed with a high degree of surface coverage and in a consistent thickness throughout the length of the column. Furthermore, it was also found that the thickness of the polymer layer was controllable through reaction time, allowing for some manipulation of

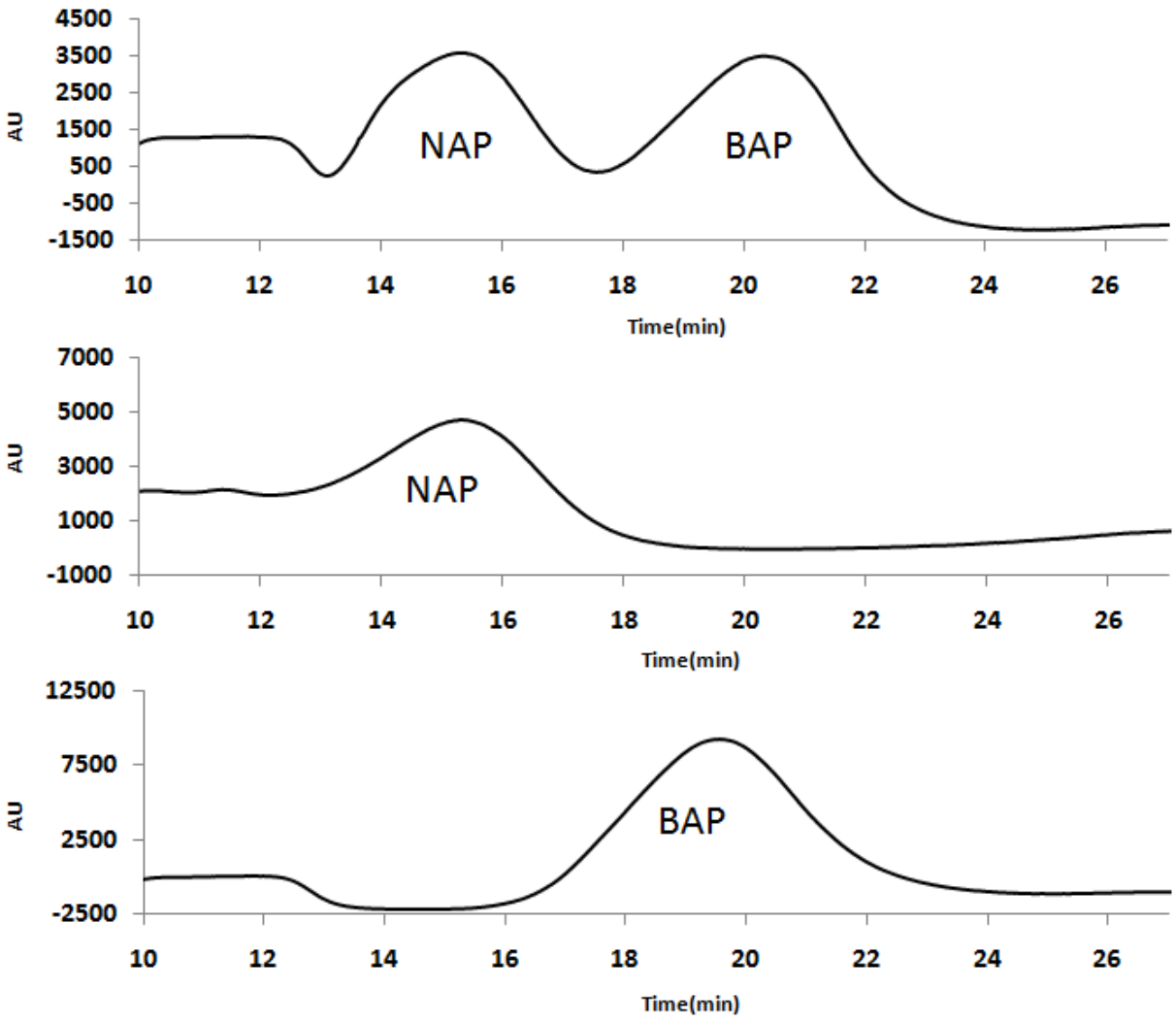


Figure 6: Chromatograms for i.d.10 μm capillary with a monochlorooctadecylsilane surface treatment as stationary phase. Analytes were naphthalene (NAP, 100 μM) and benzo[a]pyrene(BAP, 15 μM) using a 400 nL/min 10-99% ACN/H_2O gradient over 20 min

the stationary phase thickness, as shown in Figure 7. Polymer thickness increases with respect to time during the first 5 hours of polymer formation, after which it levels off. The large error bars seen in Figure 7 have to do with nodular features in the polymer layer. These nodular features were taken into account to estimate an average polymer thickness as each cut only represents a snap shot of the polymer formation throughout the column at that time. Another contributing factor to the large error in our measurements comes from our etching procedure to detach the polymer from the glass capillary. The etching away of glass was not 100% efficient for all sections of column, requiring us to estimate the location of the glass and polymer interface for some sections; which increased the uncertainty of some measurements.

Chromatographic results using different polymer thicknesses (different reaction times) and the same run conditions, shown in Figure 8, hint that there may be an optimal polymer thickness for chromatography with one hour reaction time (approximately 1μm thick polymer) columns performing the best. Although the production of the polymer layer in a consistent manner is fairly reliable (> 90% of columns formed properly), this polymer formulation, unfortunately, also seems to have periodic spells of column failures, where polymer forms across the capillary, effectively sealing it off and resulting in blocked columns as seen in Figure 9. This tends to happen in clusters with many columns failing to form an open tubular column in a single batch, the cause of this is not fully understood at this time.

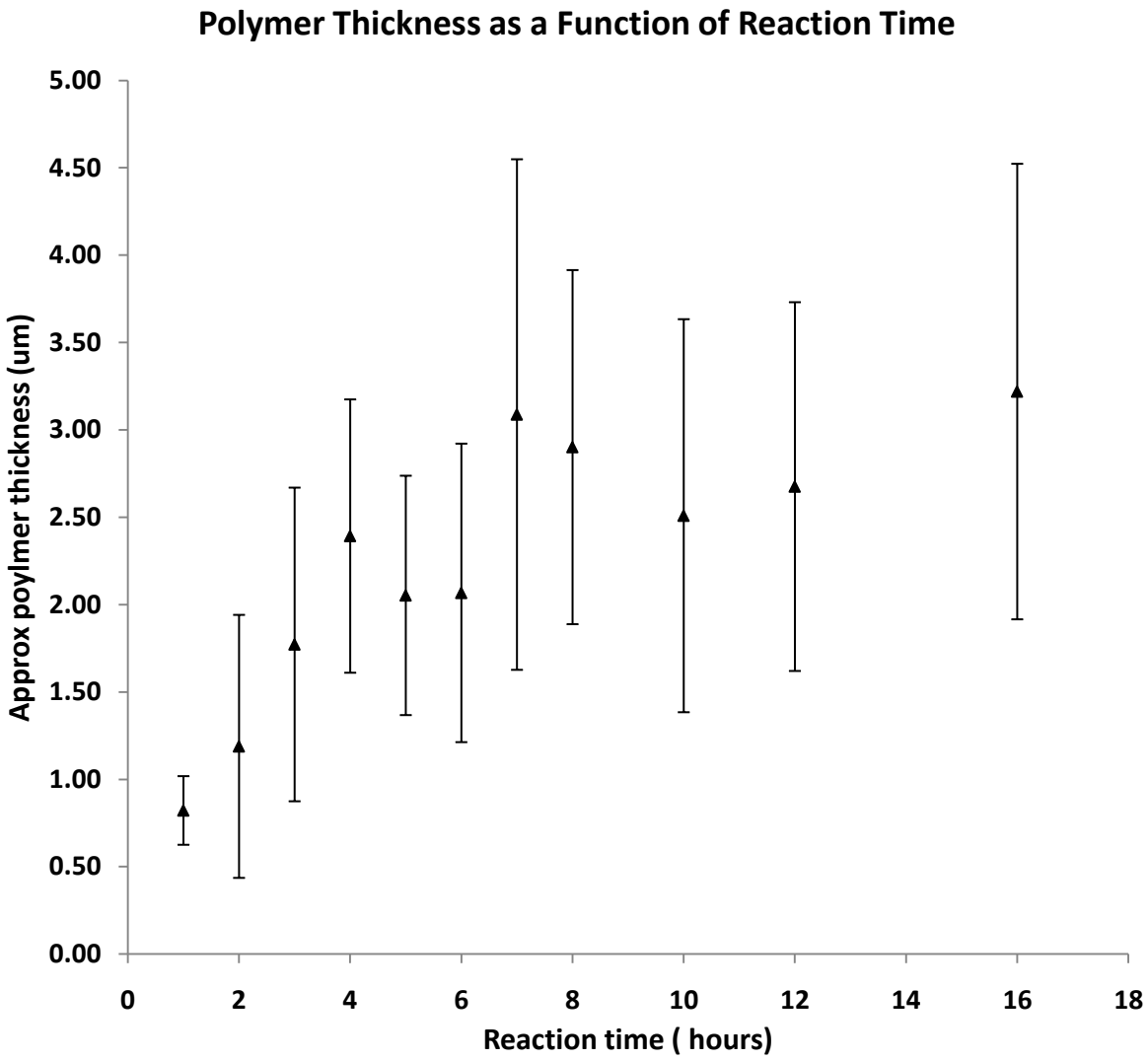


Figure 7: Relationship of polymer thickness and various reaction times. Data points are an average of six measurements of polymer thickness in 2 different sections of the same column.

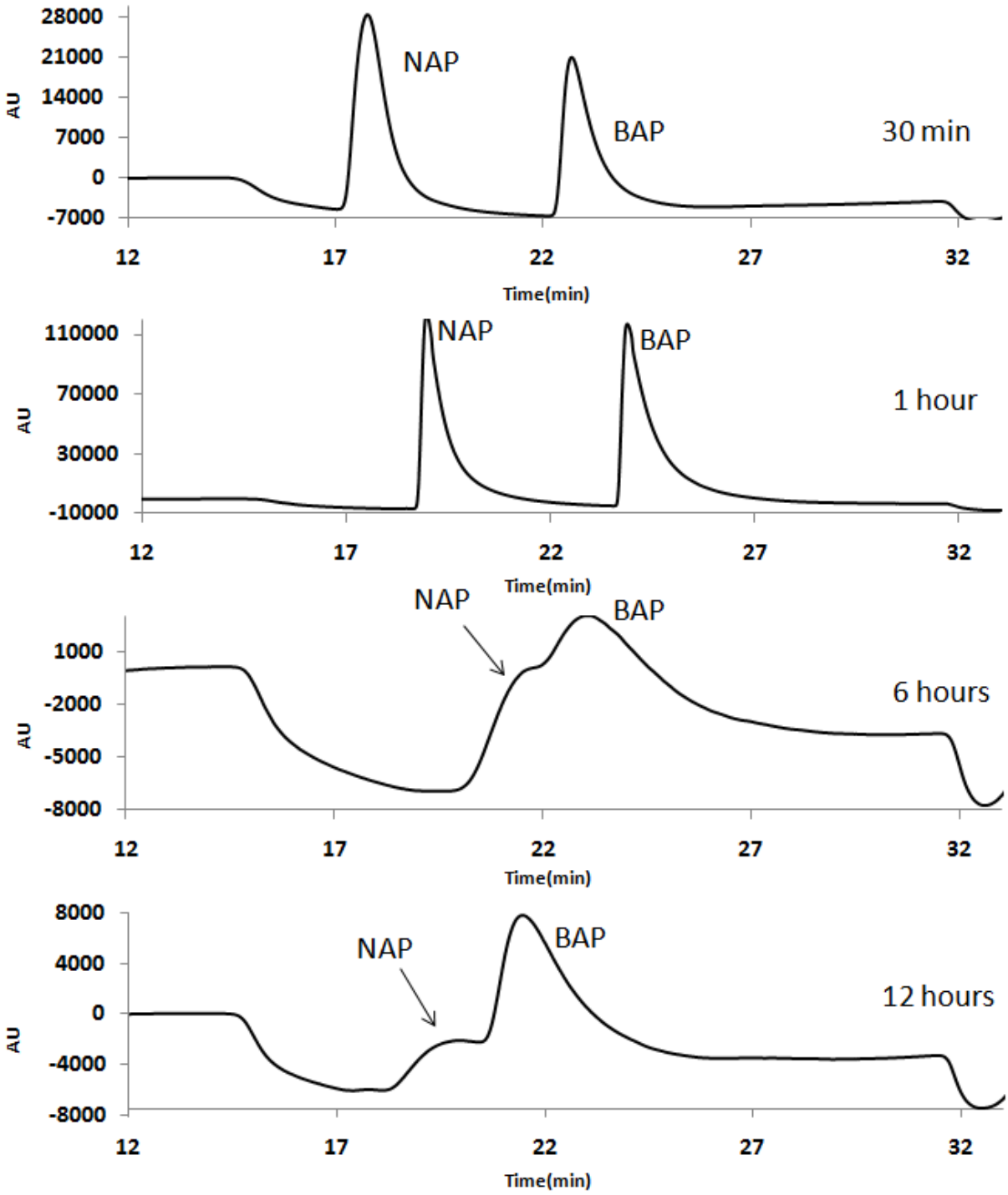


Figure 8: Selected chromatographic results of columns formed with different reaction times (12 hours, 6 hours, 1 hour and 30 min). Samples were naphthalene (NAP, 100 μM) and benzo[a]pyrene (BAP, 15 μM) in all chromatograms. All columns used a 10-99% ACN gradient at 400 nL/ min over 15 min.

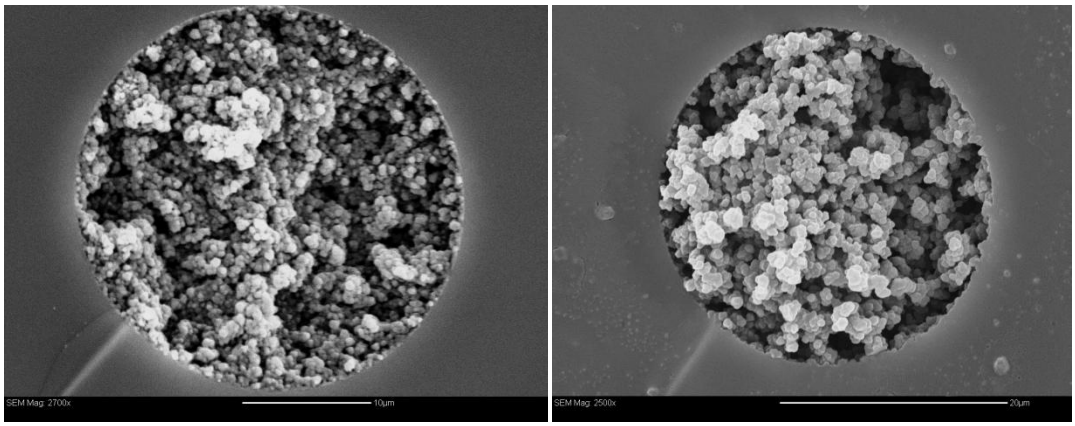


Figure 9: SEM images of two different blocked columns. Scale bars are 10 μm for left image and 20 μm for right image

While the PS/DVB column showed signs of success, it represented a significant change in the procedure for making polymer layers over those that had been tried previously with the GPTMS and γ-MPS. Many of the other methods in the literature call for toluene to be used as the solvent for the polymer mixture, while the PS/DVB PLOT procedure uses ethanol. It is known that the solvent can play a major role in the porosity of monolithic columns[52, 53]. So it was thought that perhaps the solvent was also playing a similar role in our polymer layer formations. Initially we thought that toluene may be too good of a solvent for the growing polymer chains, leading to spotty and inconsistent polymer formation on the capillary walls. A polymer formula using laurylmethcaryate (LMA) and butanedioldiacrylate (BDDA) as monomers with a methodology similar to that of the PS/DVB PLOT was used to investigate the effect of the solvent on polymer formation. The LMA/BDDA PLOT column used for this experiment was previously developed for use in MSFs, as it showed promise over early PS/DVB columns (see Ch. 3, pg 64). Shown in Figure 10, we can see the different morphologies of the polymer layer when toluene and ethanol were used. When toluene was used, the resulting polymer was more smooth in appearance and had either an irregular elliptical profile or was largely on one side of the capillary. When ethanol was used, as in the PS/DVB PLOT procedure,

the polymer was more consistently formed down the length of the column and had a rougher, more nodular surface texture.

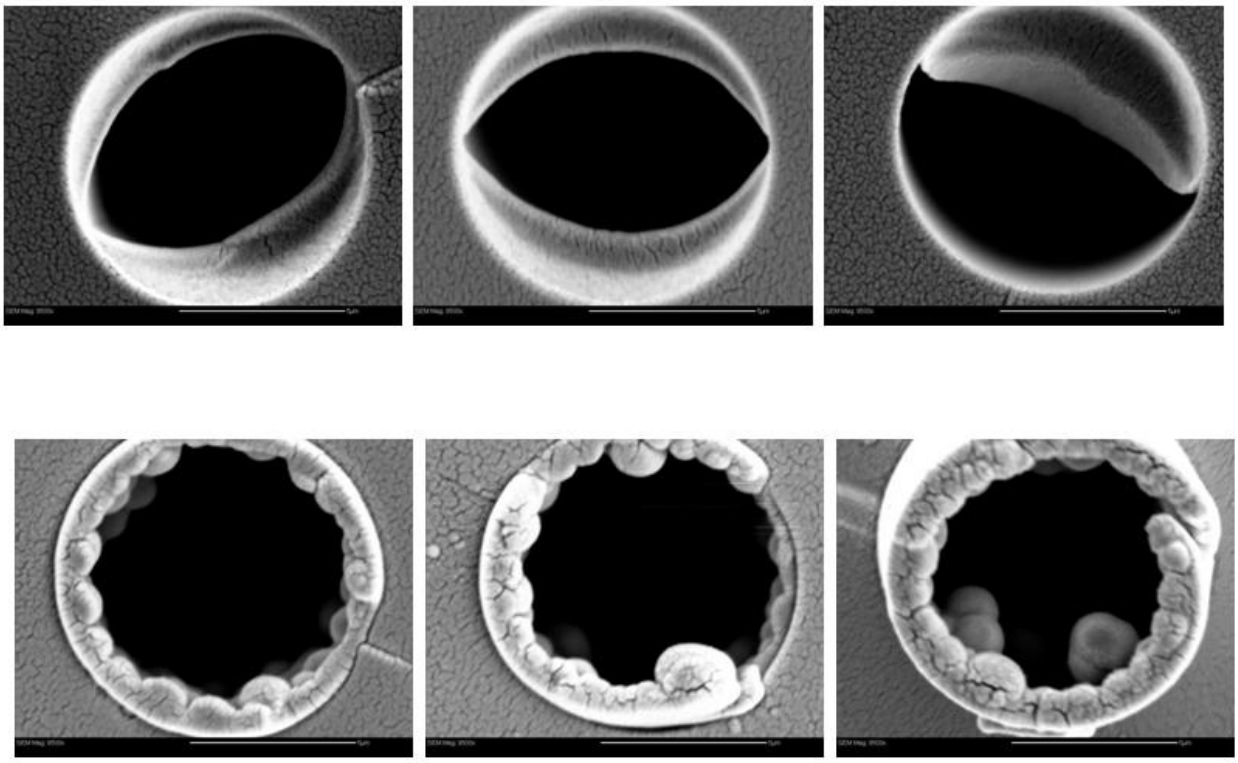


Figure 10: Different morphologies observed when a LMA/BDDA polymer layer was formed using toluene and ethanol. The top set shown is three cross sectional images of the LMA/BDDA when toluene was used and the bottom set is LMA/BDDA when ethanol was used. Scale bars are 5 μm.

With knowledge of the superior performance of ethanol as the solvent used for fabrication using the PLOT methods outlined above, a head to head comparison of the LMA/BDDA and PS/DVB columns was then carried out. Early PS/DVB columns were fabricated only using an overnight (16 hour) reaction time, emulating procedures found in literature[14, 18, 19]. These columns did not perform as well as expected, with very large broad peaks and only some resolution (≈ 0.7) between peaks. So initially, work continued with the LMA/BDDA columns as they seemed to be more efficient, yielding narrower peaks and better

resolution (≈ 2.0) than the PS/DVB columns. This comparison, however, was not quite a fair one, as the PS/DVB column were allowed to react longer (16 hours/overnight) resulting in a thicker (approx. 4-5 μm) polymer layer compared to the LMA/BDDA columns, which was only allowed to react for 1 hour (approx. 1-3 μm thick). The LMA/BDDA columns were blocked when left for longer reaction times. The performance of columns in which the PS/DVB polymer was only left to react for 1 hour far exceeded the performance of a previous PS/DVB column or the LMA/BDDA column and was vastly superior to the C18 surface treatment columns shown earlier, allowing for greater resolution (3.8) and much narrower peaks (1 to 1.5 minutes wide) as can be seen in Figure 11. This PS/DVB procedure as outlined in the experimental section, has since served as the standard polymer procedure for our OTLC work.

In order to further characterise our stationary phase material, a van Deemter plot was made. van Deemter plots are very useful for helping to optimise run conditions as they compare plate height at various flow rates, allowing one to find the flow rate at which the column will provide the highest efficiencies (smallest plate height (H))[2]. The van Deemter curve for our PS/DVB columns (using naphthalene as a test analyte) is shown in Figure 12. Our early screening of PS/DVB columns was done at a flow rate (400 nL/min) which was chosen based on other capillary based work in our lab. However we did not know if this was the best flow rate for our columns. As can be seen in Figure 12, 200 nL/min produced the highest efficiencies, although, we still used 400 nL/min as a matter of convenience for most chromatographic runs. Also noteworthy is the comparatively large minimum plate height at around 1 mm. While larger plate heights are in line with other reports for current open tubular columns, it is significantly larger than traditional (packed) columns, which are typically in the tens of micrometers[1, 2].

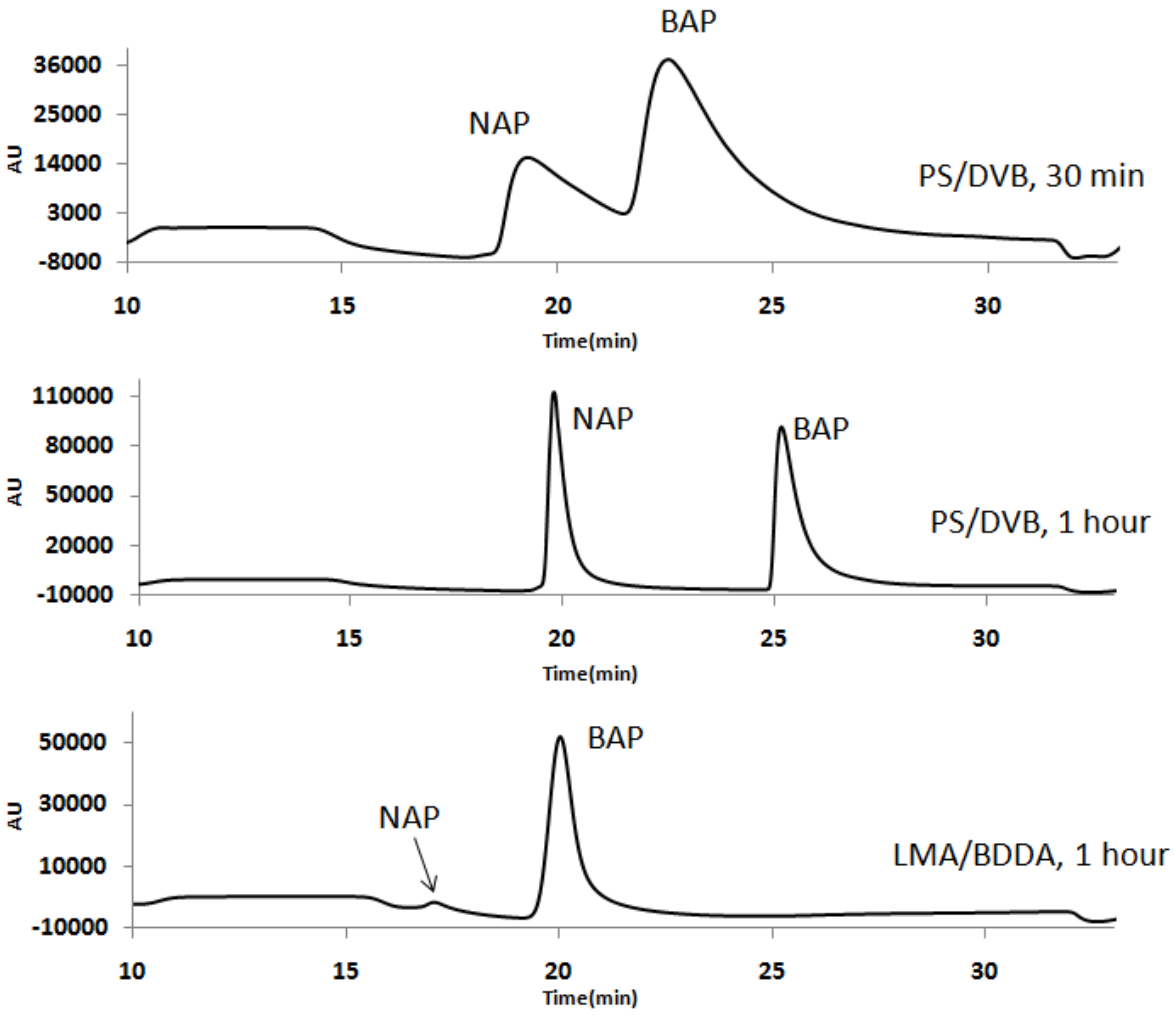


Figure 11: Comparison of polymer layers used in capillary OTLC. In all chromatograms the first peak was naphthalene (NAP, 100 μM) and the second was benzo[a]pyrene (BAP, 15 μM) using a 10-99% ACN/H_2O gradient at 400 nL/min over 15 min.

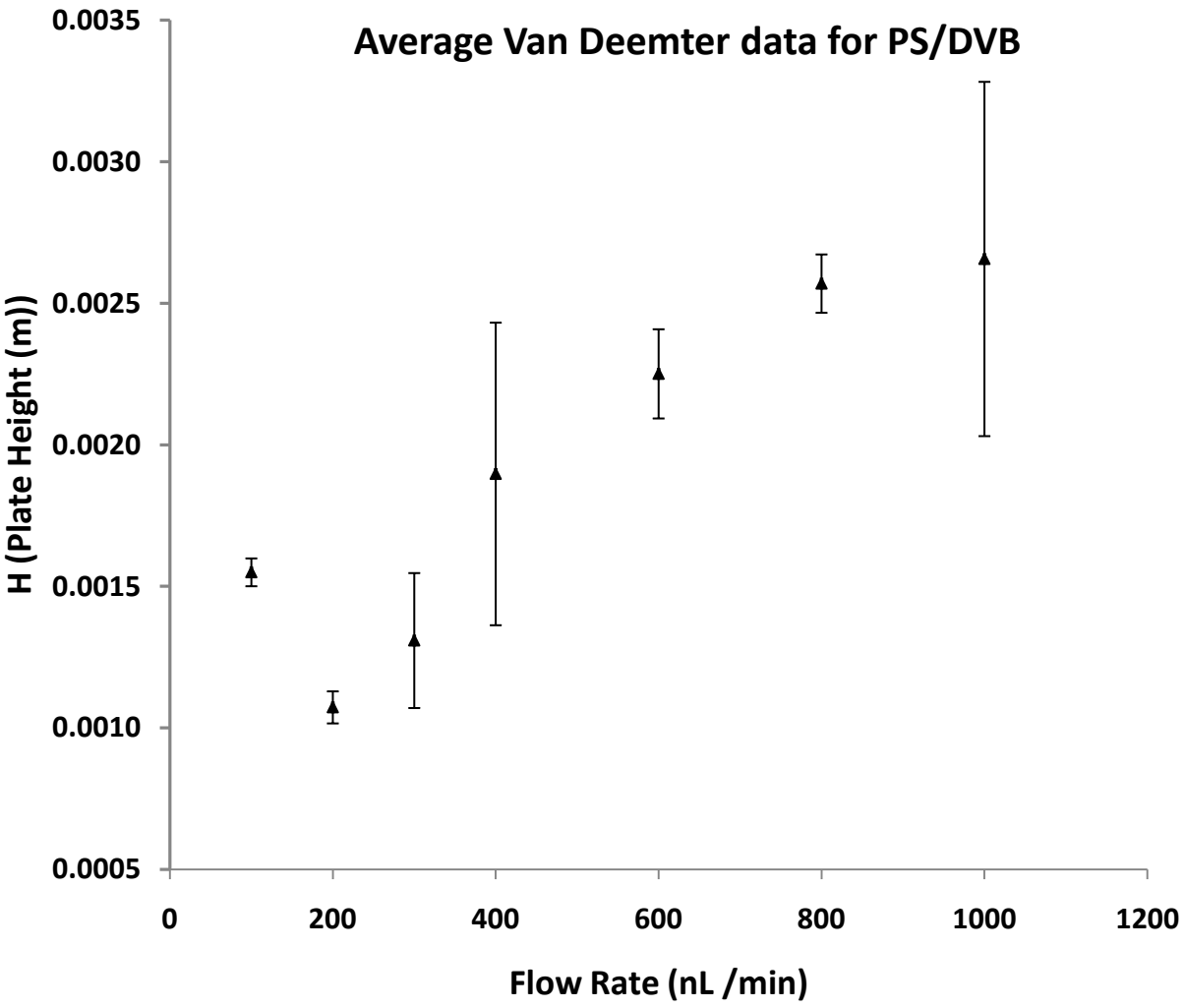


Figure 12: van Deemter plot for PS/DVB columns. This data was obtained using 25 cm long columns with a 65:35 % water/acetonitrile isocratic mobile phase, on a Eksigent nanoLC 1D plus HPLC system

However, with further development of stationary phase materials and polymerisation methods, this value will almost certainly get smaller in the future.

2.3.2 Effect of Capillary Diameter on Separation

The capillary diameter and its resulting effect on chromatographic performance were explored. Literature suggests that smaller diameter capillaries should produce better separations because of the higher surface area to volume ratio inside the capillary[12]. A higher surface area to volume ratio means that more of the sample passing through the column can interact with the stationary phase, an important consideration for OTLC since open tubular columns have significantly reduced amounts of stationary phase compared to packed or monolithic columns[8]. The higher surface area to volume ratio of smaller capillaries also allows for faster diffusion of the analyte through the bulk of the mobile phase, allowing for decreased analyte/mobile phase/stationary phase equilibration time.

The effect of capillary size was investigated with a PS/DVB column with one hour reaction time as it provided the best results of all the polymer layers tested. Columns with smaller inner diameters (10 μm) did not perform better than a larger capillary (30 μm), as can be seen in Figure 13. In all cases, the resolution (1.63 for i.d. 10 μm and 3.71 for i.d. 30 μm) and efficiency of the separation was significantly lower in the smaller diameter capillary. This comparison was also done with the LMA/BDDA polymer. Similarly, lower resolution and efficiency, was observed with these columns as well. The lower performance of smaller diameter capillaries may be due to more difficult stationary phase deposition within smaller capillaries. It is also possible, that columns with different diameters may require different optimal polymer layer thicknesses, as different polymer thicknesses had a very noticeable effect on separation

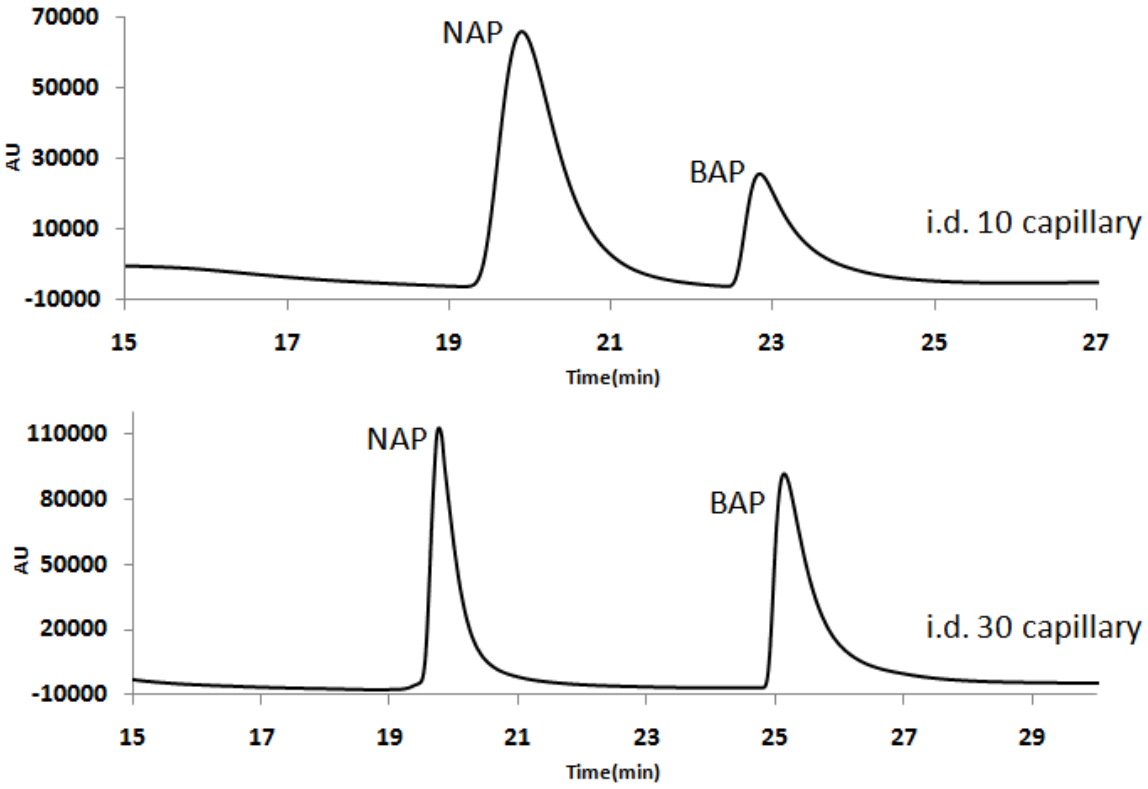


Figure 13: Difference in separation performance for the i.d. 30 μm (lower trace) and i.d. 10 μm (upper trace) columns. Analytes were naphthalene (NAP, 100 μM) and benzo[a]pyrene (BAP, 15 μM).

quality (Figure 8). In either case, the cause of reduced performance in smaller diameter capillaries is not yet well understood and may be the subject of a future study.

2.3.3 Reverse Phase Open Tubular Liquid Chromatography

With a suitable stationary phase (PS/DVB) selected and its behaviour partially characterised, further testing of the columns took place. Previous testing was centered around short (25 cm long) columns. By comparison, many other open tubular columns found in literature are significantly longer, usually on the order of 1-5 meters and often running into the tens of meters[15, 42, 54]. Building upon the stronger performance of the 1μm thick PS/DVB columns, longer two meter columns were fabricated that were more in line with what was reported in literature. Similar to the short columns, the two meter long columns were screened for chromatographic performance using a mixture of naphthalene and benzo[a]pyrene with a relatively steep solvent gradient (10-99% ACN over 15 minutes). Results were also similar to the shorter columns with a high degree of resolution between the two compunds (3.63). A more challenging mixture comprised of naphthalene, phenanthrene, pyrene and benzo[a]pyrene was then tested and again results were quite good (Figure 14). Further optimisation of the gradient resulted in improved peak resolution; however, the peak width did suffer a little as the shallower gradient seemed to reduce the analyte stacking at the head of the column, shown in Figure 14.

With a large degree of resolution between each analyte peak now being achieved, a more challenging mixture of 16 PAHs was then tried (EPA 610 mixture). Results were surprisingly good after the first attempts, with 11 out 16 possible peaks being readily identifiable.

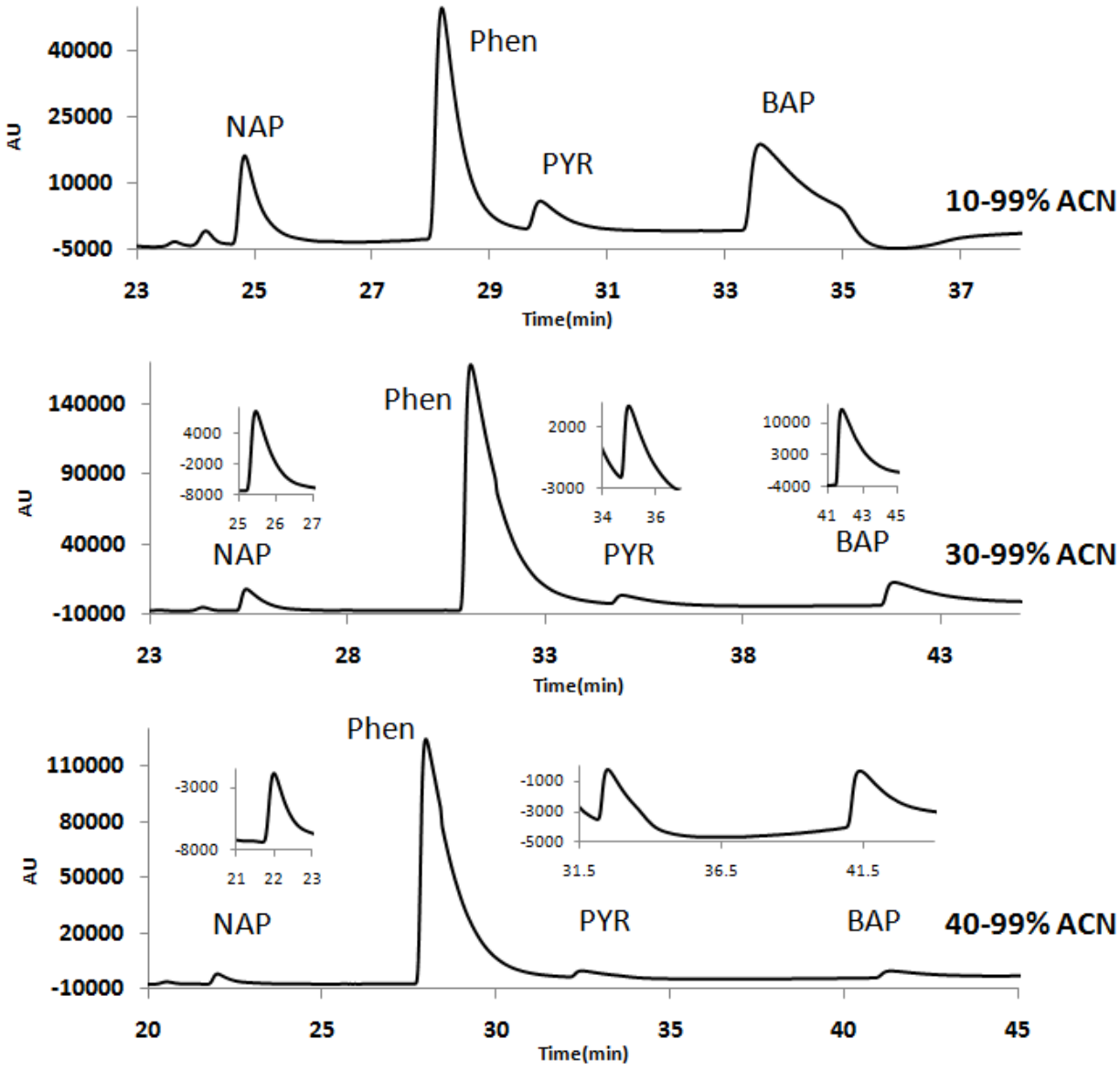


Figure 14: The separation of a 4 PAH mixture (naphthalene, phenanthrene, pyrene and benzo[a]pyrene) on an i.d. 30 μm, 2 m long PS/DVB column (top). The middle and bottom chromatograms show improvements in resolution as the gradient profile was optimised. The peaks look small due to the strong relative absorbance of phenanthrene

Following some minor gradient profile adjustments, almost all (11/16) the components of the mixture were separated and identified, with a few exceptions, shown in Figure 15a. The peak at 37.6 minutes retention time is the co-elution of benzo[a]anthracene and chrysene and the peak at 41.0 minutes is the co-elution of benzo[b]fluoranthene and benzo[k]fluoranthene. This is not surprising, based on the data presented in Table 1. Not only are these two groups of analytes very similar structurally, but they also have very similar Log K_{ow} (log P) values, making them very difficult to separate. Peaks for dibenz[a,h]anthracene and benzo[g,h,i]perylene were not observed. This is likely due to their extreme insolubility in water (both have log K_{ow} values $\geq$ 6.5).

In an attempt to push the limits of our columns separation abilities, the EPA 610 mixture was run at a much higher flow rate than our previous results, as shown in Figure 15b, it was possible to run the columns at 1μL/min and still retain the ability to see all the peaks, albeit the quality of the separation was significantly reduced. This was most noticeable for the anthracene/ phenanthrene pair where it is very difficult to distinguish between the two peaks. However, this helps demonstrate the robustness of the columns for a wide range of operating conditions, even beyond the optimum flow rate range (200 nL/min). A drug mixture was also tested as can be seen in Figure 16a. Although the efficiency for ASA and capsaicin were not as good as for naproxen, nabumetone and ibuprofen, it is thought that the higher water solubility of ASA and capsaicin might have played a role and further optimisation of analysis conditions should be carried out.

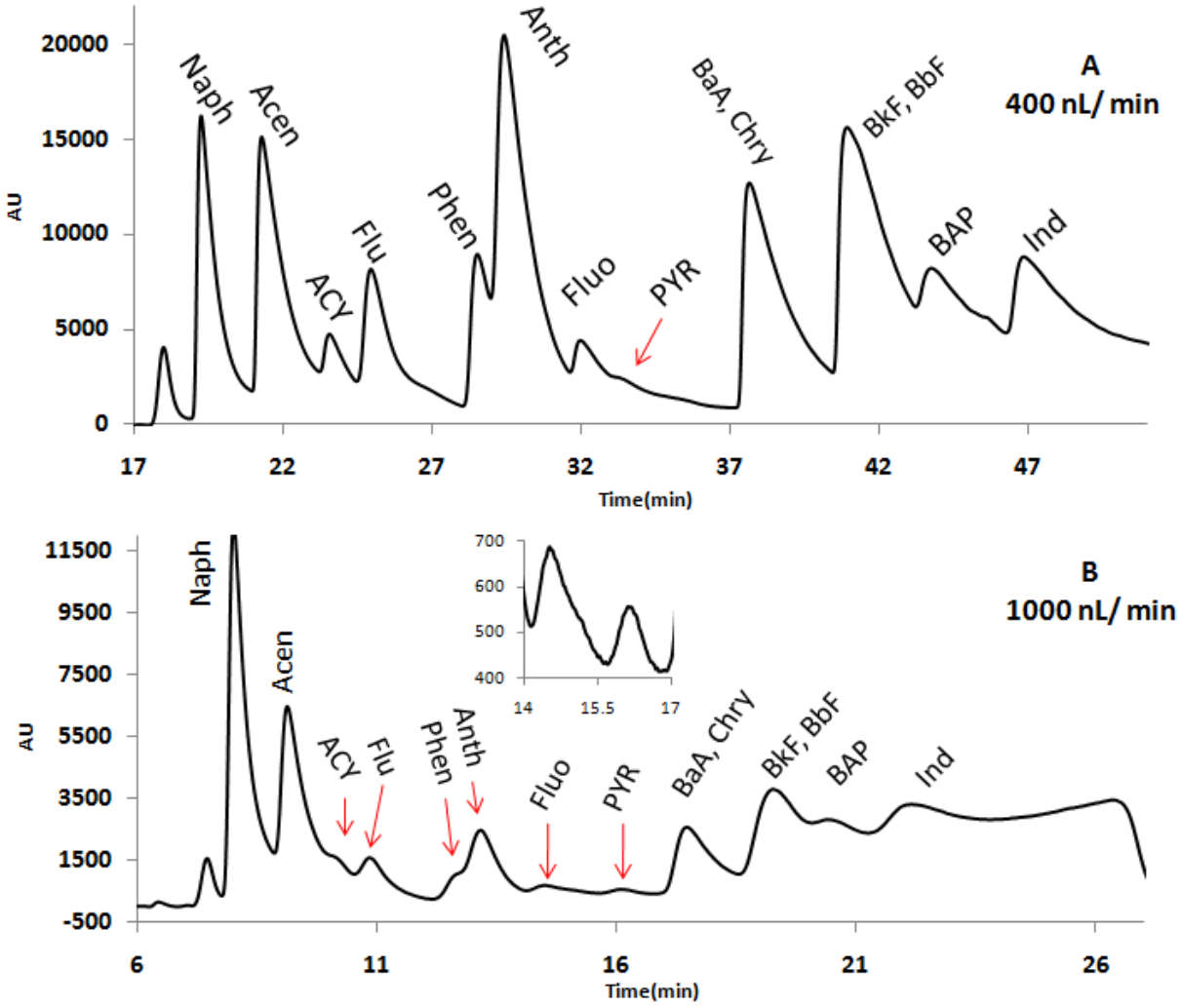


Figure 15: A) Results of the EPA 610 mixture on a 2 m PS/DVB column. The peaks at 37.6 min and 41.0 min are a co-elution of benzo[a]anthracene and chrysene and benzo[b]fluroanthene and benzo[k]fluoranthene respectively. Dibenz[a,h]anthracene and benzo[g,h,i]perylene were not soluble enough to be detected.

B) This chromatogram shows the results of a fast (1 μL/min) OTLC run of the EPA mixture. Peaks are in the same order as outlined in A, notice the reduced efficiency of separation, this is expected based on our van Deemter data, shown earlier. Inset shows a close up of the fluoranthene and pyrene peaks

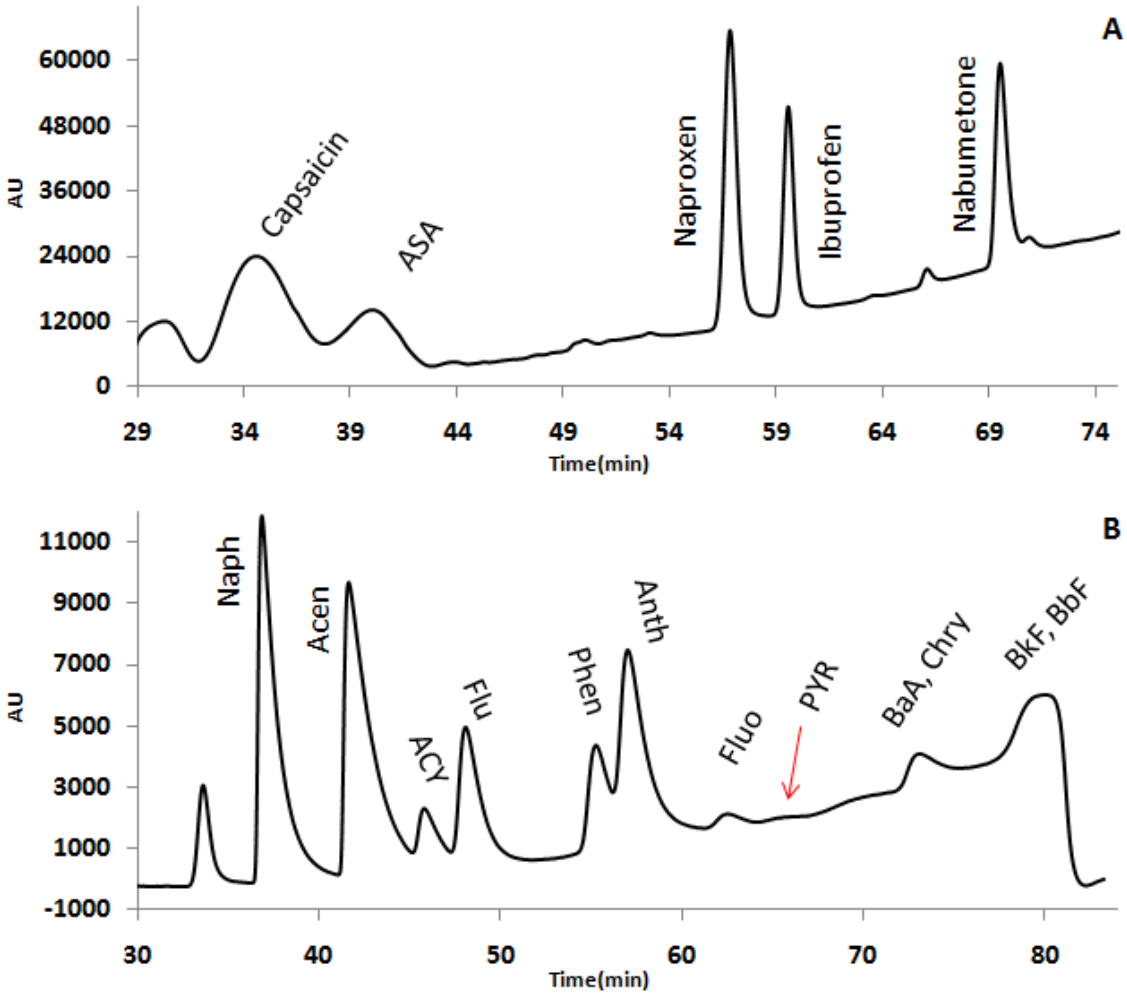


Figure 16: A) Chromatogram showing the separation of a drug mixture on the 2 m PS/DVB column, suggesting that general reverse phase OTLC may be possible. This was performed using a 10-99% ACN/H_2O gradient at 200 nL/min.

B) Results for a 5 m PS/DVB column using a 45-99% ACN/H_2O gradient at 400 nL/min. Retention of analytes was much higher than with the 2 m column.

Five meter long columns were also fabricated, and although the overall retention of all analytes increased significantly, there was no gain in efficiency which can be seen in Table 3 and Figure 16b. In all attempts with 5 m columns, no more than half of the EPA 610 mixture was observed in 1.5 hours. It is likely that with further development and optimisation of run conditions, results should improve. At this point in time, however, the upper practical limit for both analysis time and column length seems to be around two meters.

Table 3: Differences in efficiency (peak width) between two meter and five meter long PS/DVB columns for acenaphthene (Acen) and anthracene (Anth)

PS/DVB column	Peak width (min)	
	Acen	Anth
2 m	2.4	2.8
5 m	3.5	4.5

2.4.0 Conclusions

Despite the many technical issues surrounding open tubular liquid chromatography, we have shown that with careful fabrication of polymer stationary phases that reverse phase open tubular capillary chromatography is possible with modern nanoHPLC sample introduction and detection systems. We have shown that the columns are robust, functioning over a wide range of flow rates, and are able to separate complex multi-component mixtures (e.g. EPA 610, drug samples). The plate height of the OTLC columns is low at around 1 mm compared to other OTLC columns reported in the literature where plate heights are in the μm range. The other columns reported in the literature, however, require prohibitively small injection volumes (10-100's pL) and flow rates (low nL range) in order to function properly[8, 10, 14, 15]. Our columns, on the other hand, have been shown to function well with standard nanoLC equipment and comparatively large injection volumes (2 μL), alleviating the need for flow splitting assemblies

and very sensitive detection techniques. Although our results here represent a step forward for OTLC, more optimisation and further development of stationary phase materials is needed.

Chapter 3: Multi-Channel Open Tubular Liquid Chromatography

3.1 Summary

Microstructured fibres (MSFs) are a type of optical fibre, originally developed for the photonic industry that makes use of the unique light confining properties imparted by the periodic arrangement of features built into them. In an effort to alleviate some of the issues associated with capillary open tubular liquid chromatography, such as small sample volumes and high backpressure, using MSFs as a solid support for OTLC was explored. The profile of liquids flowing through an MSF is examined as well as the effects of the size and distribution of holes on chromatographic performance. Development of a suitable stationary phase for OTLC in MSFs and some of the challenges to using polymer stationary phases is also discussed.

3.2.0 Experimental

3.2.1 Chemicals/Equipment

Hydrochloric acid, acetic acid, styrene, divinylbenzene (DVB), 3-(trimethoxysilyl)propylmethacrylate (γ-MPS), chlorotrimethylsilane (CTMS), monochloro – dimethyloctadecylsilane, methyltricholrosilane, trichlorooctadecylsilane, napthalene, phenanthrene, pyrene, benzo[a]pyrene and fluorescein were all purchased from Sigma-Aldrich (Oakville, Canada, St. Louis, USA). Sodium hydroxide, toluene and HPLC grade acetonitrile were purchased from Fisher Scientific (Toronto, Canada, Pittsburg, USA). Various types of microstructured fibre (LMA-PM-15, LMA-15 and LMA-20) were acquired from NKT photonics (Birkerød, Denmark). Deionised water was obtained through an in house milliQ water purification system from Millipore (Billerica, USA). Ethanol was purchased from Commercial

Alcohols (Toronto, Canada). Azobisisobutyronitrile (AIBN) was obtained from the Cunningham lab at Queen`s University.

3.2.2 Flow Profile of Liquids in Microstructured Fibre

Lengths of microstructured fibre or capillary were cut to length (at least 20cm in length), spanning the gap over the objective lens of the microscope (Nikon Eclipse Ti-S). A syringe pump (Harvard Apparatus 11 plus) was used to pump water into the valve (VICI Cheminert 09N-0006H) and MSF fibre at 0.5 μL/ min, which were all attached using standard PEEK capillary fittings. Fluorescein was loaded into a 10 nL valve (VICI Cheminert 09N-0006H) with a syringe. The fluorescein was then injected into the MSF and video images of the fluorescent plug passing through the MSF/capillary were captured with a CCD camera. Our samples were monitored at λ_{em}520 nm, λ_{ex} 490 nm at 100x magnification.

3.2.3 Multichannel Open Tubular Polystyrene/Divinylbenzene PLOT Columns

Synthesis of the polystyrene/divinylbenzene (PS/DVB) PLOT columns was carried out as follows. A length of microstructured fibre (MSF) (10 cm, 50 cm or 1 m) with either 54 (LMA-PM-15), 84 (LMA-15) or 168 (LMA-20) individual channels was cut and pre-treated. Pre-treatment encompassed flushing the capillary for 1 hour with sodium hydroxide (0.1 M NaOH), deionized water, hydrochloric acid (0.1 M HCl), and again with deionised water using a Harvard Apparatus 11 plus syringe pump at 0.5 μL/min. The water washing steps were sometimes allowed to go for longer than 1 hour, if the eluting solution was not yet neutral when tested with pH paper. Following pre-treatment, a solution containing 50% deionised water, 20% γ-MPS and 30% glacial acetic acid was prepared and pumped through the MSF for at least 1 hour to ensure

that the MSF was completely filled with solution. Subsequently, pH paper was used to check for a mildly acidic solution eluting from the MSF. The flow rate was then reduced to 0.2 μL/min and allowed to pump overnight to minimize damage to the acrylate coating on the MSF. The MSFs were then flushed with a 95% acetonitrile/water solution using a Waters model 590 HPLC pump at 20 μL / min for 20-30 minutes. Once complete, a solution of 600 μL ethanol (EtOH), 200 μL styrene, 200 μL divinylbenzene (DVB) and 5 mg of azoisobuturylnitrile (AIBN) was pumped through the MSF with a syringe pump, until the entire MSF was filled (approximately 30 min, longer for 1m columns to ensure they were filled). The ends of the capillary were then sealed using GC septa and the MSF was then placed in an oven (Fisher Isotemp, model 281A) for 1 hour or overnight at 80^{o}C. The column was removed from the oven and flushed with 95% ACN/H_2O using the Waters model 590 pump for 30 min, after which the column was ready for use. No special storage conditions were employed.

3.2.4 Open Tubular LMA/BDDA MSF PLOT Columns

A length of MSF was cut and pre-treated. Similar to above, these columns were made by pre-treating the capillaries for 1 hour with 0.1 M NaOH, deionised water, 0.1 M HCl and again with deionised water (the water steps were sometimes left run longer if the effluent of the capillary was not near neutral when tested with pH paper after one hour). The capillaries were then flushed with a solution of 50% deionised water, 20% γ-MPS, 30% glacial acetic acid for 1 hour to ensure that the capillaries were filled with solution. Similarly to the method above, the flow rate was reduced and pumped overnight to minimize damage to the outer coating. The MSFs were then flushed with 95% ACN/H_2O for 20-30 min. The MSFs were then filled with a solution of 700 μL EtOH, 150 μL laurylmethacrylate (LMA), 150 μL 1, 3- butanedioldiacrylate

(BDDA) and 5 mg of AIBN. The MSFs were then sealed with GC septa and placed in an oven for 1 hour at 80 ^{o}C. The capillaries were then flushed with 95% ACN/ H_2O for 20-30 min, after which they were ready for use.

3.2.5 Multichannel Open Tubular Monochlorodimethyloctadecyl Columns.

A length of MSF (10 cm, 50 cm or 1 m) with 54, 84 or 168 holes was cut and pre-treated. Similar to the above method these columns were made by pre-treating the MSFs for 1 hour each with NaOH (0.1 M), deionised water, HCl (0.1M) and again with deionised water, sometimes letting the water washing steps take longer if the effluent of the MSF was not near neutral after one hour. A 10% v/v solution of chlorodimethyloctadecylsilane in toluene was prepared and pumped through the MSF overnight using a Harvard Apparatus 11 plus syringe pump at 0.2 μL/min and the column was then flushed with 95% ACN/H_2O. Following, the column was filled and flushed with a solution consisting of 10% v/v chlorotrimethylsilane in toluene overnight at 0.2 μL/ min. The column was then flushed again with 95% ACN/H_2O for 20-30 min before use.

3.2.6 Multichannel Open Tubular Trichlorodimethyloctadecyl Columns

A length of MSF (50 cm) with 54 holes (LMA-PM-15) was cut and pre-treated. The MSFs were pretreated for1 hour with NaOH (0.1 M), deionised water, HCl (0.1 M) and again with deionised water. The MSF was then placed in an oven (Fisher Isotemp model 281A) for 24 hours or more at 100 ^{o}C, to dry. After drying, the MSF was placed in a homemade controlled humidity system and held at 50% humidity for 3 hours. The MSF was then removed from the humidity box and a 3:1 mixture of tricholorooctadecylsilane and trichloromethylsilane

respectively in dry heptane was pumped overnight at 0.1 μL/min. The MSF was then flushed with 95% acetonitrile for 1 hour.

3.3.0 Results and Discussion

3.3.1 MSF Flow Profiles

Microstructured fibres (MSFs) are a type of optical fibre that was originally designed for the photonic industry that makes use of the unique light coupling properties imparted by the periodic arrangement of features built into them. Because in many cases the features are just air holes, we can flow liquid through them making the fibres good candidates as supports for liquid chromatography. An investigation into the how liquid behaves inside of the fibres was carried out. Based on some of our earliest stationary phase development work, we were not sure if a liquid flowed through the channels with similar flow velocities between each channel, and was thought to be a contributor to the observed poor chromatographic performance.

An inverted fluorescence microscope was used to monitor injections of a fluorescent dye (fluorescein (1 mM, in water)) pumped through a length of MSF (LMA-PM-15) (The complete set up is described in the experimental section). Initially, results were mixed with some samples showing the dye was confined mostly to the middle of the MSF, or alternatively having a small plug of sample confined to the center of the MSF with more dye coming through the outer channels a short time later. This flow profile would cause significant problems for LC analysis causing broad peaks and potential peak splitting, see Figure 17. It was however soon discovered, that this was caused by a capillary i.d. mismatch between the i.d. of the transfer capillary and the holey region of the MSF capillary. Table 4 shows physical properties for different MSF types

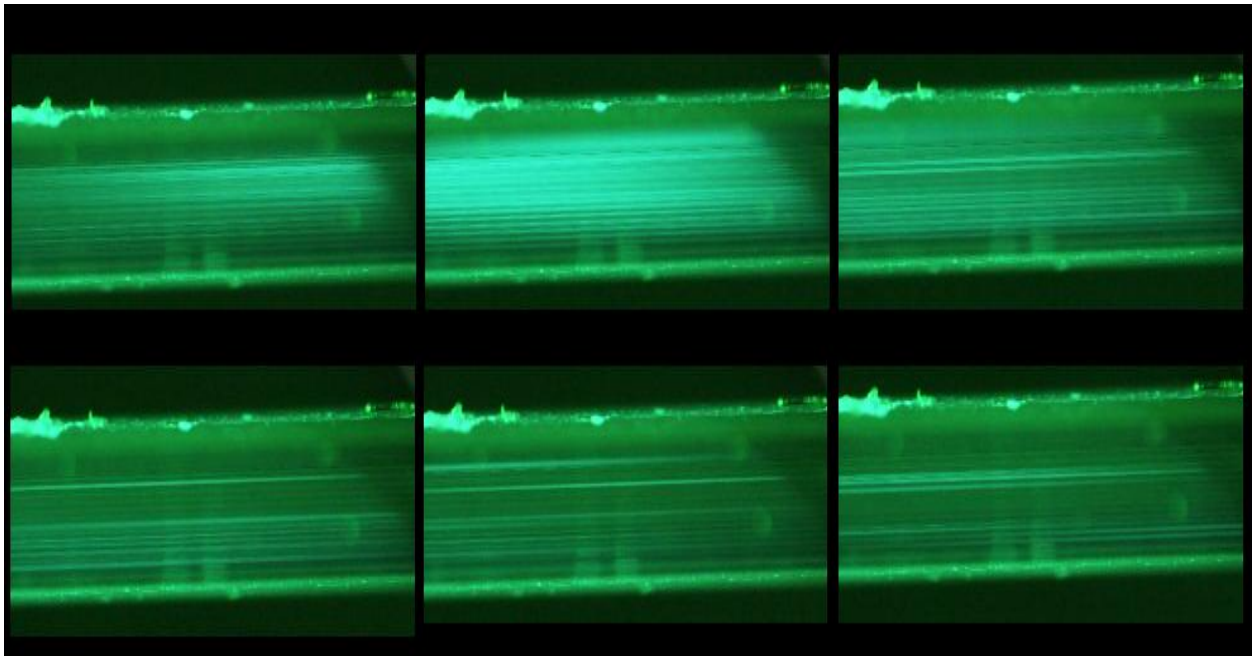


Figure 17: Snapshots of 10 nL fluorescein (1 mM, in water) plug travelling through a 168 hole MSF when the transfer capillary prior to the MSF had a smaller i.d. then the holey region (see Table 4). Images were observed about 10 cm from either end of MSF at λ_{em} 520 nm (λ_{ex} 490 nm) and 100x magnification. Images were taken at 2 second intervals.

Table 4: Physical dimensions of MSF's used for OTLC, including fibre type, number and size of microstructured holes, and the cross sectional diameter of the entire holey region. [32]

Fibre Type	Number of Holes	Hole Diameter (μm)	Diameter of Hole Region (μm)
LMA-PM-15	54	3.8	75
LMA-15	84	4.3	92
LMA-20	168	5.6	185

tested (images of the fibres can be seen in Figure 18). The diameter of the 'holey' region corresponds to the minimum capillary size needed to deliver liquid to all the holes efficiently. This problem was mostly observed when the capillary i.d. leading to the MSF was significantly smaller than the 'holey' cross sectional i.d.; likely because the central MSF channels were better aligned with the capillary than the outer set of holes. The distribution of the injected dye was not as much of an issue with capillaries where the cross-sectional areas were larger than the MSF holey region. However, matching the capillary i.d. to the cross sectional area of the holey region minimizes potential dead volume and potential interfacing problems, such as uneven pressure

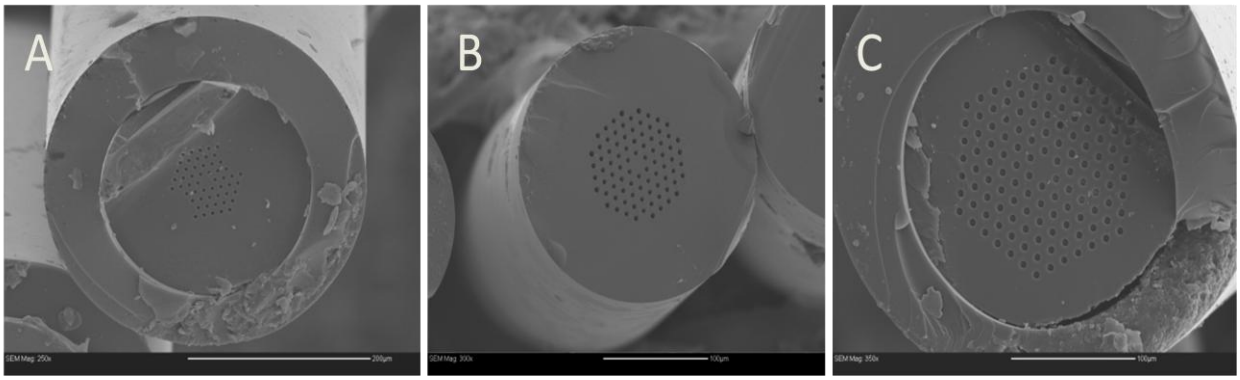


Figure 18: Images of LMA-PM-15(A), LMA-15(B) and LMA-20(C) microstructured fibre from NKT photonics. Scale bars are 200 μm for (A) and 100 μm for (B) and (C). Physical characteristics of each fibre are denoted in Table 4

across the MSF. After accounting for the cross sectional area differences, flow of the injected dye through column was much more uniform and was confined to a plug. A series of photos (taken from video images) comparing the flow profile in a 75 μm i.d. capillary (similar cross sectional area to 54 hole MSF) and 54 holed MSF are shown in Figures 19 and 20.

3.3.2 Effects of MSF Hole Size and Distribution on Chromatography

As noted above in Table 4, the physical dimensions of commercial MSFs vary from fibre to fibre, generally to exploit different properties of light[30, 32]. However, these small differences can have a significant impact on the flow of liquids through the channels and ultimately on chromatographic performance. For instance, it is known that by going to a smaller hole/particle size in chromatography a higher surface area to volume ratio is developed, which tends to help chromatographic performance by allowing for more interactions with the stationary phase[12, 23]. It follows from theory that smaller hole sizes should lead to better chromatographic performance. For example, performance should improve from the 168 holed fibre to the 54 hole fibre as the individual hole size gets smaller, see Figure 21.

One of the most significant problems with multicapillary systems is the uneven flow distribution between the individual channels, which results in broader peaks and/or poor performance[23]. These conclusions by Meyer *et al.* come from considerations like the Hagen-Poiseuille equation (3), which dictates that small differences in channel diameter can have a large impact on flow rate[23, 26]. This is a major problem when not all of the channels within a MSF are of similar size, as some of the channels will travel faster or slower than the bulk of the

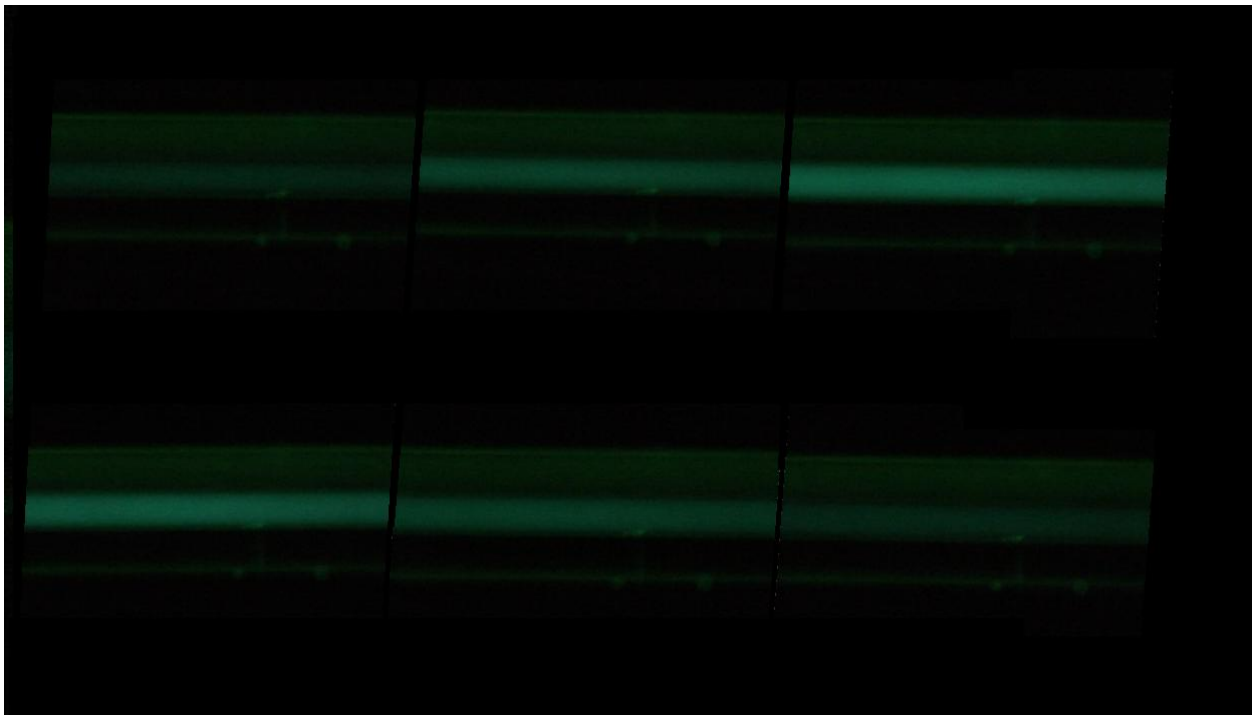


Figure 19: Snapshots of a 10 nL fluorescein (1 mM, in water) plug travelling through an i.d. 75 μm capillary. A 75 μm i.d. transfer capillary was used as it is the equivalent size to the holey region of the 54 hole fibre (shown below). Images were observed at λ_{em}520 nm (λ_{ex} 490 nm) and 100x magnification. Images were taken at 2 second intervals.

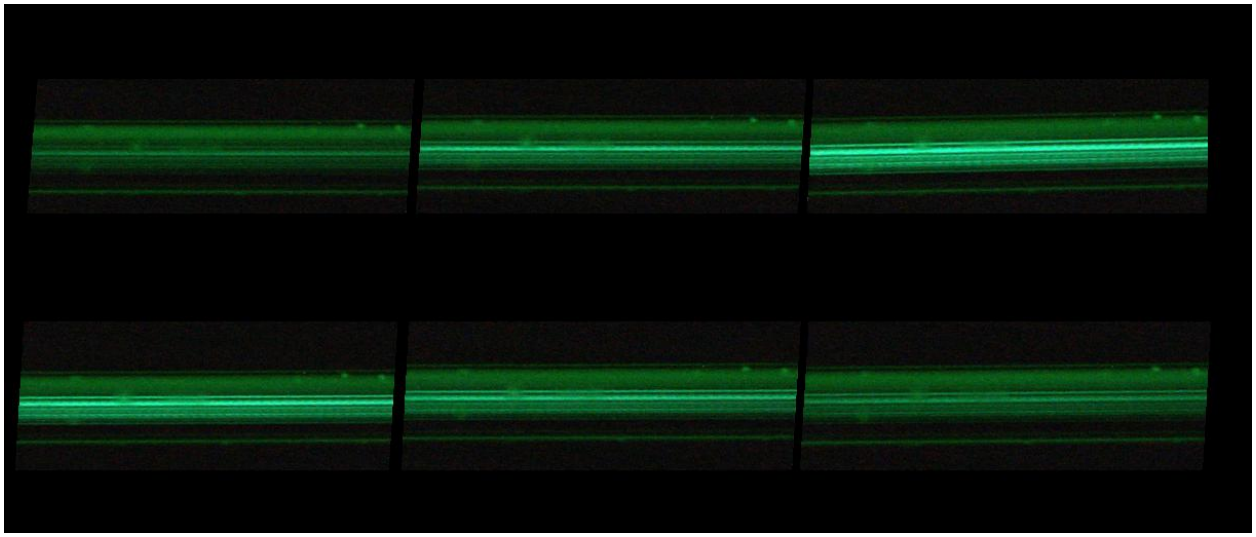


Figure 20: Snapshots of a 10 nL fluorescein (1 mM, in water) plug travelling through an LMA-PM-15 fibre. Notice how the dye travels through the channels in a plug, similar to regular capillary (shown above) when the inlet capillary is properly matched to the area of the MSFs holey region. Images were observed at λ_{em}520 nm (λ_{ex} 490 nm) and 100x magnification. These images were taken at 2 second intervals

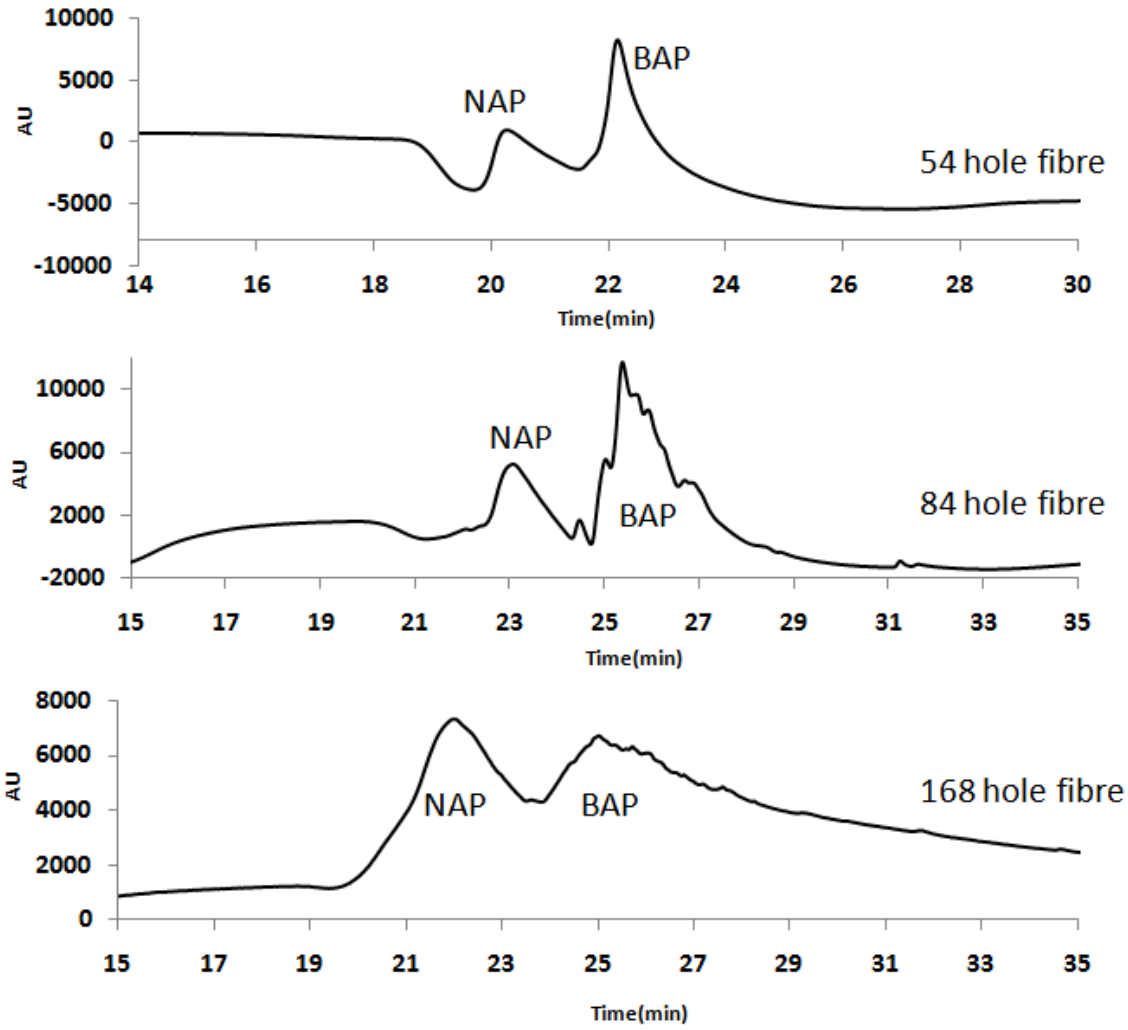


Figure 21: Chromatographic performance improves as 'hole' size decreases, as a higher surface area to volume ratio is achieved. These improvements are also likely caused by a reduction in the variance in flow rate between channels (Table 5). Analytes were naphthalene (NAP, 100 μM) and benzo[a]pyrene (BAP, 15 μM) and a 10-99% ACN/H_2O gradient at 400 nL/min over 15 min was employed. All 3 columns were functionalised with monochlorooctadecylsilane (C18)

sample plug leading to a re-introduction of an 'A' like band broadening term. This re-introduction or pseudo A term, effectively causes broader peaks, since unlike in packed columns, open tubular columns do not have random flow paths to average out these inconsistencies, since the flow is confined to a single channel for the entire length of the column. In an extreme case, this could lead to a splitting of the analyte peak into multiple peaks making analysis very difficult; as sample plugs from each individual channel arrive sequentially. Table 5 highlights the hole properties/characteristics of some of the commercial fibres tested within our laboratory. Variation appears to decrease with smaller arrays of holes. This is most noticeable when looking at the standard deviation of the theoretical flow rate.

Table 5: Hole differences between different fibre types. Variation in hole size was based on the data from 20 holes from 10 different cuts of each fibre. Flow rate data was modeled using the Hagen-Poiseuille equation (3).

Number of Channels in MSF	Mean Hole Diameter (μm)	Std. Dev. of Hole Diameter (μm)	Mean Calculated Flow Rate (m/s)	Std. Dev. of Flow Rate (m/s)
54	3.89	0.20	9.20E-06	9.52E-07
84	4.05	0.25	8.94E-06	1.10E-06
168	5.52	0.18	1.71E-05	1.13E-06

3.3.3 Stationary Phase Development

Stationary phase development for MSF OTLC columns was done in conjunction with the work done in single channelled capillary (Chapter 2). Similarly, we started with polymer layers varying in the amount of branching possible (3-glycidoxypropyl-trimethoxysilane (GPTMS), 3-(trimethoxysilyl) propyl-methacrylate(γ-MPS)) and a C18 layer type stationary phase. The results were similar to those in a single channelled capillary, where polymer formation was very inconsistent, not only down the length of column, as observed in capillary (see Chapter 2, page 27), but also between channels of the microstructured fibre, which can be seen in Figure 22.

Utilizing a simpler triethoxyoctadecylsilane C18 surface treatment, did not result in significantly improved chromatographic performance, instead broad peaks and very little resolution resulted. A switch to chlorinated octadecylsilanes, specifically chlorodimethyloctadecylsilane, was then pursued based on the ease with which chlorosilanes have been used in the past for the functionalization of silica particles and glass surfaces [2, 47-50].

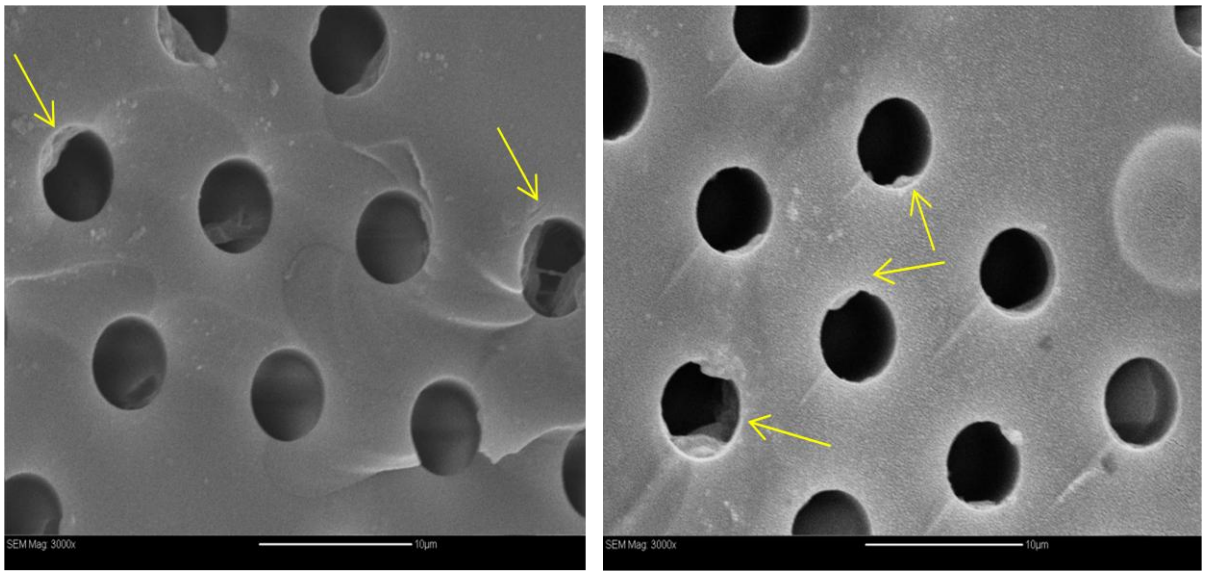


Figure 22: SEM images showing spotty formation of early polymer stationary phases (GMTPS and γ-MPS based) within a 54 holed MSF, scale bars are 10 µm

Initial results were again discouraging, however, with some changes to the pre-treatment procedures, allowing for longer acid and base treatment of the MSF glass; results improved and allowed for two PAHs (napthalene and benzo[a]pyrene) to be partially resolved which can be seen in Figure 23. The resolution was fairly poor (0.47) and the efficiency was also quite poor with broad peaks spanning almost 4 minutes. One other notable feature was the smaller peaks on the tailing side of the bezno[a]pyrene peak. These small secondary peaks are a result of the pseudo 'A' term and are connected to issues surrounding the different flow rates between the channels, discussed above. These secondary peaks are also related to homogeneity problems with the stationary phase, which also contribute to the pseudo 'A' term problem.

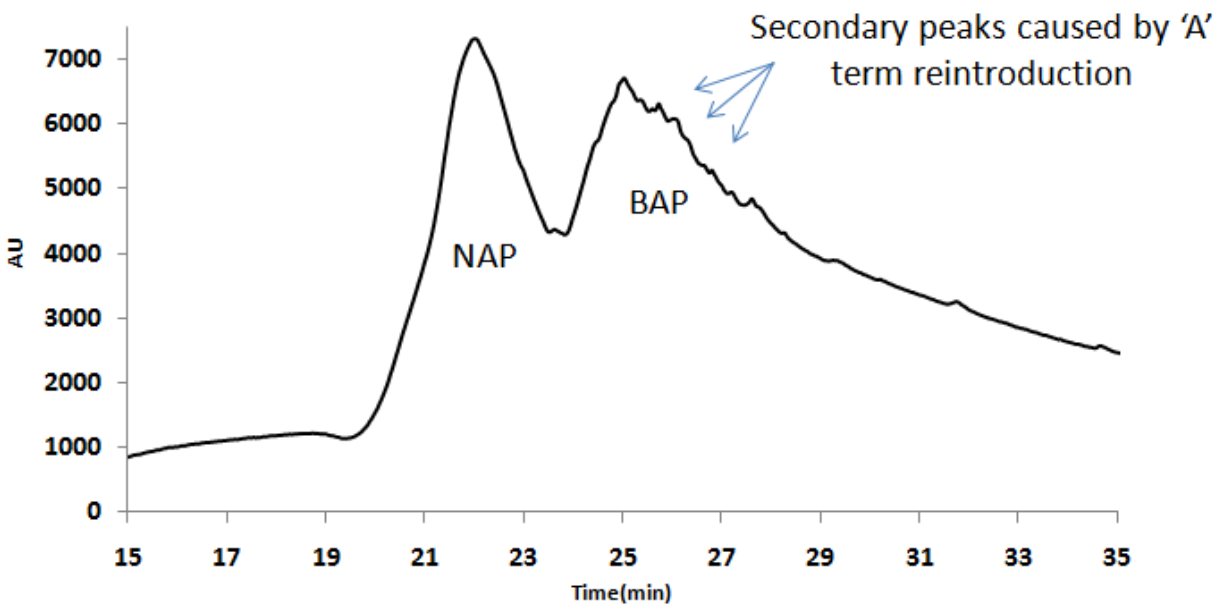


Figure 23: The first successful separation achieved with a MSF, using a C18 layer stationary phase. One notable feature is the smaller peaks on the tailing side of the bezno[a]pyrene (BAP) peak. These small secondary peaks are a result of the pseudo 'A' term issues. Run conditions were: 10-99% ACN/H_2O gradient over 15 minutes at 400 nL/ min

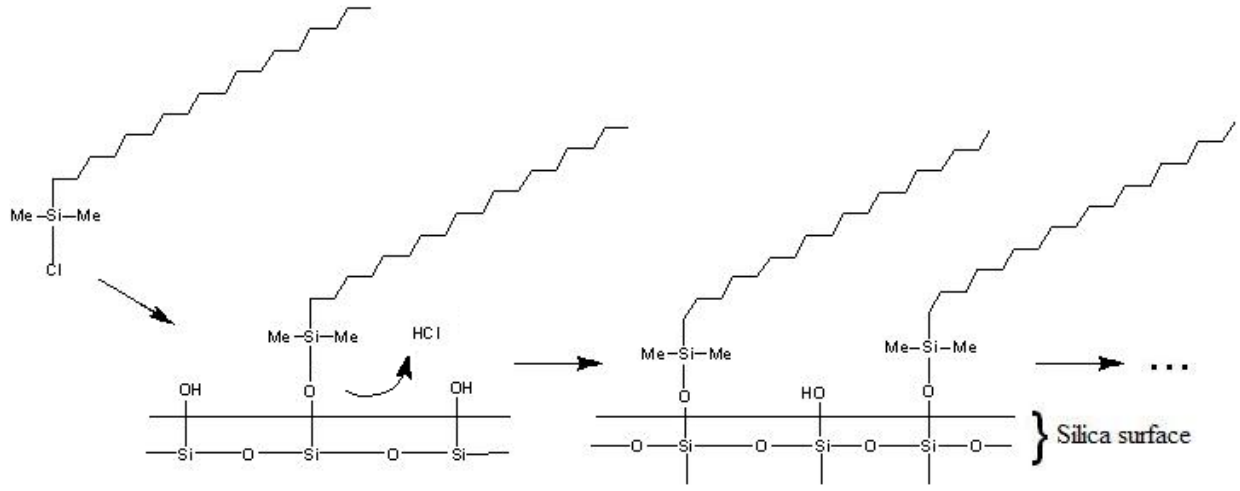


Figure 24: Schematic reaction scheme for the deposition of C18 on a silica surface. The bulky side chains can sterically impede complete coverage of a surface resulting in free Si-OH, which can reduce stationary phase stability and chromatographic quality

Further refinement of our coating protocols using chlorodimethyloctadecylsilane, included moving to the 54 holed fibre (LMA-PM 15), since it had the least variability of the holes within the fibre (see Table 4) and the addition of chlorotrimethylsilane (CTMS) to the regular chlorodimethyloctadecylsilane treatment. The addition of CTMS was explored since it is known that due to the bulky C18 side chains, that coverage of a surface, in this case, the inside of an MSF, is not 100% efficient and there are always some unreacted silanol (Si-OH) groups left on the glass surface, see Figure 24 [2, 55]. These unreacted silanols can negatively impact the quality of a separation by causing some parts of the column to retain analyte more strongly than others and as such contributes to band broadening [2,55]. This was explored by treating columns first with C18 followed by chromatography. After which the same column was then treated with CTMS, and the same samples run again. This was done to minimize differences observed between columns used previously where one column was made with C18 and the other with C18/CTMS. With the addition of CTMS, we were able to achieve about a 40% reduction in the peak width for benzo[a]pyrene, which is shown in Figure 25. This amount of reduction was not observed for the naphthalene peak; however, naphthalene is one of the least retained PAHs, so it may be less affected by the addition of CTMS. With the addition of CTMS to the procedure and carefully controlled fabrication of columns, reproducible separations were achieved with MSFs. Further optimisation of the LC conditions to a step gradient, allowed for the separation of a 4 component PAH mixture, see Figure 26. The plate heights of the columns are on par with other multichannel OTLC columns in the literature, providing efficiencies in the low hundreds of plates per meter[58]. Our columns however, were able to separate a more complex mixture of analytes (4 PAHs compared to 2 structurally different drugs (phenobarbital and codeine)) used in other reports of multichannel columns[58]. Our columns were also able to function with current nanoLC equipment. While the resolution and efficiency could still be greatly improved

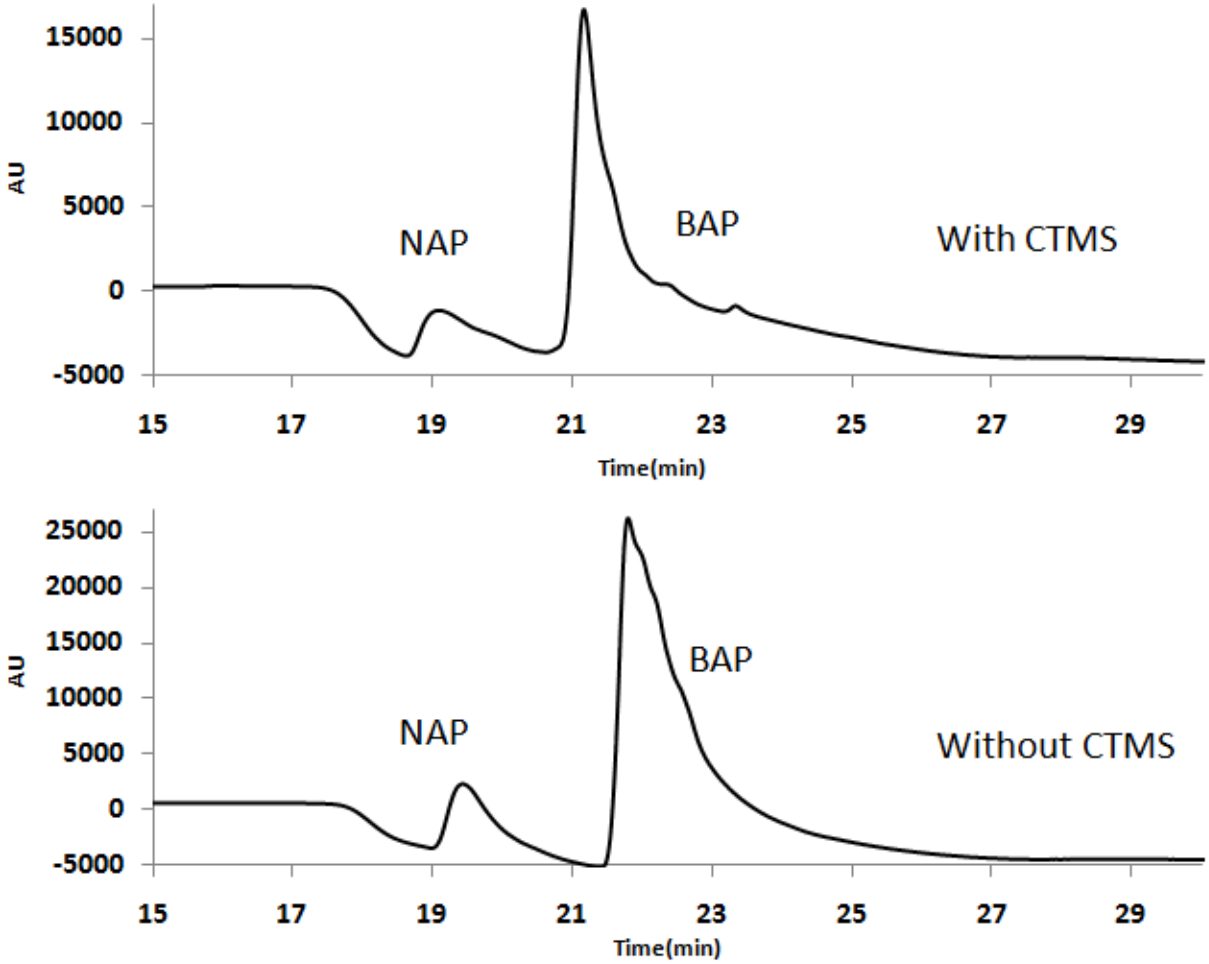


Figure 25: The effect of using CTMS to eliminate free Si-OH groups on the surface of the MSF. Using CTMS we achieved about a 40% reduction in the peak width at half height for benzo[a]pyrene (BAP).

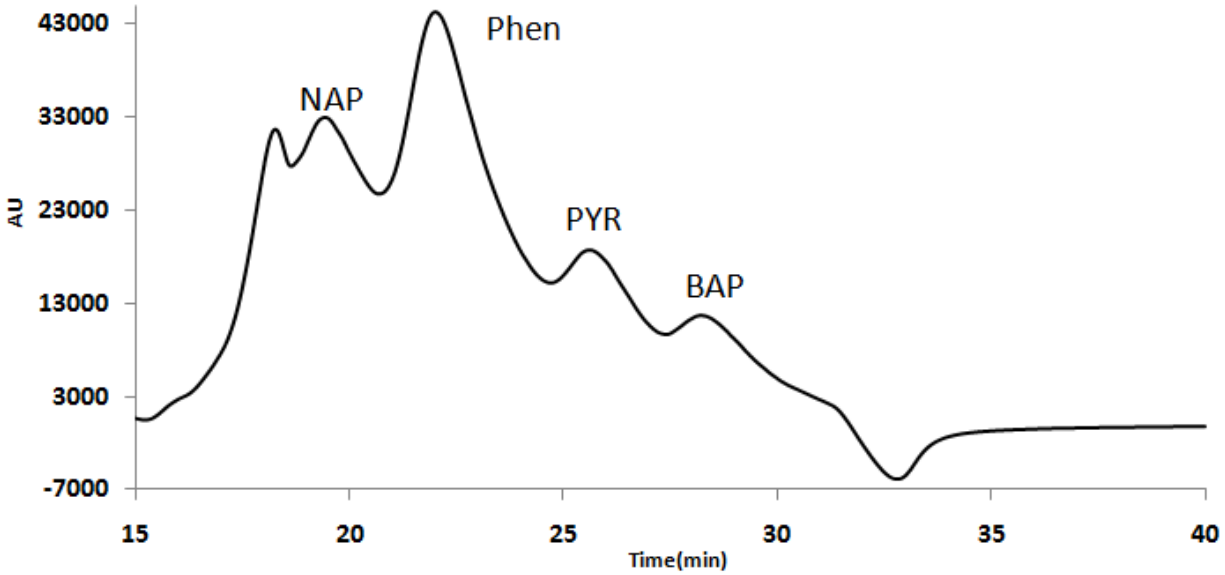


Figure 26: Chromatogram showing the separation of a 4 PAH mixture with a 1 meter 54 hole MSF with a C18/CTMS stationary phase, using a 2 µL injection, 400 nL /min, 1-20% ACN at 1 min, 20-30% at 5 min, 30-40% at 9 min, 40-50% at 13 min, 50-80% at 16 min, 80-1% at 19 min

with further development of stationary phase materials, multichannel OTLC may yet have a bright future.

3.3.4 Use of Trichlorosilanes for Stationary Phase Formation in MSFs

Trichlorosilanes were also investigated in an effort to further improve the characteristics of the C18 layer stationary phases. Trichlorosilanes have seen a lot of use in treating silica particles for chromatography; they are widely used because of the ability to crosslink with other silanes (Figure 27), resulting in a more complete and uniform layer being deposited when compared to monochlorosilanes[47, 55, 56]. Using trichlorooctadecylsilane to make columns in MSFs proved to be quite difficult. These columns were very prone to blockage and did not provide us with any improvement in chromatography, in fact, it was more often than not, worse. This is partly due to the pseudo 'A' term discussed previously, where different flow rates in each channel negatively impact the quality of chromatography, since some of the analyte will travel faster or slower than the bulk of the analyte plug, as a result of a distribution in the sizes of the holes within an MSF. However, the quality of the stationary phase is the most dominant problem with trichlorosilane use in MSFs. In MSFs, the problem arises with the cross linking abilities of the trichlorosilane, this results in highly variable amounts of stationary phase being deposited in each channel. This, in turn, results in very complicated chromatograms with the analytes being split up into many peaks over time, effectively amplifying the problems caused by the pseudo 'A' term as now analytes in some channels stick more than in other channels.

As a modification to using just trichlorooctadecylsilane, a procedure known as horizontal polymerisation, where trichloromethylsilane is used as a spacer between C18 groups to reduce steric interference, similarly to how CTMS is used with monochlorinated C18, was also tested.

It has been reported that horizontal polymerisation also provides very uniform layers on chromatographic packing materials[47, 55-57]. This procedure needs to be carried out at a specific humidity (50%) as it has been shown previously in the literature that the amount of surface adsorbed water can have a significant impact on the quality of monolayer formation (Figure

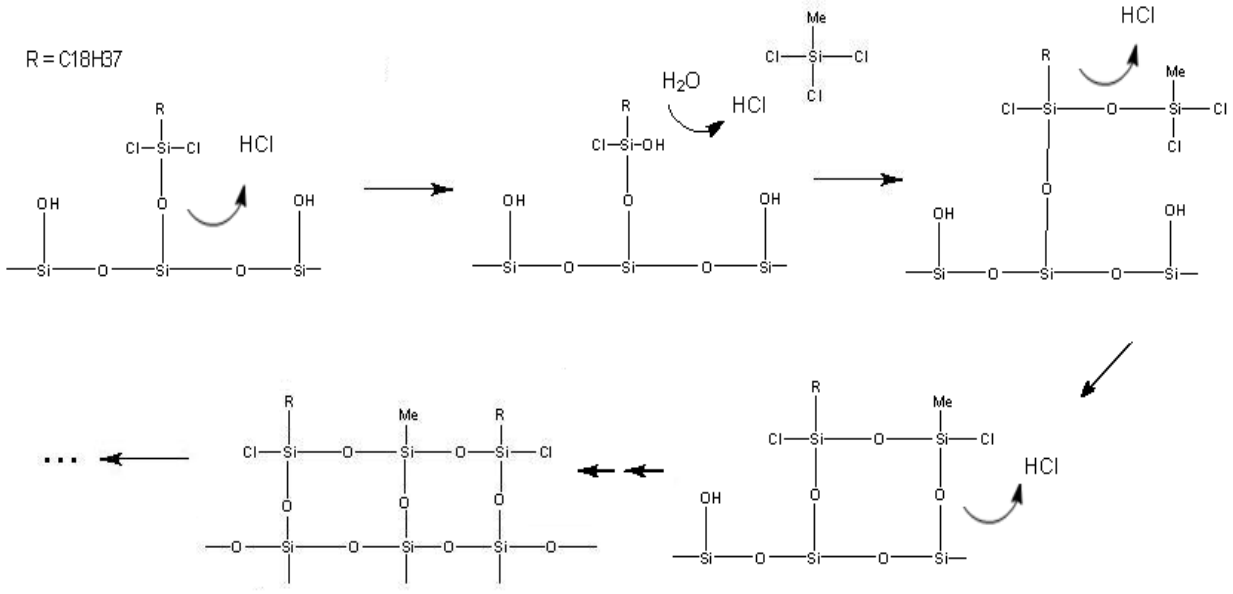


Figure 27: Idealized schematic reaction scheme of horizontal polymerization using trichlorosilanes. Note how water (humidity) needs to be present as it can aid in the crosslinking steps.

27)[58]. If the humidity is too low (<50%), poor coverage of a silica surface results and if the humidity is too high (>60%), then small micro particles of stationary phase can form, once again limiting the quality of the stationary phase deposited on the silica surface[58]. While this procedure may be useful for things like silica spheres, where the environment is easily controlled, it did not yield any better results when used in MSFs. Similar to standard tricholrosilane polymerisation procedures, columns prepared with horizontal polymerisation produced very broad peaks and peak splitting. It is possible however, that better results may be produced with further polymerization refinement.

3.3.5 Polymeric Stationary Phase Materials in MSFs

Polymer layers were also attempted in MSFs; Initial results of MSF OTLC with the same polystyrene/divinylbezene copolymer used in regular capillary were discouraging. As can be seen in Figure 28, the polymer seemed to form in a consistent manner, with a high degree of surface coverage down the length of the column and across all the channels. However, the chromatographic performance was poor, with broad peaks, spanning over 5 minutes and very low retention of test analytes, see Figure 29-A. Revisiting this polymer mixture after refining the coating procedure for capillary OTLC (see Ch 2, pg 28) produced similar or worse results. These new columns produced chromatograms that were plagued with multiple peaks, rendering analysis of mixtures next to impossible, which is also shown in Figure 29-B.

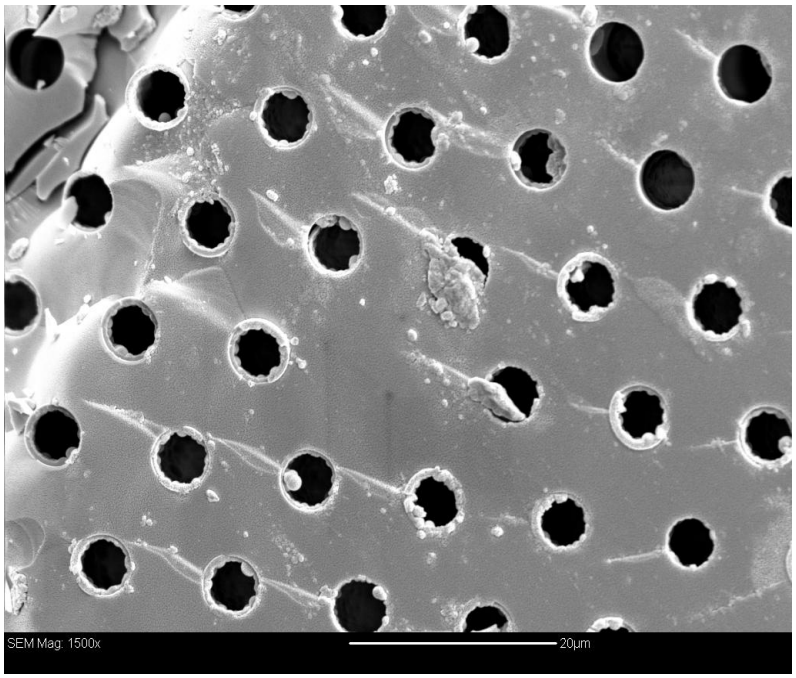


Figure 28: SEM image showing PS/DVB formation in MSF. Scale bar is 20 µm.

A second polymer blend similar to the PS/DVB PLOT procedure was also tried with MSFs. This was the lauryl methacrylate (LMA) / 1,3-butanedioldiacrylate (BDDA) polymer used for investigating the effect of the solvent on polymer formation discussed in chapter 2. This polymer was based upon methods found in the literature that seemed to have some success when used in CEC[14, 15, 59, 60]. Initially, this polymer mixture showed promise over the early PS/DVB MSF columns, with different retention for the two test PAHs (naphthalene and benzo[a]pyrene). With some refinement to the procedure, we were able to separate a 2 component mixture, although, the peaks were still fairly broad, spanning almost 4 minutes, complete with some of the multiple peak issues described earlier. A chromatogram of the LMA/BDDA columns is shown in Figure 30. However despite the better performance of the LMA/BDDA polymer over that of the PS/DVB polymer, it was still quite poor compared to the C18/CTMS procedures discussed above. A comparison of chromatograms produced by the two methods is also shown in Figure 30.

Polymer layers, as with the tricholrosilanes, still have a few technical issues to work out before they can become useful. As discussed previously, the dominant problem with polymer layers (including trichlorosilanes) is the quality of the stationary phase. Not only does a heterogeneous stationary phase cause peak broadening, but in the case of MSFs it can cause different retention of analyte as well; since the stationary phase coverage seems to be slightly different in each channel. This, along with pseudo 'A' term issues described earlier, further compounds the problems with polymer stationary phases, leading to complex chromatograms

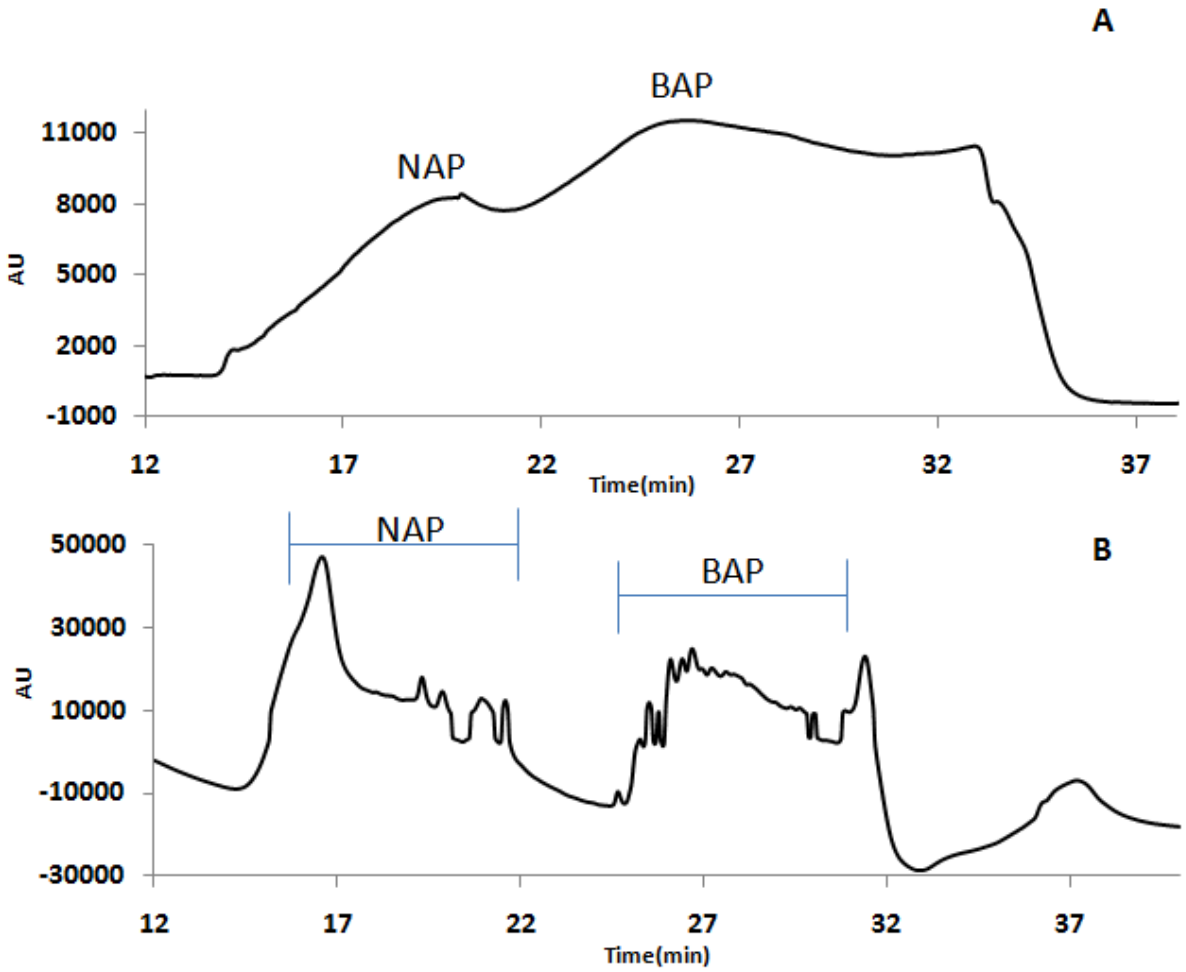


Figure 29: A) Representative results of early (overnight (16 hour) reaction time) PS/DVB PLOT MSF columns. The resolution and efficiency of these columns was quite poor despite consistent and regular polymer formation as shown in Figure 28.

B) Results of a 1 hour reaction time, PS/DVB layer in MSF. Notice the multiple peaks for the two analytes as a result an amplified pseudo 'A' term effect from different amounts of polymer being deposited in each channel, similar results were obtained for tricholosilane stationary phases.

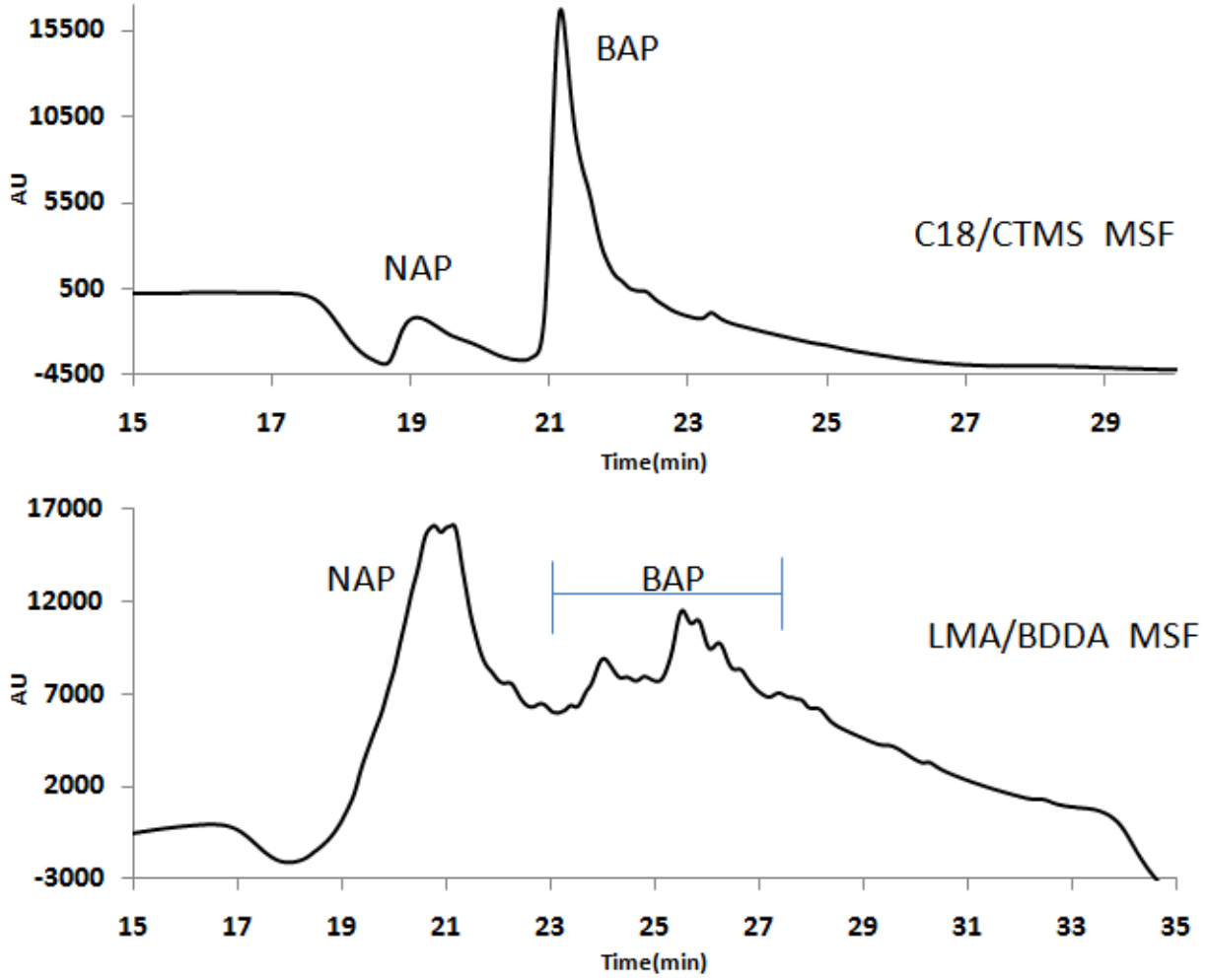


Figure 30: Comparison between the C18/CTMS MSF column (top) and the LMA/BDDA MSF column (bottom).

which produce multiple peaks per analyte, making interpretation almost impossible. A future goal for MSF OTLC work should be to find new polymerisation procedures that allow for the reliable deposition of polymer in all the channels as well as potentially new and refined stationary phase materials to try and eliminate this problem.

3.4.0 Conclusions

In order to alleviate some of the issues associated with open tubular liquid chromatography, such as small sample volumes and high backpressure, columns were made using MSFs as a solid support. We showed that these columns have great potential even when simple C18 layers are used as stationary phases; which was able to separate 4 PAHs. While the potential advantages of these types of columns, such as increased surface area, relatively low pressure and potentially higher sample capacity, is great, some technical problems remain with the manufacturing of MSFs as well as with the use of more extended stationary phases. Current manufacturing tolerances lead to a distribution of the MSF hole sizes, which causes differences in flow rates between the channels, effectively broadening peaks via a re-introduction of the A term in the van Deemter equation. Compounding this problem, is the quality of extended stationary phase materials, which can cause further inhomogeneity between the channels of an MSF causing broad peaks and the splitting of analytes into multiple peaks. However, as new stationary materials emerge and deposition procedures are further tailored to MSFs results should improve.

Chapter 4: Conclusions and Future work

Open tubular liquid chromatography has historically suffered from many technical challenges that have hindered its' development. Many of these challenges have been the result of technological limitations. Sample volume and detector sensitivity were long standing issues as well as a limited ability to reliably manufacture small diameter (micrometer scale) columns. Despite many of the advancements in the manufacturing of capillary, sample introduction and detection techniques, open tubular chromatography still suffers from some technical limitations such as small sample volumes and high backpressure and has remained an elusive goal in chromatography. We have shown that with careful fabrication of polymer stationary phases that reverse phase open tubular capillary chromatography is possible with modern HPLC sample introduction and detection systems. We have shown that these columns are robust and can function over a wide range of flow rates and separate complex multi-component mixtures (EPA 610, drug samples). We have also shown that these columns are useful for different analytes types and may be useful for general reverse phase chromatography in the future.

In an effort to alleviate some of the technical issues associated with open tubular liquid chromatography, such as small sample volumes and high backpressure, columns were made using microstructured fibre as a solid support. We have shown that the potential of multichannel open tubular columns is great, even when relatively simple stationary phases like C18 are used, with which, a separation of 4 PAHs was achieved. While other potential advantages of these types of columns, such as increased surface area, relatively low pressure and potentially higher sample capacity are also great, some technical problems remain with the manufacturing of MSFs as well as with the use of more extended stationary phases. Current manufacturing tolerances lead to a distribution of the MSF 'hole' sizes, which in turn, causes differences in flow rates

between the channels. This distribution of hole sizes leads to a broadening of peaks, via a re-introduction of the A term in the van Deemter equation. The quality of deposited stationary phases further compounds this problem for extended stationary phase materials such as polymers and trichlorosilanes; where inhomogeneities in stationary phase coverage between the channels of an MSF can cause peak broadening as well as peak splitting.

While our results represent a step forward for OTLC, there remain some key areas that could greatly benefit from further study. A future focus of capillary OTLC should centre on further stationary phase development. Further refinements of the PS/DVB polymer procedure should be carried out, focusing on increasing the consistency of the polymer deposition as well a more reliable production process. Continued searching for new and/or better stationary phase materials should also be a major focus of future work, as other stationary phase materials may very well out perform the current stationary phase materials being employed. Future work involving MSFs should primarily focus on finding a reliable way to coat all the channels of an MSF with the same amount of stationary phase as this has been a major limiting factor for multichannel OTLC. Another key focus should be on issues surrounding the use of smaller capillaries. Future work should further investigate why smaller capillaries continually perform worse than the larger capillaries. Furthermore, possible connections to polymer thickness and how it impacts chromatographic performance should also be in investigated more thoroughly. Further advances in multichannel OTLC will also come from improved manufacturing of MSF's. However, this is mostly out of our control experimentally, but as manufacturing practises improve so should the future of OTLC.

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