

The Design and Fabrication of Microfluidic Reactors and Their Application to Small Molecule Organic Synthesis

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by

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Abstract

In the area of analytical chemistry, great interest has been generated by the potential advantages of fluid handling devices with feature sizes in the low micron range, termed micro-Total Analysis Systems (μ -TAS). This thesis describes the application of the same precept of miniaturisation to synthetic organic chemistry, where mixing of reagents over reduced distances and high heat transfer may lead to a reduction in by-product formation and increased yields. An introduction discussing issues relating to mass and heat transfer in microfluidic regimes is followed by a brief literature review of work on the application of microreactors to hazardous reactions, heterogeneous catalysis and compound library generation.

The experimental studies presented fall into two main sections. The first details work carried out on the design and fabrication of microfluidic devices and interconnects. Various methods for the coupling of microfabricated structures to the macroenvironment of the real world (e.g. capillary tubing, sample reservoirs and pumps) are examined in detail. These include facility of use, exclusion of dead volumes and the effects of solvents on microfluidic devices and interconnections. The development and testing of fully integrated interconnection devices for glass microreactor applications is also described and discussed. In addition, a novel microfluidic mixing device for biphasic or two component reaction systems is designed and fabricated. Quantitative data on mixer performance is obtained and compared with conventional microfluidic mixing methodology.

The second part of this thesis concerns the effect of microenvironments on the synthesis of small molecules. A range of reactions involving unstable or dangerous intermediates are performed including the formylation of phenols by the Reimer-Tiemann and Vilsmeier-Haack reactions and the synthesis of diazonium salts. Furthermore, the use of Raman spectroscopy is demonstrated for dynamic feedback control and online optimisation during synthesis on a microfluidic reactor for the first time.

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Work and play are words used to describe the same thing under differing conditions.

--- Mark Twain

I don't want to achieve immortality through my work
I want to achieve it through not dying.

---Woody Allen

Abbreviations

AJM	abrasive jet machining
CD	circular dichroism
CI	chemical ionisation
CVD	chemical vapour deposition
DCM	dichloromethane
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DWL	direct-write lithography
EDM	electrical discharge machining
ee	enantiomeric excess
EI	electron ionisation
ESI	electrospray ionisation
EPD	electrophoretic deposition
FEP	fluoroethylenepropylene
FSC	fused silica capillary
GC	gas chromatography
HF	hydrofluoric acid
HTS	high throughput screening
ID	inner diameter
LC	thermochromic liquid crystal
LIGA	lithographie, galvanofornung, und abformung
MS	mass spectrometry
μ -TAS	micro-total analysis system
NMR	nuclear magnetic resonance spectroscopy
OD	outer diameter
ORD	optical rotatory dispersion
PCR	polymerase chain reaction
PDMS	poly(dimethylsiloxane)
PEEK	poly(etheretherketone)
PET	poly(ethyleneterephthalate)
PFA	perfluoroalkoxy
PMMA	poly(methylmethacrylate)
PMT	photomultiplier tube
PTFE	poly(tetrafluoroethylene)
SEM	scanning electron microscopy
UV	ultra violet

Preface

The current interest in DNA amplification, separation, and characterisation has been at the forefront of academic and industrial research into micro-Total Analysis Systems. [1][2] By combination of sample handling and analysis into a single unit, these devices offer a high level of automation, ideal for high throughput screening in chemistry and biochemistry. Furthermore, a number of advantages are associated with miniaturisation, including increased efficiency, low reagent consumption, low unit cost and disposability. From this initial impetus, microfluidics is rising to the forefront of modern science in the area of chemical and biological analysis. However, in addition to analytical applications, chemical synthesis systems are also emerging as an important application of microfluidics.

The use of microfluidic reactor technology to combat reactive chemical hazards is a topic of increasing interest in current research.[2] The phrase ‘microfluidic reactor’ herein has been used to denote reaction systems with feature sizes most conveniently measured in microns and total volumes in the nano to microlitre range. Microfluidic reactors are expected to have a large number of advantages over traditional synthetic methods on both a bench scale as well as in full-scale industrial synthesis. High surface to volume ratios and short mixing distances culminate in extremely efficient heat dissipation and mass transfer. Due to the small scale of the reactors and the minute instantaneous reaction volumes involved in the continuous flow syntheses, manipulation of hazardous (e.g. toxic, pyrophoric, explosive or unstable) compounds allows chemical reactions to be undertaken with increased safety. Moreover, accurate manipulation of reagent concentration, temperature and reaction times are possible by operating within laminar, continuous-flow regimes. This reaction control is achievable due to the large surface area to volume ratios encountered on the microscale that allow ultra-fast heating and cooling of reactants. In the typically low Reynolds number environments encountered on microfluidic devices, precise characterisation of heat and mass transfer may also allow reactions to be performed under more uniform conditions and in many instances lead to improved reaction selectivities.[3]

The scale-up of processes from the laboratory to the production plant is an intricate procedure where precautions against loss of selectivity and increase in reactive hazards must be taken. In contrast to traditional production scaling, increased throughput in microfluidic systems can be achieved by operation of a number of reactors in parallel to provide a simulated large scale flow reactor – ‘scale out’ as opposed to ‘scale up’.[4] In this way, the high capital costs of redesign and pilot plant experiments are eliminated by the mass replication of the microfluidic reactor units used in the developmental stage. With mass production techniques for microfabrication in metals, glass and plastics already well established, significant timesavings could be attained during transfer from the laboratory to commercial production. This approach may be especially advantageous to the pharmaceutical and fine chemical industries (with annual productions of less than a few tonnes per year) which may benefit from a modular, highly flexible facility with on-demand production capabilities.

1.

INTRODUCTION

This chapter provides a theoretical background of reaction miniaturisation. The concept of mixing, mass and heat transfer are discussed and examples of mixing methods are provided. The theory of miniaturisation is followed by a practical demonstration of its use in microfluidic chemistry in the form of a concise literature review. The work reviewed herein focuses on specific examples of reaction miniaturisation which show a distinct advantage of utilizing microfluidic reactors over traditional bulk scale flasks and reaction vessels. This chapter will detail factors that must be accounted for in the design process, specifically detailing those pertinent to microfluidic mixers.

1.1. Mass transfer

When performing a chemical reaction the first step is the bringing together of the reagents to intimate contact. The execution of this fundamental process has a great effect on the efficiency of a reaction and therefore requires detailed examination when designing new reaction environments. The transport of a component from one place to another along a concentration gradient by molecular diffusion and turbulent convection is known as mass transfer. From a chemical engineering point of view, the transfer of mass through an interface between various media or phases of the same medium is extremely important, since chemical reactions are often coupled with the mass transfer efficiency of the reactor in question. The mass transfer coefficient (Equation 1.1) is used to quantify the efficiency of mixing in macrofluidic systems where C_S and C_B are determined experimentally.[5]

Equation 1.1

$$k_c \equiv \frac{N_A}{C_S - C_B}$$

Where k_c is the mass transfer coefficient (ms^{-1}), N_A the molar flux ($\text{molm}^{-2}\text{s}^{-1}$), C_S the concentration at the phase boundary (kgm^{-3}) and C_B the concentration in bulk solution (kgm^{-3}).

Since the analytical prediction of mass and heat transfer for forced laminar convection in a channel is quite complex, (the analogy between heat transfer and mass transfer being first proposed by Reynolds in 1912[6]) analysis of transfer coefficients can be simplified by employing Sherwood's number (Sh). Sherwood showed that for circular channels with fully developed laminar flow, where the channel length is such that the distance for full flow development is insignificant, the transfer coefficient is linked to the diffusivity of the solute and the characteristic diffusion dimension. Thus by the application of the Sherwood number, the mass transfer coefficient (k_c) can be estimated from the channel geometry (Equation 1.2).[7]

Equation 1.2

$$Sh = \frac{k_c d_h}{D}$$

Where Sh is the Sherwood number (dimensionless), k_c the mass transfer coefficient ($\text{kg s}^{-1} \text{m}^{-2}$), d_h the hydraulic diameter (m) and D the diffusion coefficient ($\text{m}^2 \text{s}^{-1}$).

Due to the nature of microfabrication techniques, microfluidic channels are seldom of circular cross section. In place of the circular diameter, the hydraulic diameter (Equation 1.3) is used as the characteristic length for each of the following equations.[8]

Equation 1.3

$$d_h \equiv \frac{4A_c}{P}$$

Where d_h is the hydraulic diameter (m), A_c the cross sectional area (m^2) and P the wetted perimeter (m).

The contribution of convection and diffusion in mass transfer can be characterised by the dimensionless Peclet number (Equation 1.4).[9] A ratio of convective to diffusive mixing, the Peclet number represents the relative importance of the two regimes in mass transfer. Where $Pe \gg 1$ indicates convection as the dominant regime responsible for mass transfer, whilst $Pe \ll 1$ indicates that diffusion is dominant. Due to the nature of microfluidic devices, Peclet numbers are characteristically small (1-100).[10]

Equation 1.4

$$Pe = \frac{ux}{D}$$

Where u is the average linear velocity (ms^{-1}), x the diffusion dimension (m) and D the diffusion coefficient ($\text{m}^2 \text{s}^{-1}$).

1.1.1. Turbulence

On the macroscale, bulk solutions of reagents are brought into intimate contact homogeneously by turbulent or eddy flow. In turbulent flow, fluid elements of varying sizes called eddies, travel randomly creating a mixture that is highly segregated with a finely dispersed structure. The eddies are a product of vortices or areas of circulatory motion in the fluid created by the inertial forces of flowing liquids (Figure 1.1).

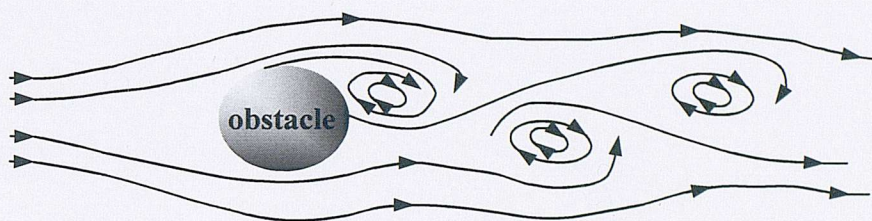


Figure 1.1 Vortices generated by flow around an obstacle, $Re \approx 100$. [11]

The mixture that is produced, although finely dispersed, is still heterogeneous at the molecular level. Mixing of these fine fluid elements is then achieved by the random motion of the component molecules of the solutions or molecular diffusion. The degree of turbulence in a system depends heavily on its scale and the density and viscosity of the fluid. In large systems such as fluidic jet streams, where turbulence is high, different fluid elements can move great distances and therefore mixing is much faster than mixing by molecular diffusion alone.

1.1.2. Diffusion

Diffusion is a slow process and can be described as the flux of particles down a concentration gradient, the proportionality of which is described in Fick's First Law of diffusion (Equation 1.5). [12]

Equation 1.5
$$F = -D \frac{\partial c}{\partial x}$$

Where F is the rate of transfer per unit area of section ($\text{kgm}^{-2}\text{s}^{-1}$), c the concentration of diffusing substance (kgm^{-3}), x the space coordinate measured normal to the section (m).

From this fundamental equation, Fick's Second Law (Equation 1.6), describing the distribution of concentration as a function of time and position, can be calculated.[12]

Equation 1.6

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}$$

Where c is the concentration (kgm^{-3}) and t the time (s).

In microfluidic reactors, fluidic streams occur along the axis of the channel. These streams are differentiated from one another by dissolved solute, concentration, or simply by their location. It is between these streams that mixing must occur to perform a reaction, extraction or dilution. The dimension along which diffusion occurs between streams, x , is critical in the design of a diffusion based microfluidic device. The diffusion dimension determines the extent of interdiffusion between streams with time and thus is intrinsically linked to the other primary parameters, such as channel length, width, and flow rate. As with conventional batch reactors, the length of time that the reagents spend in contact in a continual flow system is known as the residence time. Unlike batch reactors, a continual flow system has a finite residence time, determined by the length of the reactor and flow rate of the system.[13] To allow diffusion to proceed sufficiently for a reaction to occur in microreactors, these primary parameters must be adjusted such that mixing is considered to be complete within the mean residence time of the devices (Equation 1.7).

Equation 1.7

$$t = \frac{x^2}{2D}$$

Where t is the average diffusion time (s), x the diffusion dimension (m) and D the diffusion coefficient (m^2s^{-1}).

Since the diffusion coefficient in Equation 1.7 is fixed by the reaction system under examination, the mixing time is determined by the diffusion distance, x . In conventional mixing vessels, devices such as stirrers are employed to induce turbulence. Stirring increases the number of eddies which in turn shortens the distance for molecular diffusion. By increasing the efficiency of mixing, the likelihood and speed of reaction is also increased. As will be demonstrated in section 1.1.3.1, microfluidic devices are unable to generate turbulence. For microfluidic devices to perform efficiently as reaction vessels, alternative methods for achieving rapid mixing must be examined.

1.1.2.1. Diffusion coefficient

As with the diffusion dimension, the diffusion coefficient is extremely important in determining the mixing time in diffusion-based mixing systems. The rate at which a molecule diffuses is dependent on parameters such as size or molecular mass, the viscosity of the local environment and the system temperature. Due to the diffusion coefficient's dependence on the physical parameters of the system, diffusion coefficients are usually determined experimentally. However, the accurate determination of diffusion coefficients is very difficult. Small variations in environment such as temperature or slight mechanical vibration can significantly effect the rate of mixing compared to molecular diffusion alone. Consequently, a wide variety of methods for the determination of diffusion coefficients are available.[14] A commonly used method employs a sintered glass diaphragm separating two solutions of different compositions. Each solution is stirred to maintain a uniform concentration in the two compartments and to preserve a steady state condition. This method does not provide an absolute diffusion coefficient, but has to be calibrated against a system whose mutual diffusion coefficient is known. In layer analysis methods, a concentrated solution is introduced beneath a more dilute solution which is contained in a vessel whose diameter is small compared to its height. Diffusion continues until concentration changes have occurred throughout the column, which is then separated into equal portions for analysis. In the above set-up, before extensive diffusion can take place, the initial diffusion boundary is sharp. The

concentration change can then be followed by measuring changes in the refractive index or absorption spectrum as a function of distance and time. In the absence of experimental data, the diffusion coefficient (D) can be estimated using the Stokes-Einstein equation (Equation 1.8), which allows the calculation of diffusion coefficients from viscosity measurements and vice versa.[15]

Equation 1.8
$$D = \frac{kT}{6\pi r_0 \eta_B}$$

Where k is the Boltzmann constant (JK^{-1}), T the absolute temperature (K), η_B the liquid viscosity ($\text{kgm}^{-1}\text{s}^{-1}$) and r_0 the molecular radius (m).

Equation 1.8 assumes that the molecule in question is spherical, that it is not small in comparison to the solvent molecules and that the speed of the molecule is inversely proportional to the viscous drag. These relationships have been detailed by Hobbie[16] and are illustrated in Figure 1.2.

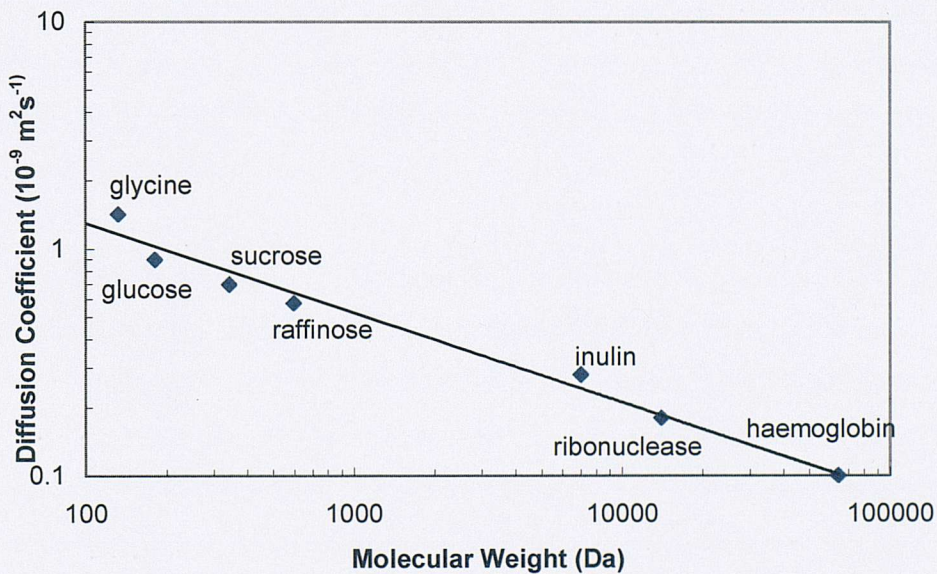


Figure 1.2 The relationship between molecular weight and diffusion coefficient illustrated by a series of biologically important compounds.[16]

As previously demonstrated, in macrofluidic systems, the application of the mass transfer coefficient is most useful in the assessment of mixing efficiency. In a diffusion controlled mixing environment however (see section 1.1.3.1), the rate of transfer is solely dependent on the rate of diffusion. Thus the extent of mixing, known as the Fourier number (τ), can be estimated from the geometry of the system, the diffusion coefficient and the residence time (Equation 1.9).[17]

Equation 1.9
$$\tau = \frac{t_r D}{x^2}$$

Where τ is the Fourier number, t_r the residence time (s), D the diffusion coefficient (m^2s^{-1}) and x the diffusion distance (m), where a Fourier number of 0.1 indicates good mixing and a value of 1 equates to complete mixing.

As well as assessment of the efficiency of a reaction vessel in terms of its mass transfer capabilities, the efficiency of a mixer can be evaluated by determination of the quality of the final mixture obtained. The mixedness or homogeneity of a solution is measured by how uniform the final solution is at the molecular level. Consequently, as the level of mixedness increases, the inhomogeneities become increasingly more difficult to measure.

As described in section 1.1.3, the mechanisms related to the efficient mixing of solutions in laminar flow conditions are derived from decreasing the individual striation thickness until the rate of diffusion is significant. This continuity for mixing in laminar systems allows the measure of mixedness to be evaluated by the scale and intensity of the segregated striations.[18] The scale of segregation refers to the size of the unmixed regions, assuming no diffusion has acted to enlarge them, and the intensity of segregation refers to the concentration variations that occur in the streams as mixing increases. However, despite the clear definition of these parameters, their experimental determination is extremely difficult. As the streams become increasingly small and the mixture approaches homogeneity, the sample volume and sensitivity of the detector must

become increasingly small and sensitive respectively. Several methods to address these difficulties and to determine mixing efficiency have been reported.[18] These methods include employing solutions of differing conductivity, colour and temperature as well as fast reactions where product yields and distribution are examined.

1.1.3. Mixing on the microscale

1.1.3.1. Laminar flow

Fluid flow in the absence of turbulence is described as laminar. In this case viscous forces outweigh inertial forces and eddies are no longer present. Laminar fluid flow can be represented as concurrently flowing parallel layers or streams with little or no mixing between streams. Since the same viscous forces that restrain the fluid streams in laminae also dampen out irregularities and streamlines, laminar flow is not disturbed by obstacles in its path and instead maintains a smooth path (Figure 1.3).

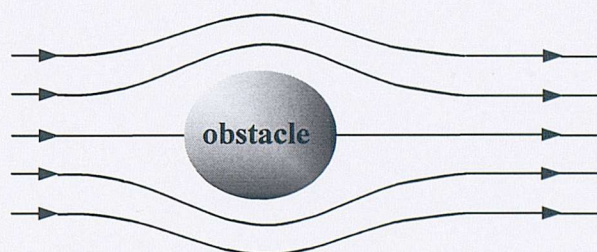


Figure 1.3 At low Re (<0.5), laminar flow dampens stream irregularities and the fluid elements are undisturbed by an obstacle in its path.

With the removal of the highly segregated structure created by eddies, mixing must be achieved by molecular diffusion alone. From Equation 1.7 it can be seen that the rate of diffusion is proportional to the square of the diffusion dimension (Figure 1.4).[17]

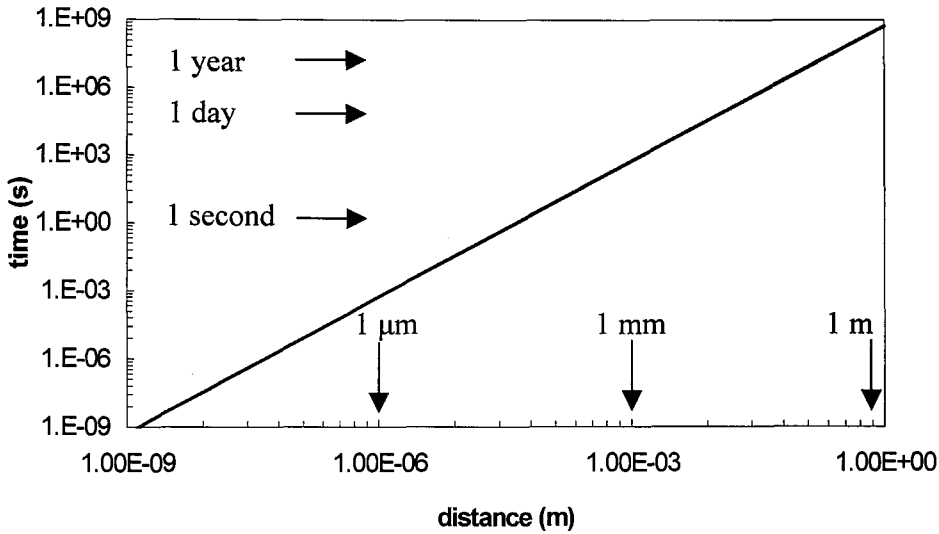


Figure 1.4 The relationship between time and distance travelled for a molecule with a diffusion coefficient of $1 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ in water at room temperature.

The boundaries between the regimes of turbulent and laminar flow are not precisely defined. They are simply terms to define the two extremes of flow behaviour. The progress of turbulent flow to laminar flow via a transitional flow regime can be delineated by use of Reynolds number (Re), a unitless description of the ratio of inertial to viscous forces (Equation 1.10). At Reynolds numbers greater than 2000 inertial forces dominate and turbulent flow can be expected. At Reynolds numbers less than 1, viscous forces will dominate and the flow will be laminar.[19]

Equation 1.10

$$Re = \frac{\rho(4(A/P))u}{\mu}$$

Where ρ is the density of the fluid (kgm^{-3}), u the average linear velocity (ms^{-1}), A the cross sectional area of the channel (m^2), P the wetted perimeter (m) and μ the absolute viscosity ($\text{kgm}^{-1}\text{s}^{-1}$).

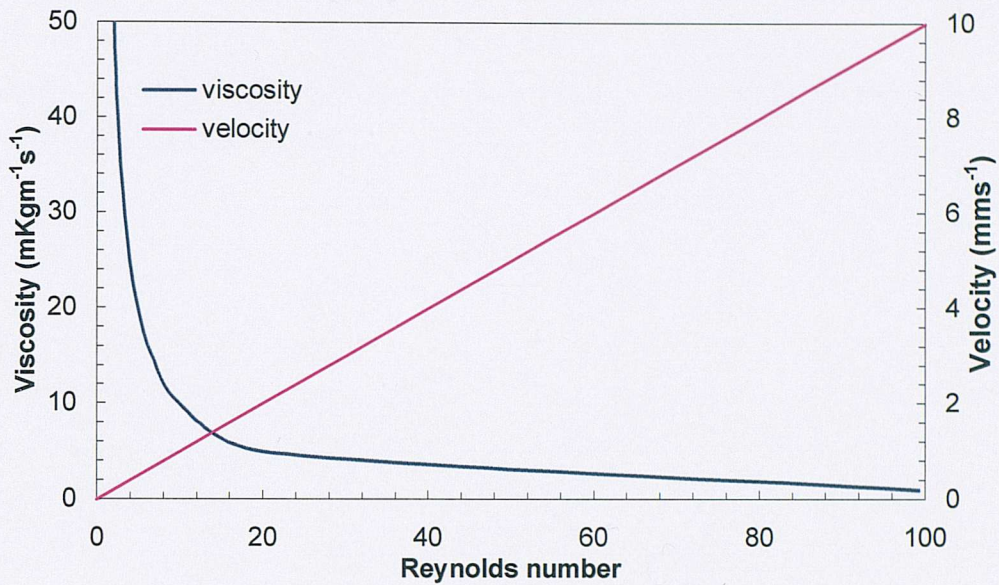


Figure 1.5 The effect of viscosity and velocity on Reynolds number for a pipe 1 m long and a diameter of 10 mm.

As illustrated in Figure 1.5, the Reynolds number shows a linear dependence on fluid velocity and an exponential dependence on fluid viscosity. As the vessel dimension and flow rate are reduced, or fluid viscosity increased, mixing occurs by diffusion only. However, as the rate of diffusion also scales with the square of the vessel dimension, mixing on a small scale by diffusion only can be extremely fast. For example, although a small molecule such as glycerol (MW 180) would take 500 seconds to diffuse just 1mm in water at room temperature, diffusion of the same glycerol molecule across a channel of 10 microns occurs in just 50 milliseconds.

The diffusion of a solute between an area of high concentration and an area of low concentration is driven by the concentration gradient between the two areas. Therefore diffusion will occur until the concentration gradient gradually decreases and a uniform concentration is reached. From Fick's law (Equation 1.5), the concentration of a solute at time t , at a certain distance x from its originating position can be determined. In laminar flow regimes where turbulence is absent, alternative methods of achieving rapid mixing must be

examined for efficient microreactors to be successfully fabricated. As already illustrated in Figure 1.4, diffusion alone can afford rapid mixing over very short distances. Moreover, additional flow phenomena exist that may be manipulated to achieve rapid and efficient mixing unobtainable in macroscale systems.

1.1.3.2. Shear flow

In pressure driven or hydrodynamic flow systems, the flow is often described as parabolic. Parabolic flow arises from an inequality in driving and friction forces across the cross section of a pipe. In the middle of a pipe, the fluid experiences very little friction and is therefore able to flow unimpeded. However, close to the walls of a pipe or channel, friction occurs between the channel wall and the fluid. This friction acts to retard the fluid streams in those regions close to the channel walls, which in turn restricts neighbouring fluid streams. This creates a variance in velocity over the cross section of the channel and the fluid streams travel past each other at different rates. The shear force acting between the streams as they flow past one another distorts the fluid elements leading to increased interfacial areas and a reduction in striation thickness and ultimately increases mixing rates.[18] Therefore, by altering channel dimensions and controlling the surface roughness of the channel walls, a degree of control over the extent of shear and therefore mixing is possible. The velocity profile for a simple shear flow system in a circular tube is illustrated in Figure 1.6, where r represents the channel radius u the average linear velocity and $u_{\max}$ the maximum linear velocity. The average linear velocity can be written as Equation 1.11.[20]

Equation 1.11

$$u = \frac{\Delta P}{8\eta L} r^2$$

Where u is the average linear velocity (ms^{-1}), ΔP the pressure gradient (Nm^2), η the viscosity ($\text{kgm}^{-1}\text{s}^{-1}$), L the length (m) and r the radius (m).

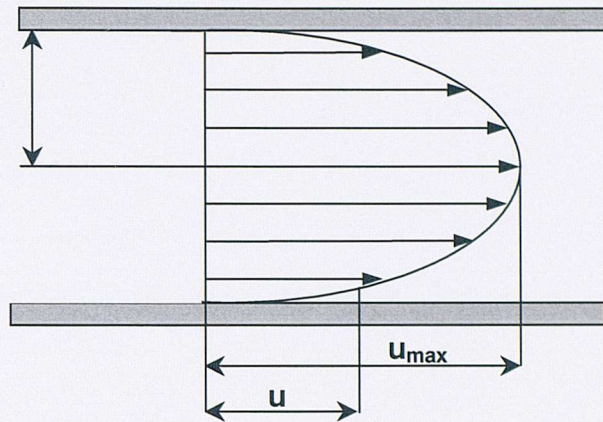


Figure 1.6 A parabolic velocity profile in a simple shear flow system.[20]

1.1.3.3. Extensional flow

As a stream is forced into a channel of reduced dimensions, the fluid stream decreases in width. Assuming the fluid is incompressible, to maintain a constant volume the length of the stream must be extended. This increase in length is known as extensional flow and is illustrated in Figure 1.7.

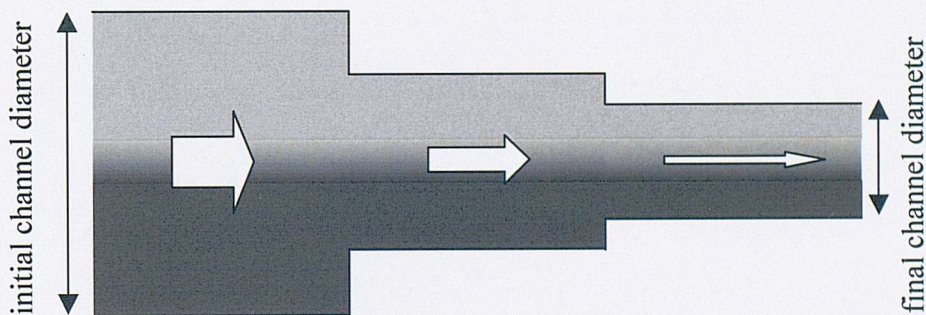


Figure 1.7 Reduction of channel width leads to a narrowing and extension of fluid elements represented by the flow direction arrows.[18]

Although the stream is deformed, it is not rearranged. A stream composed of a set number of concurrently running laminae continues to have the same arrangement upon entering a narrower channel but the lamina thickness is reduced. This influence over laminae thickness allows direct control over diffusion distances and thus mixing times.

1.1.3.4. Distributive mixing

As discussed in the previous section, influence over mixing on the microscale may be gained by altering the diffusion distance by simple modification of single channel geometry. However, the number of dimensions in which the two solutions may mix is not affected by changes in channel dimensions. To perform mixing in multiple dimensions, several channels must be incorporated to physically split and redistribute the two parent streams. By splitting and redistribution of the parent streams into multiple smaller elements, a highly segregated mixture with a finely dispersed structure can be created artificially (Figure 1.8). By employing so called distributive mixing, it has been shown that by splitting two streams into n smaller parallel elements, the rate of mixing is increased in proportion to n^2 times faster than two unsplit streams.[21]

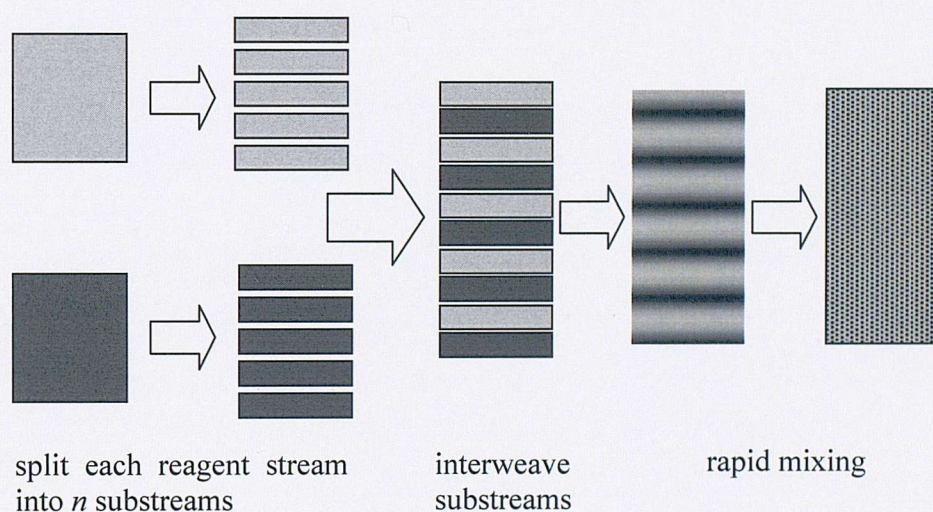


Figure 1.8 Solutions A and B are split into n substreams, subsequent interweaving leads to rapid mixing and diffusion to produce a homogenous mixture of A and B.

1.1.4. Mixing and chemical reaction

The effect of mixing on the extent of a chemical reaction and its product distribution is an extremely important area in chemical reactor design and

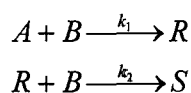
analysis. It is generally recognized that first order irreversible reactions are not affected by local turbulent mixing, but by the residence time of the system and the conversion of such systems can therefore be easily calculated.[22] However, in the case of fast reactions where two or more reagents are initially present in separate streams, reaction rarely occurs throughout the whole volume uniformly. As the mixture is initially highly segregated with a heterogeneous dispersed structure the reaction is stifled until micromixing occurs. In this situation, the rate of reaction is no longer related to the rate constant but instead is limited to the rate of diffusion.[22] In the case of fast reactions where a single reaction product is produced, the yield can be regarded as a direct measure of the degree of mixing in the reaction vessel. In the case of fast reactions with two or more products, the product distribution has similar connotations. In this way the product distribution can be manipulated by increasing or decreasing the amount of segregation or the efficiency of mixing within the reactor.

The relationship between the rate of a reaction and the rate of mixing in a reaction falls into one of three main categories. These are the *chemical* regime, the *diffusional* regime and the mixed *chemical/diffusional* regime. The interaction between chemical reactions and fluid dynamics is described by the Damkoehler Number which represents the contribution of these two systems.[23] The Damkoehler can be represented in either of two forms, N_{DaI} and N_{DaII} where N_{DaI} is a ratio of the chemical reaction rate to the bulk mass flow rate and N_{DaII} is the ratio of the chemical reaction rate to the molecular diffusion rate.

In the *chemical regime*, the mixing time is fast in comparison to the reaction rate and is represented by a Damkoehler number that is close to zero. In this situation the chemical reaction is slow compared to the mass transfer and the reaction system can be regarded as an unreactive fluid. That is to say that mixing is complete before significant amounts of products are present and once mixed, the reaction proceeds uniformly throughout the reaction volume. In the case of a competitive-consecutive reaction as detailed in Scheme 1.1, the smallest amount of secondary products are formed. In the *diffusion regime*, ($N_{Da} \approx \infty$), the

chemical reaction is very fast and thus the reaction is limited by the rate of mixing. In this condition the area available for reaction is reduced from the volume of the entire reaction vessel at $N_{Da} \approx 0$ to a plane between reacting streams at which each reagent has zero concentration. When a chemical reaction occurs, microscopic concentration gradients are introduced increasing segregation, which in turn leads to a decreased level of mixedness. The reaction rate is then independent of the rate constant and the formation of secondary products in this situation is the greatest for the reaction system detailed in Scheme 1.1. In a mixed *chemical/diffusional* regime ($N_{Da} = 1$), the greatest interaction between chemical reactions and fluid dynamics occurs and the product distribution depends on both the chemical factors, (e.g. rate constants) and also on the diffusional factors (e.g. efficiency of mixing). In this situation the amount of secondary product formed lies between the extremes of the *chemical* and *diffusional* regimes.

Some practical examples where micromixing has a profound effect on the product are fast consecutive competing reactions, polymerisations and precipitations. In competitive-consecutive reactions, a reaction occurs between two reagents (A and B) to produce a product (R) at rate k_1 . The product in this situation is then able to react with one or more of the initial starting reagents in a competitive manner at rate k_2 to form a by-product (S), leading to a reduction in the initial product (Scheme 1.1).



Scheme 1.1 The competitive reaction of the product R with the initial reagent B leads to the formation of by-product S and a reduction in the overall yield of R

In the case of fast competitive-consecutive reactions k_1 is much larger than k_2 and k_2 is still large (Scheme 1.1). If a small amount of B is mixed with a large excess of A, R is instantaneously formed. If the fluid is completely mixed, R and B are dispersed throughout the reaction volume, B is consumed by the excess of

A and R is preserved. If a small amount of B is mixed with a large excess of A and the fluid is a segregated mixture, R stays at the fluid interface of B. In the presence of B, R is immediately converted to S, the concentration of which then may be used as a measurement of segregation or unmixedness in the vessel.

Precipitation is a special case of crystallisation. Initially, the mixing of two reagents gives rise to the supersaturation of a solution, which in turn initiates crystal nucleation and growth. The rate of crystal nucleation and growth is determined by the efficiency of mixing of the reagents, which in turn determines both the ultimate size and the size distribution of the crystals. Control over size and polydispersity of crystals is of considerable scientific and commercial interest in the field of electronic materials and devices. Nanocrystalline semiconductors have tuneable optical and electronic properties determined by the physical size and shape of the crystallites.[24] Since these properties are ultimately determined by the macro and micromixing effects, their production a key area of interest in the area of microreactor technology.[25-28]

1.1.5. Backmixing

In conventional tubular reactors the ideal plug flow model is preferred. In this situation rapid mixing by turbulence results in an averaging of solute concentration across the width of the channel and generates a flat concentration profile. Most closely approximated under conditions of fully developed turbulence ($Re \approx 10,000$), any deviation from the plug flow model can have a detrimental effect on reactor performance. If the bulk velocity decreases, the flow becomes more laminar and the concentration profile becomes increasingly parabolic. In this situation back mixing may occur. As with other flow systems, reagent streams take an independent forward path through the reactor but the overall forward flow is countered by a large backward component (Figure 1.9).[29]

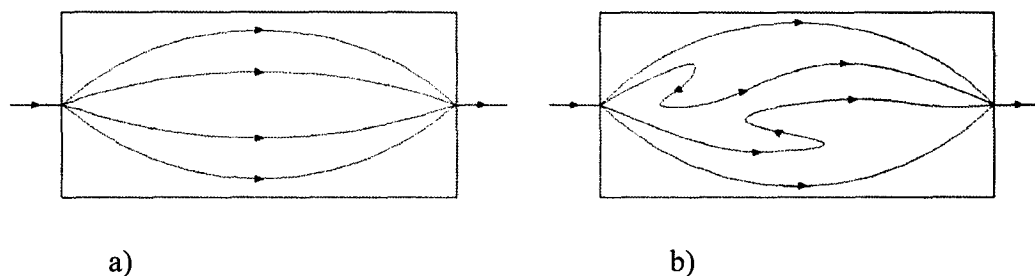


Figure 1.9 a) Forward flow with no back mixing b) bulk forward flow is countered by a large backward component leading to back mixing.[29]

As microfluidic reactors operate almost exclusively in the laminar regime, it is naturally assumed that backmixing is high in these systems. However, as the laminar nature of the flow is attributed to the scale of the channels and not the bulk velocity, the linear forward velocity remains high compared to the rate of diffusion and backmixing is low. The amount of back mixing in a tubular reactor can be estimated by the Bodenstein number, π , (Equation 1.12).

Equation 1.12
$$\pi = \frac{ul}{D}$$

Where π is the Bodenstein number (unitless), u the average linear velocity (ms^{-1}), l the reactor length (m) and D the axial mass diffusivity (m^2s^{-1}).

π can be regarded as the average linear flow rate divided by the average rate of longitudinal diffusion. For a small molecule in water in a microfluidic channel $50\text{ }\mu\text{m}$ wide and $50\text{ }\mu\text{m}$ deep operating under continuous flow at $10\text{ }\mu\text{Lmin}^{-1}$, a typical Bodenstein number is in the range of 1×10^6 . This indicates extremely low backmixing despite operating under laminar flow conditions.

1.1.5.1. Taylor-Aris dispersion theory

As previously described in section 1.1.3.2, in microfluidic systems under hydrodynamic flow conditions, molecules in the centre of a channel move twice the average bulk velocity ($u_{\max}$), whilst molecules near the walls move much slower due to friction effects. This differential velocity results in dispersion that increases linearly with time. However, if the channel is sufficiently narrow such that the time for the particles to diffuse across the radius (r) of the tube is less than $r/u_{\max}$, no molecules are primarily trapped in either the fast or slow streamlines. In this way, counter intuitively, diffusion acts to keep the plug together and microreactors are able to operate in laminar flow conditions with no axial dispersion.. This behaviour is described by the Taylor-Aris dispersion coefficient (Equation 1.13).[30-32]

Equation 1.13

$$\kappa = \frac{u_{\text{sup}}^2 d^2}{192D} + D$$

Where κ the Taylor-Aris dispersion coefficient (m^2s^{-1}), u_{sup} the superficial velocity (ms^{-1}), d the inner capillary diameter (m) and D the diffusion coefficient (m^2s^{-1}).

1.1.6. Heat Transfer

Heat exchange in a microfluidic device is most typically between a wall and a fluid where the flow is forced along the wall by external means, e.g. pressure or gravity. This form of heat transfer is known as forced convection heat exchange. If a fluid enters a tube at a uniform temperature that is less than the surface temperature, convective heat transfer occurs and a thermal boundary layer begins to develop. If the surface conditions are fixed, i.e. assuming a constant heat flux, a fully developed thermal condition is reached. The main constituent of the resistance to heat transfer is concentrated in the thin boundary layer immediately adjacent to the wall surface. Once heat has penetrated this boundary layer, it is easily carried away and distributed by the surrounding material. Since heat exchange by convection is promoted by the transfer between the bulk solution

and the boundary layer, heat transfer in turbulent flow systems are traditionally considered higher than in laminar flow systems. However, as previously shown by Fick's law (Equation 1.5), when the overall distance is reduced, transfer in laminar systems can be very fast.

If a fluid flowing inside a pipe is being heated, its temperature will increase in the axial direction at a rate that is proportional to the heat transfer rate (q). The rate of heat transfer from the fluid to the walls of the device is characterised by the channels convective heat transfer coefficient (h), a quotient of the thermal conductivity and film thickness (Equation 1.14).

Equation 1.14
$$h \equiv \frac{q}{T_s - T_\infty}$$

Where h is the convective heat transfer coefficient ($\text{Wm}^{-2}\text{K}^{-1}$), q the heat flux ($\text{Km}^{-2}\text{s}^{-1}$), T_s the surface temperature (K) and T_b the temperature of the bulk fluid (K).

Since the mechanisms of heat and mass transfer are inexorably linked, the analysis of heat transfer coefficients can be simplified in a similar way by employing Nusselt's number (Nu).[33] Essentially, the Nusselt number is a dimensionless heat transfer coefficient and can be expressed as a ratio of the characteristic length and the thermal boundary layer thickness, assuming a constant surface flux (Equation 1.15). Thus by the application of Nusselt's number, the heat transfer coefficient can be estimated from the geometry of the channel (Table 1.1).

Equation 1.15
$$Nu \equiv \frac{h \cdot d_h}{k}$$

Where Nu is the Nusselt number (unitless), h the convective heat transfer coefficient ($\text{Wm}^{-2}\text{K}^{-1}$), d_h the hydraulic diameter (m) and k the thermal conductivity ($\text{Wm}^{-1}\text{K}^{-1}$).

Cross Section	Aspect ratio	Uniform q_s	Uniform T_s
	-	4.46	3.66
	1.0	3.61	2.98
	2.0	4.12	3.39
	3.0	4.79	3.96
	4.0	5.33	4.44

Table 1.1 Nusselt numbers for uniform heat flow (q_s) and uniform surface temperature (T_s) for a range of channel aspect ratios (width/height) under laminar flow conditions.[33]

Since boundary layer thickness is inversely proportional to Reynolds number and the heat transfer coefficient is inversely proportional to the boundary layer thickness, the rate of heat transfer should be low at low Reynolds numbers. As microfluidic devices operate almost exclusively in the laminar regime, it is naturally assumed that microfluidic devices are unsuitable for efficient heat transfer. However, despite thick boundary layers, heat transfer in microfluidic devices can be efficient. As the characteristic diffusion dimension is extremely small, rapid molecular diffusion occurs between the bulk solution and the boundary layer. In this way efficient heat transfer can be achieved by thermal convection.

Where the *convective* heat transfer coefficient is a description of the heat transfer from the bulk solution across the boundary layer, the *conductive* heat transfer coefficient is concerned the dissipation of heat from the inner wall of the channel to the outer regions of the channel wall. The efficiency of heat transfer by conduction is governed by the size and thermal conductivity of the vessel walls. The combination of the convective and conductive heat transfer coefficients allows the overall heat transfer coefficient (U) to be determined (Equation 1.16).

Equation 1.16

$$\frac{1}{U} = \frac{1}{h} + \frac{x}{k} + \frac{1}{h_x}$$

Where U is the overall heat transfer coefficient ($\text{Wm}^{-2}\text{K}^{-1}$), h is the convective heat transfer coefficient ($\text{Wm}^{-2}\text{K}^{-1}$), x the reactor wall thickness (m) h_x the convective heat transfer coefficient of the external environment ($\text{Wm}^{-2}\text{K}^{-1}$), and k the thermal conductivity ($\text{Wm}^{-1}\text{K}^{-1}$).

For a typical glass microreactor with channel dimensions of 100 μm , values of U ($750 \text{ Wm}^{-2}\text{K}^{-1}$) are of the same order as traditional bulk-scale (50 L) reaction vessels ($500 \text{ Wm}^{-2}\text{K}^{-1}$). However, when the significantly larger surface area to volume ratio of the microreactor is taken into account, it is clear that the heat transfer coefficient for a glass microreactor is sufficient for near isothermal operation. Fabrication of microreactors from more thermally conductive materials commonly employed for microfabrication, such as silicon, allow values of U as high as $54,000 \text{ Wm}^{-2}\text{K}^{-1}$, far higher values of U than normally encountered in conventional chemical reactor technology.[3]

1.1.7. Summary

In the previous sections, the theory of fast mixing in microfluidic environments has been introduced. The efficiency of mixing between reagents has been demonstrated to affect the rate of simple reactions, and in the case of competitive-consecutive reactions, the product distribution and yield. The aim of the remainder of this thesis is to investigate the fabrication of microfluidic reactor devices and their application to demonstrate that miniaturisation may improve both the speed and quality of chemical reactions.

1.2. Literature survey of microfluidic reactor research

In the following section a short summary of microfluidic reactor research will be presented. Excellent reviews on the application of microfluidic reactors and chemical synthesis have previously been presented by Erhfeld, Jensen and Wootton.[4, 34, 35] This review will focus in particular on the advantages of

microfluidic reactors in combination with hazardous reactions, new reaction methodology and heterogeneous catalysis.

1.2.1. Hazardous reactions

The application of microfluidic devices to the synthesis of extremely toxic substances and the development of novel synthetic methods is a subject of ongoing interest. The synthesis of particularly hazardous and toxic chemicals is dictated by the economies of large-scale production. The processing of these compounds often requires specialised cylinder storage, environmental enclosures, pipelines and emergency contamination or breach facilities. With the storage, shipping and transport of these compounds comes serious safety and environmental concerns. This is demonstrated succinctly by the Bhopal disaster in which water leaking into a methyl isocyanate storage tank led to the deaths of nearly 4000 people. In order to produce a derivative for safe transportation, extra synthetic processes must be undertaken, which in turn implies additional cost and experimental complexity. By moving manufacture to small plants at the site of consumption, production may be performed on-demand, removing transport issues and reducing chemical storage to nearly zero. For this purpose a number of microfluidic reactors may be employed in parallel to produce a flexible chemical production plant. Since the volume of each reactor is small, the failure of a single reactor would lead to the releases of negligible amounts of chemicals leading to increased safety.

1.2.1.1. Toxic Gases

The Andrussov process for the production of hydrogen cyanide from methane, ammonia and air is a well-known industrial synthesis. To prove the concept of mobile production for small amounts of highly hazardous gases, a microfluidic reactor was fabricated by Hessel *et al.* at the Institut für Mikrotechnik Mainz (IMM) (Figure 1.10).[36] Capable of operating at temperatures exceeding 1000°C, the reactor was used as a tool for the study of process regimes and reaction performance.

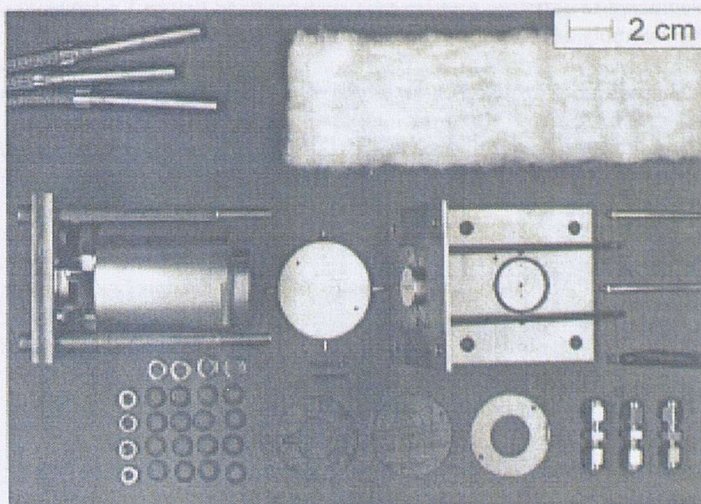


Figure 1.10 The components and supplementary equipment for the mobile production of small amounts of highly hazardous gases by IMM.[36]

The microfluidic reactor was designed to operate using similar process parameters as in the traditional technical scale process and incorporated a platinum catalyst and a micro heat exchanger. In comparison to metal and ceramic monolith laboratory reactors, yields were increased and comparable to those achieved in the industrial synthesis. However, although yields were adequate, the selectivity of the microfluidic reactor was decreased over that of the industrial process. The authors assigned this to the microfluidic reactor design, and it is believed that the selectivity and scope of the reaction could be improved by implementation of a new microfluidic reactor design.

Phosgene (COCl_2) is used throughout the chemical industry for the production of aniline dyes, polycarbonate resins, pesticides, isocyanates, polyurethane as well as pharmaceuticals. Manufactured from gaseous chlorine and carbon monoxide over activated carbon, phosgene is a toxic gas that targets the pulmonary system. The manufacture of phosgene on a microfluidic reactor has previously been investigated by Ajmera *et al.*[37] who employ a silicon packed bed microfluidic reactor incorporating a thick passivating layer of silicon dioxide by heating in a wet oxidation furnace (Figure 1.11).

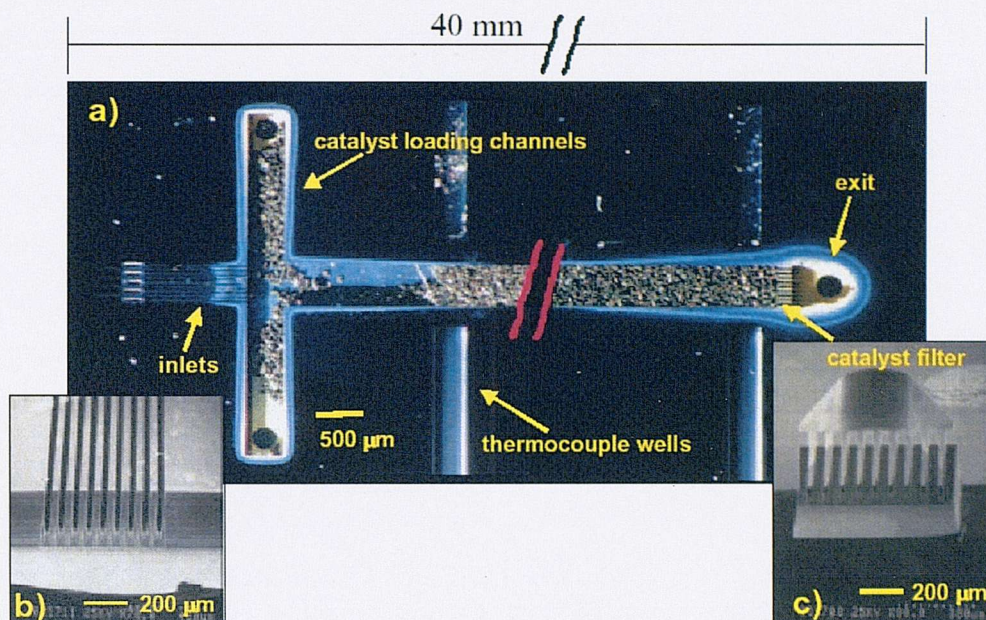


Figure 1.11 Microfabricated silicon packed-bed reactor for production of phosgene. a) Top-view of reactor partially loaded with 60 μm activated carbon particles. b) SEM of the 25 μm wide interleaved inlets. c) SEM of the catalyst filter structure.[37]

Due to the high thermal conductivity of silicon and the large surface to area volume of the chip, precise temperature control is possible. In addition, the formation of hotspots is reduced leading to a reduction in by-product formation. The effect of temperature on conversion was also examined, operating at 200°C, a productivity of 3.5 kg/yr (0.40 g/h) of phosgene per single channel reactor is projected.

The ability for microfluidic reactors to suppress the generation of hotspots not only reduces by-product formation, but also prevents thermal runaway of a reaction. In open vessels, a runaway reaction can result in the boiling over of the reaction mass or rapid gas generation. In addition, elevated temperatures may initiate secondary reactions, which may lead to yet more hazardous runaways or decompositions, either of which can lead to catastrophic failure of the reaction vessel. By reducing or removing the risk of thermal runaway, reactors can be run under more aggressive conditions or in regimes that would otherwise be

considered unsafe in larger scale systems. In this way, microfluidic reactors open the way to entirely new chemical syntheses and methodologies. The direct fluorination of aromatics on a microfluidic reactor with fluorine gas is an attractive route to fluorinated compounds in comparison to, for example the multi-step Schiemann process. Despite direct fluorination being the target of much research, it remains an unsolved technical problem.[38-41] Early experiments with conventional reactors led to formation of unspecified compounds which resulted in explosion due to excessive heat release. Jahnisch *et al.*[39] compared the direct fluorination of toluene in two different microfluidic reactor designs (Figure 1.12) and compared the space-time yield and conversion to a conventional laboratory bubble column (Figure 1.13). The first microfluidic reactor, a miniaturised falling film reactor, generates a falling film (tens of microns thick), flowing over a platelet comprising of many micro channels by means of gravity. The second, a microbubble column, consists of a mixing area, to bring together the gas and liquid flows, and a microcolumn for mixing and reaction.

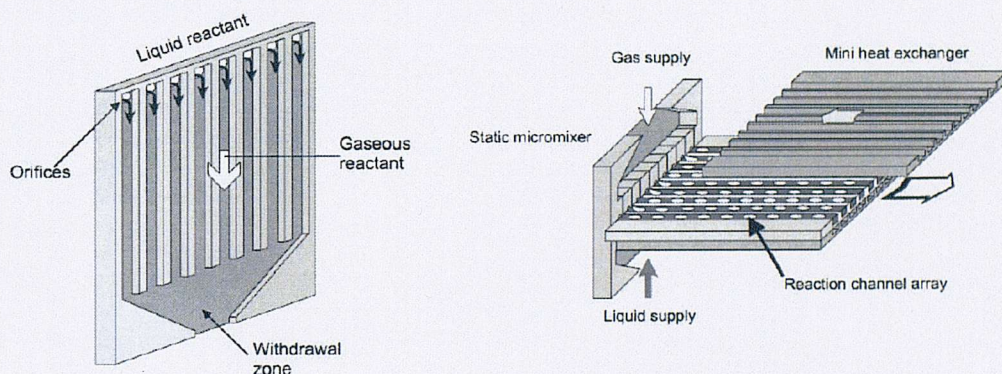


Figure 1.12 Schematic of a) a falling film microfluidic reactor b) microbubble column used for the direct fluorination of toluene.[39]

When expressed as a space-time yield and selectivity conversion diagram, the performance of the microfluidic reactors was far superior to the laboratory process, and of the same order as the technical Schiemann process.

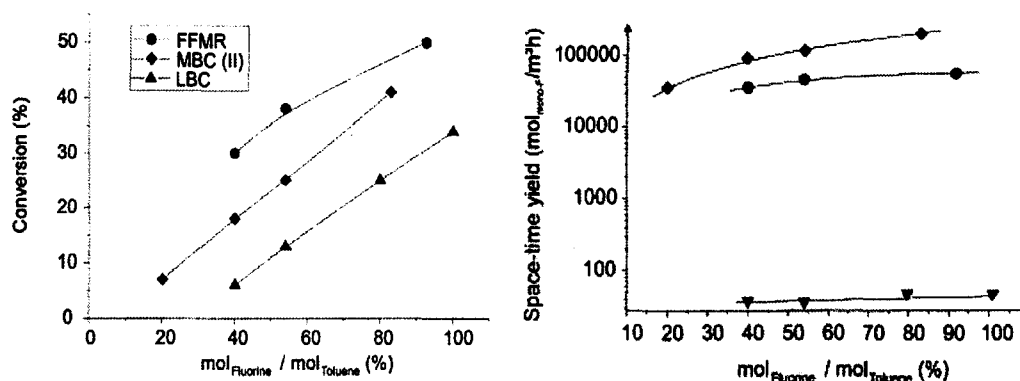


Figure 1.13 Comparison of a) conversion and b) space-time yield for a falling film microreactor (FFMR) and a microbubble column (MBC) to a laboratory bubble column (LBC).[39].

1.2.1.2. Oxidations

The partial oxidation of gases is a particularly interesting area of ongoing research. Although partial oxidations are in general, only mildly exothermic reactions, failure to remove hotspots or to properly quench the partial oxidation can lead to full oxidation. Full oxidations are extremely exothermic processes resulting in rapid decomposition and explosion. Typically, partial oxidations have short reaction times and are constrained by heat transfer. This makes them ideal targets for the application of microfluidic reactors and are an example of one of their earliest applications. The partial oxidations of many compounds have been investigated. These include methane[42], ammonia[43-47], ethane,[48] isoprene[49] and 1-butene[50].

More recently the partial oxidation of ethylene has been investigated by Kestenbaum *et al.*[51]. The partial oxidation of ethylene yields an extremely useful product; ethylene oxide, employed in the production of ethylene glycol. The world trade in ethylene oxide alone being worth approximately \$10 billion a year. Lithographie, galvanofornung, abformtechnik (LIGA) is a specialised form of microfabrication whereby metal devices, ideal for operation at high temperatures and pressures, can be produced (see Chapter 2). Laser-LIGA has

been utilised by Kestenbaum *et al.* for the fabrication of a microfluidic reactor for partial oxidations. A stack of 14 silver foils is used as a catalyst, whilst gold is deposited electrochemically to prevent any catalytic activity of the mixing unit (Figure 1.14).

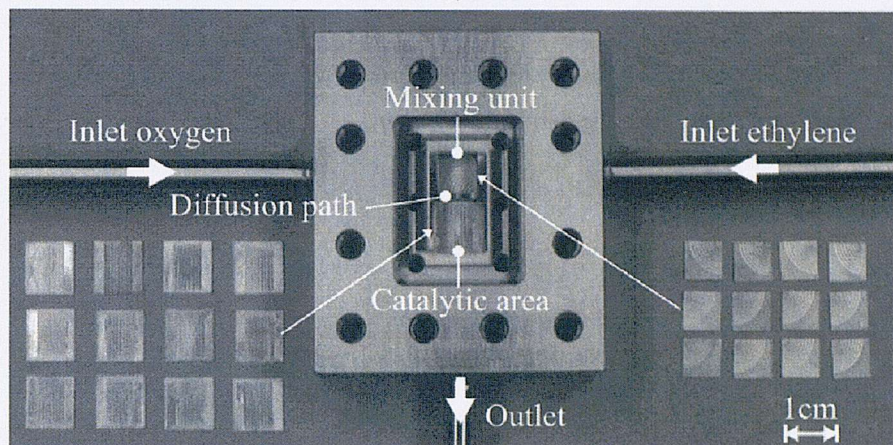


Figure 1.14 Overview picture of the stainless steel housing containing a mixing unit on the top and silver foils realized by Laser-LIGA for partial oxidation of ethylene. The reactor consists of nine parallel shallow channels of 500 μm width, 50 μm height, and 9.5 mm length.[51]

Previously it has been reported that using higher oxygen concentrations has a positive influence over both selectivity and conversion.[52] The use of lean gas compositions (oxygen rich reaction mixtures), is of great importance as operation in these explosive regimes could lead to significant process improvement. Oxygen rich mixtures, which are in the middle of the explosive regime in a bulk format, may be performed in complete safety in a microfluidic reactor.

The ability of a microfluidic reactor to suppress explosive reactions is not only due to efficient heat dissipation, but also due to the efficient quenching of free radicals. Radical species are crucial as chain carriers for explosive gas phase reactions and are quenched by binding to free surfaces. Since microfluidic reactors have inherently high surface areas in comparison to their volume, they are extremely efficient at reaction quenching. This has been demonstrated by experimental and theoretical investigations of hydrogen oxidation in a high-

temperature catalytic microfluidic reactor (Figure 1.15) by Göktz Vesper[53] and co-workers at the Max Planck Institut für Kohlenforschung.[54]

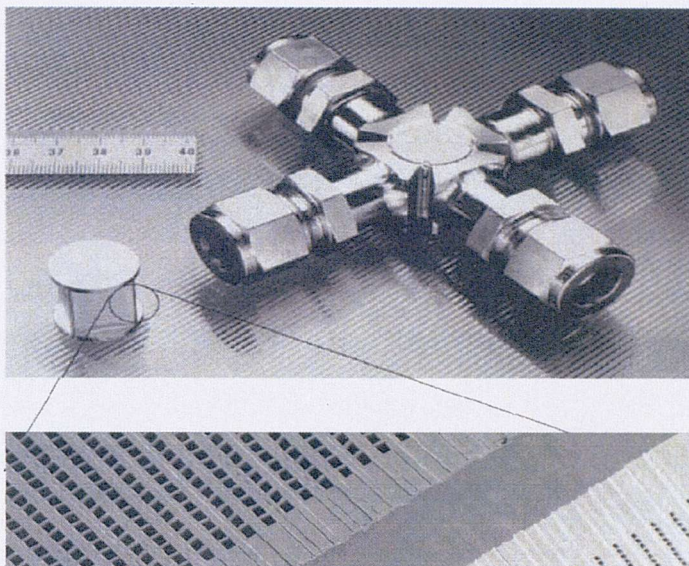


Figure 1.15 A high temperature catalytic reactor for the oxidation of hydrogen with and without pipe fittings. The lower picture is an SEM micrograph of the corner of the catalytic structure incorporating many microchannels.[54]

The oxidation of hydrogen to water is explosively exothermic over a broad range of hydrogen in oxygen from 4% to 94%. Ordinarily, large, expensive equipment is required to deal with a reaction of this type and the safety issues of such an experiment cannot be overstated. While the hypothesis of radical quenching by free surface binding has been examined in the specific case of hydrogen oxidation, similar quenching of chain carriers can be expected for other reactions operating within explosive regimes.

1.2.1.3. Nitrations

Nitrations are one of the fundamental reactions of aromatic chemistry, employed in the synthesis of a wide range of compounds in every area of chemistry including pharmaceuticals, agrochemicals, pigments and polymers. Nitrations are highly exothermic with explosive potential. This combined with the

corrosivity of the nitration agent, makes the nitration process potentially very hazardous. In addition, rapid warming, combined with short reaction times, leads to large numbers of secondary decomposition reactions resulting in low selectivity and yields. For these reasons nitration reactions within microfluidic reactors have been investigated by several groups.[55-57] Loebbecke *et al.*[58] employed microstructured devices fabricated from silicon, titanium or glass for the nitration of simple hydrocarbons, fused aromatic systems and heterocyclic compounds under pressure driven flow. Selective single or multiple nitrations were achieved in both liquid and gas phases using nitric pentoxide (N_2O_5) at ambient temperature. This is in contrast to the low temperatures (-80 to -20 °C) normally required for these syntheses. In contrast, at The University of Hull, mobilisation of the reacting solutions is achieved by the use of an electric field termed electroosmotic flow (EOF).[59] The nitration of benzene and toluene was achieved by employing an EOF enabling microemulsion of surfactant (sodium dodecyl sulfate) and co-surfactant, butan-1-ol (Figure 1.16).[56]

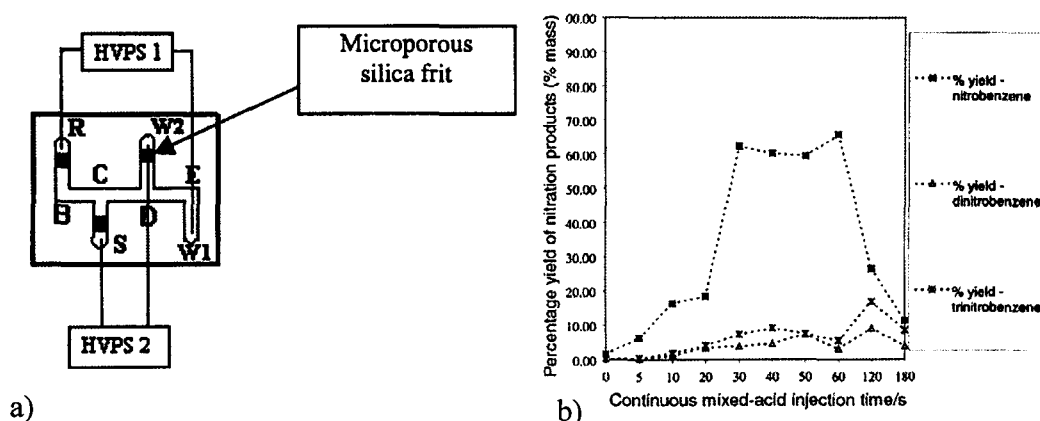


Figure 1.16 a) A microfluidic reactor for the EOF mobilisation of solutions. b) A comparison of yield of single and multiple nitration products of benzene with injection time.[56]

Studies were performed on the effect of flow and feed rates of the reacting species. In this case, the reaction is perturbed by the presence of water shifting the equilibrium away from the formation of the nitronium species and an interaction between the EOF enabling surfactants and the strong acids present.

1.2.2. Electro-osmotic flow

The use of EOF for fluid motivation has a number of advantages over traditional pressure driven flow systems in microfluidic reactors and has been used for a number of synthetic processes.[60-64] These advantages include ease of miniaturisation of electronic components and the ability to move materials selectively through complicated microfluidic networks, without the need for additional valves and control components. Although the miniaturisation of pumping mechanisms has been examined by several groups,[65-67] the proposed solutions are currently inflexible and not widely available. Despite the advantages of EOF, its application to chemical synthesis is severely restricted, EOF being dependent on particle size and charge as well as the surface properties of the microfluidic device. The difficulties of EOF motivation are well illustrated by Haswell *et al.* who determined nitrite concentration by means of an azo coupling.[68] The problems encountered during this reaction included poor reagent motivation, joule heating and bubble formation. To overcome these problems, it was necessary to pre-mix the first reagents prior to introduction to the microfluidic reactor. In addition, the concentration of the reagents had to be maintained within a narrow range and the pH of the system carefully maintained by reagent solution buffering.

1.2.3. Heterogeneous catalysis

The catalysis of a reaction generally refers to the reduction in the energy required to initiate the reaction. A reduction in the activation energy of a reaction corresponds to lower reaction temperatures and pressures. In addition to savings in plant and process costs, new processes previously unavailable are made possible and thus heterogeneous catalysis is one of the most valuable processes in industrial chemistry.

Unlike generic micromixing devices, which are broadly applicable to any homogenous reaction mixture (including homogeneous catalysis),[69] heterogeneous catalysis microfluidic reactors are by their nature highly specific

devices. The combination of micromixers and heterogeneous catalysis has been approached in several different ways. These include parallel microchannel reactors, chip-based single microchannel reactors, and monolithic reactors acting as multichannel reactors.[70] By permanent incorporation of a solid phase catalyst, miniaturized catalytic devices can be used to conveniently screen for new reactions. Catalytic materials can be incorporated into microfluidic devices by a wide range of techniques including electrophoretic deposition (EPD),[71] chemical vapor deposition (CVD),[54] anodic oxidation of aluminium,[72, 73] nanoparticle washcoating[74] and sol-gel processes.[75] The permanent incorporation of a catalyst for the screening of catalytic activity is well illustrated by Greenway *et al.*[60] through the permanent fixation of palladium into the microchip using a fused microporous silica bed. A modified Suzuki cross-coupling of an aryl halide and an organo boron compound was performed and contamination of the product due to palladium leaching was reduced.

The design, development and synthesis of a new catalyst is often a complex and time consuming process. Catalyst consumption during testing may be minimised by the incorporation of the catalyst into microfluidic reactor devices. In addition, the reaction at a catalyst's surface is often extremely sensitive to temperature. Due to the excellent thermal characteristics of microfluidic reactors (see section 1.1.6), catalytic microfluidic reactors may be operated under isothermal conditions, prolonging catalyst life and increasing reaction efficiency. The ability of microfluidic reactors to offer large surface area to volume ratios and work under isothermal conditions is discussed by Wörz *et al.* in the synthesis of formaldehyde from methanol by oxidative dehydrogenation.[76] Industrially important, the formation of formaldehyde from methanol suffers from a dramatic loss in selectivity from 95% to 40% due to adiabatic temperature increases when the size of the production vessel is increased. A silver microfluidic reactor was fabricated with a heat transfer coefficient an order of magnitude higher than a pan reactor, leading to a reduction in the hotspot temperature from 160°C to 0°C (Figure 1.17).

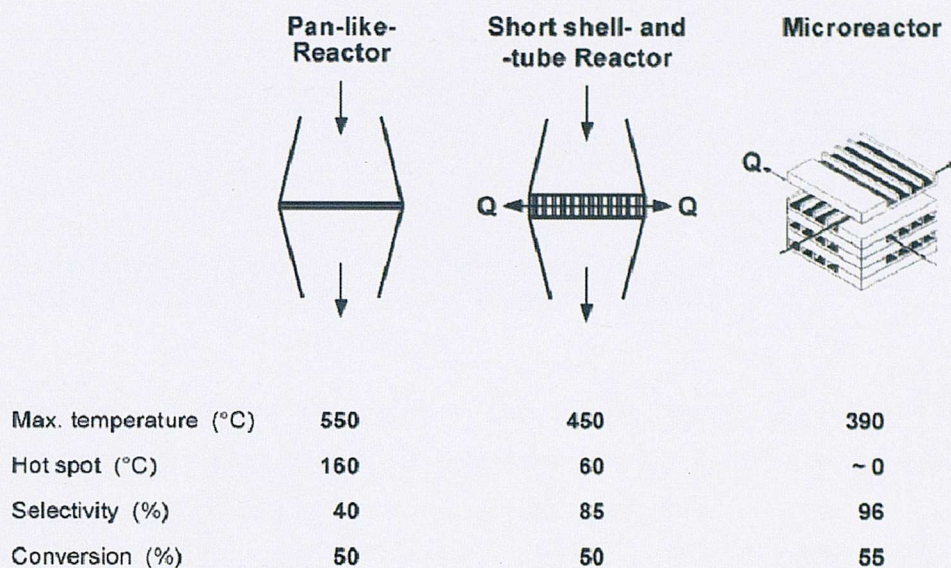


Figure 1.17 Comparison of scales of a microfluidic reactor to laboratory scale reaction vessels, Q represents the flow of coolant through the reaction vessel.[76]

The effect of a decreased hotspot temperature was evident by an improvement in both selectivity and conversion, despite a reduced residence time.

For the efficient scale-up of a chemical reaction, information on the kinetics of a reaction gained in the laboratory is invaluable. For kinetic data to be reliable it is important that the catalyst and the support on which it is deposited are as close as possible in nature to those used in the final process. By employing microfluidic reactors for catalyst screening, information may be gained about factors such as temperature and pressure gradients and mass transport limitations. By employing a cross flow design, researchers at the Massachusetts Institute of Technology (MIT) incorporated a catalyst similar to those used in industrial applications, reversibly into a silicon microfluidic reactor (Figure 1.18). Catalyst retainers act as a filter to hold the catalyst in place whilst 64 channels provide an even flow across the packed catalyst bed.[77, 78] The incorporated catalyst was then used to study the oxidation of carbon monoxide and the kinetic data gained were compared to literature values.

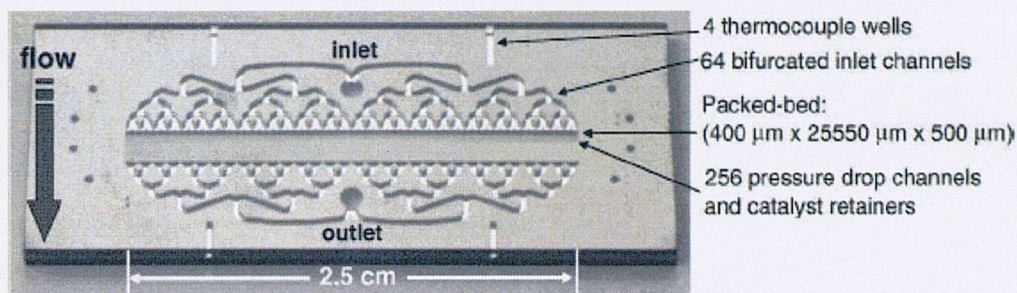


Figure 1.18 A silicon microfluidic reactor chip for catalyst screening. Catalyst retainers act as a filter to hold the catalyst in place. 64 channels provide even pressure flow across the packed bed.[78]

By utilising multiple microfluidic reactors in parallel, a rapid combinatorial style screening can be employed either with respect to the catalyst, (to determine catalyst activity), or with respect to the reaction. Reaction parallelisation has been described by Ouyang and Besser[79] who employ an iron catalyst surface in a silicon/Pyrex® chip for the Fischer-Tropsch synthesis of methane (Figure 1.19).

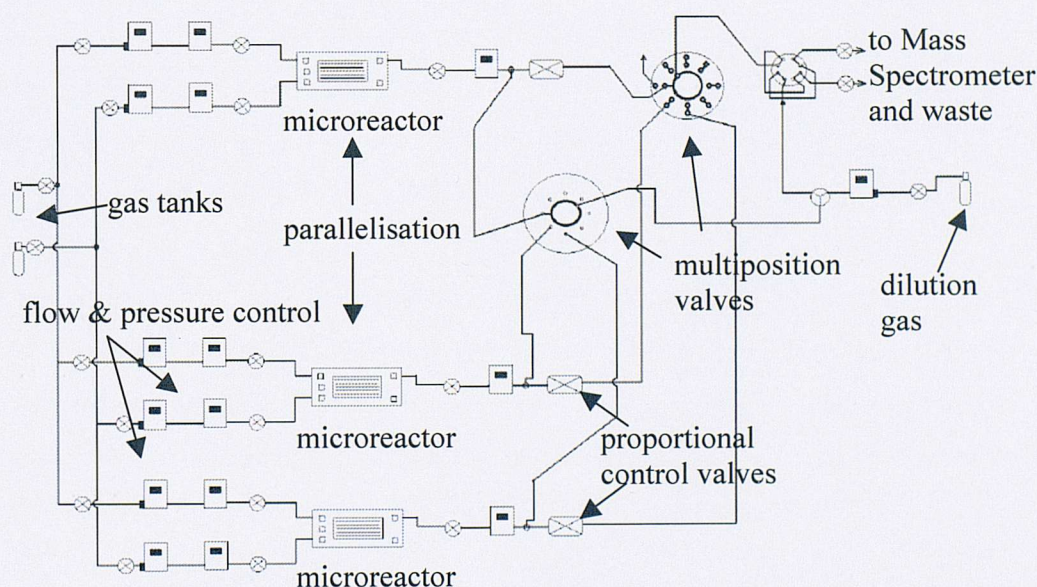


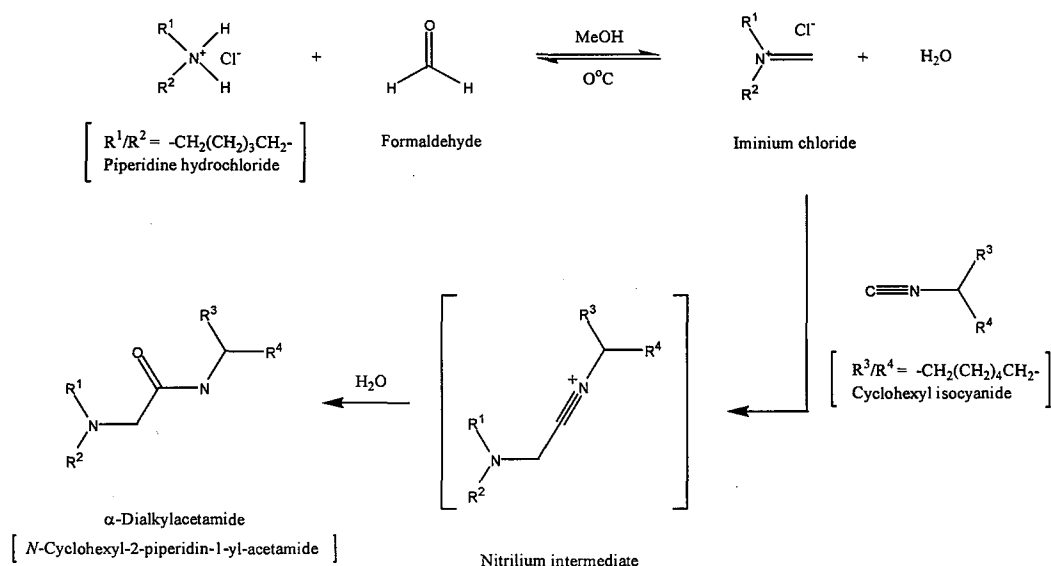
Figure 1.19 A schematic for a complete microfluidic reactor-based parallel-catalyst analysis system incorporating gas flow controllers, pressure gauges and proportioning solenoid valves, resistive heaters and thermocouples.[79]

Multi-position valves are used to connect an array of microfluidic reactors with a mass spectrometer. Gas flow controllers, pressure gauges/proportioning solenoid valves, resistive heaters and thermocouples are incorporated for flow, pressure and temperature control respectively. In addition to the advantages for catalyst and target screening gained through miniaturisation, microfluidic reactors can lead to significant yield improvements over bulk scale methodology. This was demonstrated by Wilson *et al.*[80] who studied the dehydration of alcohols using sulfated zirconia. A glass/poly(dimethylsiloxane) (PDMS) chip incorporating an immobilised activated catalyst and electrodes for heating and electroosmotic flow motivation yielded ethene and hexene from ethanol and hexanol with conversion efficiencies approaching 100 %, a significant improvement over the 30 % yields often observed in large-scale reactors.

1.2.4. Compound libraries

The demand for faster and more economical synthesis for the rapid generation of compound libraries has led to increased interest in the areas of parallel synthesis and high throughput screening (HTS). However, issues surrounding the dependence of HTS on heterogeneous methodologies such as resin bead and ‘tea-bag’ technologies are numerous. These include efficient attachment and detachment of the solid support, filtration, separation and washing steps that are time consuming and difficult to automate.

The generation of a solution phase compound library has been examined by Mitchell *et al.* utilising a distributive microfluidic reactor.[81, 82] Using serially switched and parallel reaction modes, a series of Ugi multicomponent reactions was performed (Scheme 1.2). In addition to compound library generation, high throughput screening is also examined by the combination of continuous flow synthesis with electrospray ionisation mass spectrometry (ESI-MS). In addition to the obvious benefits of on-line monitoring for real-time reaction optimisation, mechanistic and kinetic information was gained by the first experimental observation of the proposed nitrilium intermediate.



Scheme 1.2 The Ugi multicomponent reaction (MCR) of piperidine hydrochloride, formaldehyde and cyclohexylisocyanide.

The generation of a small array of compounds in a systematic fashion on microfluidic reactors has also been examined by Warrington *et al.*[83] The synthesis of a series of cycloadduct products was performed on a glass microfluidic device under hydrodynamic flow conditions and analysed off-line without reaction quenching by liquid chromatography-mass spectrometry (LC-MS). Four different cyclo adducts were prepared in addition to a three member array produced in a single chip. Yields obtained were comparable to those on the bulk scale with high reproducibility and low cross contamination.

Similarly, Bellefon *et al.* demonstrate the use of microfluidic reactors for the HTS of an air sensitive organometallic catalysts for the asymmetric reduction of acetophenone into (R)- and (S)-phenylethanol.[69] A library of 60 chiral ligands covering 11 structural families was combined with three metal precursors of ruthenium and iridium giving rise to 180 ligand/metal couples. A total number of 3200 tests were performed and analysed resulting in the discovery of a new class of ligand for this reaction with an enantiomeric excess (ee) of 93% (Figure 1.20).

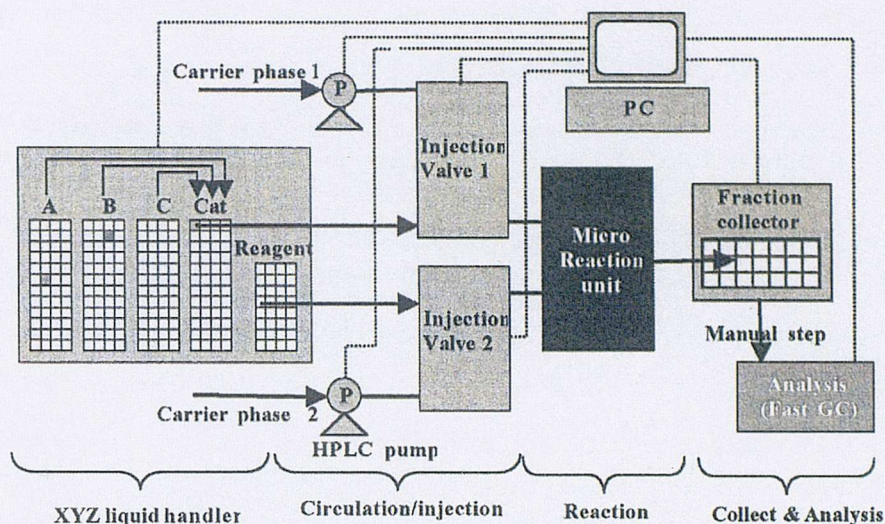


Figure 1.20 Experimental set-up for the HTS of organometallic catalysts for the asymmetric reduction of acetophenone.[69]

1.2.5. Photochemistry

Photochemical reactions are extremely useful for a number of transformations including simple cleavage, decomposition and rearrangements. The move to larger production methods however, suffers from the inherent difficulties of scale-up. Industrial scale photochemical processes are performed in large tubular reactors. To provide adequate illumination, high intensity light sources are required, which can lead to unwanted sample heating and therefore may require additional refrigeration. The benefits of photochemical reaction miniaturisation has been addressed by several groups.[84-86]

In the industrial generation of singlet oxidation, illumination difficulties are further complicated by achieving sufficient oxygen saturation and the extreme explosive hazard presented by oxygen saturated organic solvents. The generation of singlet oxygen on a microfluidic reactor for the synthesis of ascaridole has been performed by Wootton *et al.*[84] Oxygen saturation of the system was performed on the microfluidic reactor, resulting in a volume of

oxygen saturated solvent measured in nanolitres. Due to the small cross section of the device, effective irradiation of the photosensitiser dye was achieved using a low power light source. This led to reduced sample heating and reduced radical recombination, resulting in efficient transformations of >80% in 5 seconds.

The application of microstructured reactors for the photochemistry has also been performed by Jähnisch *et al.*[85] A falling-film microreactor for gas/liquid reactions was demonstrated by the photochemical chlorination of alkylaromatics (Figure 1.21). Use of the microfluidic reactor led to remarkably higher selectivities over that of a conventional batch reactor and an increase in space time yield of over 300 times. Using data gained from performance of the reaction on the microreactor, a kinetic model for the chlorination and by-product formation was suggested and used to predict product selectivity.

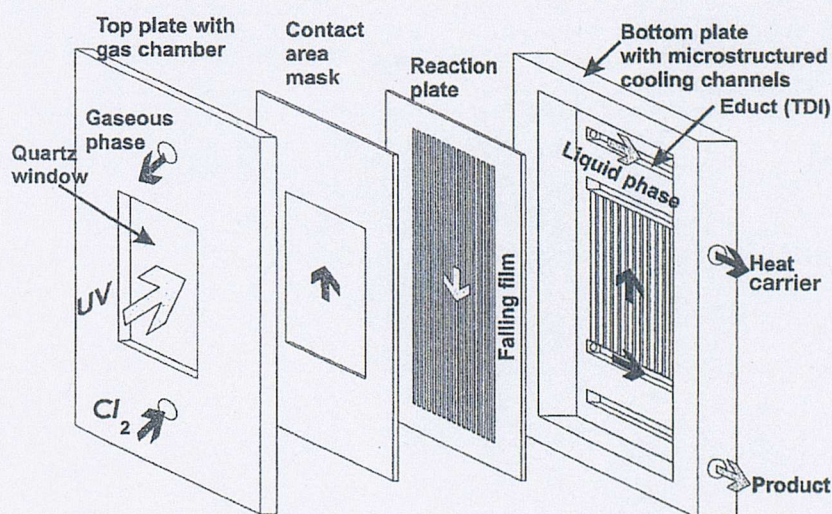


Figure 1.21 A microstructured falling film gas/liquid reactor for the photochlorination of alkylaromatics.[85]

1.2.6. Summary

From the brief description of some current research in the field of microfluidic reactors, it is clear that such devices may have an important role in future

chemical research and development. This is demonstrated by the application of microfluidic reactors to new process regimes, catalyst and reagent screening where increases in reaction selectivity and yield are observed. Whilst some of the presented reactors show great potential for application to organic chemistry, in general each reactor is designed to perform a specific function and is not directly applicable to any other. If this premise is applied to all microfluidic reactors, the cost of each reactor and the timescale of reactor development may work to counter any benefits received by miniaturisation. For research and development of organic chemistry on microfluidic reactors, a system will be required to perform each of the fundamental steps required for synthesis, currently fulfilled by Quickfit® glassware and other laboratory equipment. The user should require no specialist knowledge for microfluidic reactor operation and interfacing must be rapid to implement and consistent in performance. Microfluidic reactor systems should also be flexible over a number of dimensions. For example, the number of serial additions and parallel operations should not be fixed, nor the order in which they are performed or their duration. In addition, the microfluidic reactor system should be amenable to integration with existing technologies such as analytical spectroscopy for *in situ* monitoring, without extensive re-engineering of either system.

2.

DEVICE FABRICATION AND INTERFACING

The efficient design and fabrication of microfluidic devices is key to the successful creation of microfluidic reaction systems. This chapter details the design and evolution of microfluidic devices for chemical synthesis. Fabrication techniques including photolithography, chemical etching, bonding and fluidic interfacing are detailed and their optimisation described. In addition, the crucial operation of sample introduction and removal from microfluidic devices is examined. Subsequently a series of interface prototypes are demonstrated, and their respective advantages and disadvantages discussed in relation to issues such as chemocompatibility, dead volume and cost.

2.1. Device fabrication

As has been demonstrated in Chapter 1, there are many factors that must be taken into consideration when designing a macroscale reaction vessel. This can be evinced by the number of standard texts available on macroscale chemical reactor vessel design and technology.[87] In some respects, the design of microfluidic reactors is simplified, in comparison to macroscale reactor vessels, by the limitations imposed by the microfabrication facilities available. However, microfabrication techniques also impart their own additional design complications as will be demonstrated below. The variety of microfabrication techniques available to the microchemist is vast. Based on technology carried over from silicon chip processing, microfluidic chips can be fabricated from a host of materials including silicon,[88] glass,[89] quartz,[90] diamond,[91] plastics,[92] metals[93] and ceramics.[94] Channel fabrication generally falls into two categories; wet chemical etching and dry etching. Wet etching involves the submersion of the substrate in a bath of aqueous chemical etchant, whereas dry etching employs either a vapour phase etchant or a stream of reactive ions. These etching methods are then differentiated on the basis of whether they act in an isotropic or anisotropic manner.

2.1.1. Wet etching

Wet chemical etching is the simplest technique commonly available for the production of microchannels in glass or silicon. Typically, a design is transferred to the surface of the substrate by photo exposure of a polymer photoresist coating on the substrate surface. When glass is submersed in a buffered solution of hydrofluoric acid, the surface is etched in an isotropic manner to give a near semicircular profile. However, some crystalline materials such as silicon, exhibit anisotropic etching characteristics in certain chemicals. Due to the crystal lattice orientation, anisotropic etching can occur resulting in a pyramidal or a trapezoidal shaped profile instead of a semicircular profile. Significantly cheaper than dry etching techniques, the disadvantage of wet chemical etching is that conventional isotropic processes generally yield sloping walls and relatively low

aspect ratio features. As each channel must be at least twice as wide as its depth, fluidic networks in isotropically etched devices are generally restricted to channels that are both wide and shallow.

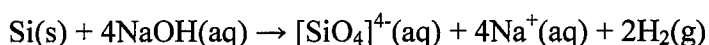
2.1.2. Dry etching

There are three main dry etching techniques; sputtering,[95, 96] vapour phase etching[97] and reactive ion etching (RIE).[98, 99] In RIE, an O_2 plasma is used to generate $F^\bullet$ radicals from SF_6 . These $F^\bullet$ radicals chemically etch the silicon by forming volatile SiF_4 . Oxygen dissociates to $O^\bullet$ radicals, which combine with the silicon surface to form a passivating SiO_xF_y layer. SF_x^+ ions then remove the SiO_xF_y layer at the bottom of the etched trenches by forming volatile SiO_xF_y . The ability of RIE to fabricate channels of high aspect ratios makes it one of the most commonly used anisotropic etching methods.

2.2. Substrate materials

2.2.1. Silicon

Silicon is a popular substrate material for microfluidic devices, most probably due to its extensive use in microfabrication in the electronics industry and the wide range of etching techniques available. At room temperature, silicon reacts with air to form silicon dioxide. This leaves the surface and channels inert to water and to further oxidation at temperatures up to 900°C. Despite the inertness of silicon to oxidation, it reacts vigorously with some acids, including nitric and acetic acid, and most bases to form silicate anions (Scheme 2.1). These chemical incompatibilities allow silicon only strictly limited roles in microfluidic synthetic chemistry.



Scheme 2.1 Chemical etching of silicon by sodium hydroxide.

2.2.2. Metals

The fabrication of microfluidic reactors from metals has several advantages. Firstly, metals have high thermal conductivities and, as with silicon, three-dimensional channels of varying sizes and aspect ratios can be fabricated. Metal microstructuring can be achieved by a number of methods including laser-assisted wet chemical etching[100] and focused ion beam milling.[101] An alternative method for the production of laminated metal microdevices is by LIGA technology. LIGA derives its name from the German acronym for “lithography, electroplating, and moulding” and is conventionally used for the production of moulding tools for high aspect ratio imprinting in polymers.[102] Firstly, a thick polymer resist is irradiated with laser light or X-rays through a mask to define a two-dimensional structure. Upon development of the irradiated areas, the structure’s depth is defined and the polymer resist coated substrate is electroplated to create a metallic copy of the design. Once fabricated, these metallic foils can be clamped together to create complex designs capable of operating at extreme temperatures and pressures.[93]

2.2.3. Polymeric materials

Plastics and polymers are universally used for the mass production of almost any component imaginable. Frequently chosen for reasons of cost and disposability, plastics offer these same advantages to microfluidic systems. In addition, the wide variety of plastics available makes them suitable for any number of applications from DNA analysis[103] and clinical diagnostics[104] to spectroscopic methods.[105] Fabrication methods fall into two categories, either direct fabrication or replication moulding. Direct fabrication, as with silicon or glass processing, involves removal of material from the surface of the individual substrate. This is achieved, either by etching methods such as RIE, or by techniques such as laser ablation and mechanical milling. In the mass production of plastic products, replication methods such as injection moulding, hot-embossing and compression moulding are the norm. These replication methods employ a master mould or template from which an identical copy can be

fabricated. Although replication methods can suffer from high start-up costs for the precision fabrication of complex master moulds and templates, they also benefit from extremely short cycle times of just a few seconds. Unlike glass or silicon, whose physical characteristics are broadly similar across subtypes, the properties of polymeric materials such as hardness, rigidity and thermal resistance can differ widely from one another. Such is the variation of properties within polymeric materials, it is difficult to succinctly summarise the characteristics of polymers and plastics. Suffice it to say, that due to the sheer number of polymers available, for a specific application there is almost certainly a polymer that is suitable. Thus far in the field of microfluidic devices, structures have been fabricated from a vast range of polymers including; Mylar,[106] poly(dimethylsiloxane) (PDMS),[107] acrylic,[108] polyimide,[109] parylene,[110] Zeonor,[111] poly(ethyleneterephthalate) (PET),[112] poly(methylmethacrylate) (PMMA),[113] poly(tetrafluoroethylene) (PTFE) and fluoroethylenepropylene (FEP).[114, 115]

2.2.4. Glass

Glass has long been favoured as the main constituent of the developmental chemist's toolkit. From reaction vessels to chromatography columns and separating funnels, glass offers chemical inertness that few materials can compete with. Rigid and hard, if not particularly strong, glass also has the advantage that it is optically transparent. In silicon, metal and some polymeric devices, once assembled the performance of the reactor can no longer be observed directly and the efficiency of the reactor can only be established by the examination of the products post-reactor. The visual observation of a reaction system is one of the most basic and important methods of reaction monitoring available. Operating in opaque microfluidic reactors effectively blindfolds the operator and a large amount of potentially useful information is lost. In addition to visual information, the availability of *in situ* monitoring by spectroscopic methods such as IR, UV-Vis and Raman is removed. For these reasons and the applicability of simple fabrication techniques such as wet etching, glass was

chosen as the primary material for the fabrication of microfluidic reactors in this thesis.

2.3. Design patterning

To allow channels to be fabricated using wet etching techniques, a protecting layer or mask must be employed such that only the desired areas of glass are etched (and the remainder left) to create a channel. This is most commonly achieved by photolithography. Photolithography utilises a light sensitive polymer or photoresist to protect the glass from the etching solution. Those areas of photoresist exposed to light energy in the desired pattern will no longer protect the substrate from the etching solution and thus defines a channel pattern. Patterning of photoresist can be performed directly using direct-write lithography (DWL) systems. This employs a Helium-Cadmium laser to define images of a design at high resolution (minimum feature size of 5 μm) on photosensitive substrates. With care, it is possible to produce a number of designs on a single substrate as long as certain fabrication criteria, such as inlet hole spacing and inter-chip distance are met. The cost of such design flexibility and high resolution is lengthy production times with typical exposures taking up to 6 hours.

Once a channel has been defined in the masking material by the DWL method, it can be combined with an unfocused UV source to produce copies. In comparison to the DWL technique, use of a UV light box allows designs to be transferred from a mask to a fresh substrate in a matter of minutes, such that pattern exposure no longer determines the rate of chip production. However, in comparison to the high-resolution raster scan employed by the DWL, an uncollimated UV source exposes the photoresist to photons from a variety of angles. This results in features approximately 10 microns wider than produced by the DWL method. In addition to using masks created in-house by DWL exposure, masks can be purchased commercially. The use of commercially produced, photo-plotted high-resolution (32,000 dpi) polyester film masks,

allows the production of microfluidic devices without loss of quality or resolution, at higher unit cost but with lower capital expenditure. Throughout this thesis, chromium-on-glass masks created by DWL were used as standard, with polyester film masks used when the DWL was unavailable.

2.4. Channel development and etching

To produce channels of uniform depth, roughness and regularity, a range of hydrofluoric acid etching solutions were tested. The concentration of acid, the use of buffer and additional components, such as hydrochloric acid, were altered to provide the best etch. Hydrochloric acid is included to prevent the build up and precipitation of insoluble fluoride salts from being deposited on the surface of the channel. If allowed to precipitate, these salts act to partially mask the channel from the etching solution creating a rough channel of irregular width and depth. The etch rate for a 5% HF solution on green sodalime glass is approximately $0.275 \mu\text{mmin}^{-1}$ (Figure 2.1).

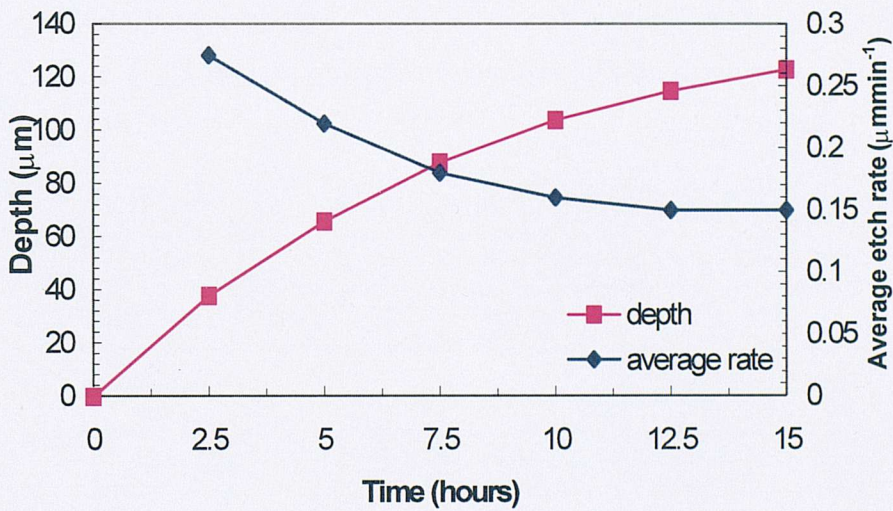


Figure 2.1 Variation in depth and etch rate with time for a 5% 7:1 $\text{NH}_4\text{F}:\text{HF}$, 10% HCl glass etching solution.

However, as the channel becomes deeper, the etch rate begins to decrease. As refreshment of the HF solution at the surface of the channel in an unstirred solution is governed by diffusion, as the channel becomes deeper, the time for diffusion rapidly increases (see Chapter 1). This, in combination with the consumption of the HF, leads to a decrease in the etch rate with time.

2.5. Commercially produced devices

Glass microfluidic devices were also commercially produced for this thesis by Micronit Microfluidics B.V. (Enschede, The Netherlands). The most economical method, powder blasting or Abrasive Jet Machining (AJM), was used for channel fabrication.[116] AJM employs a jet of aluminium oxide particles 3 – 30 microns in diameter for mechanical removal of surface material. The channel is defined by an electroplated copper mask and the jet nozzle is scanned over the target repeatedly to achieve a uniform etched surface. The AJM method has the additional advantage of higher aspect ratios (up to 2.5) than those achieved by wet etching (0.5) (Figure 2.2). However, due to the aggressive nature of the process, the surface roughness of powder blasted channels is very high, typically 2 - 2.5 microns, although this could be smoothed by additional wet etching.

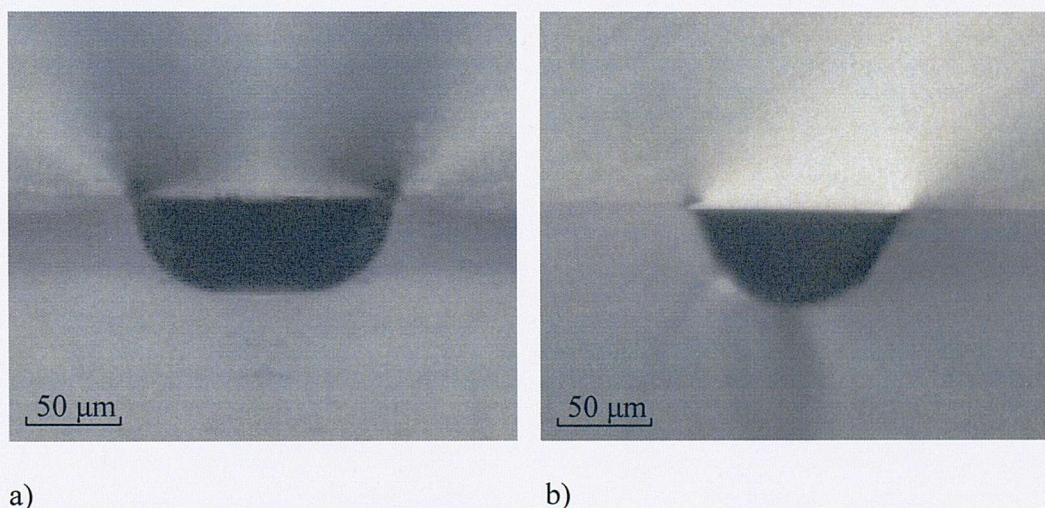


Figure 2.2 Comparison of aspect ratios for glass channels created a) in this laboratory by HF wet etching b) by Micronit Microfluidics B.V. by powder blasting.

2.5.1. Channel profiling

In the fabrication of wet etched channels, the channel depths were routinely determined by comparison to a calibrated microscope graticule. Based on the supposition that etching is isotropic, the depth can be assumed to be half of the observed width less the exposed width. Observations were performed at regular intervals during etching to ensure the correct etch rate was maintained. Knowledge of channel width is important for a range of calculations such as diffusion time and linear velocity which allow characterization of the device for properties such as the Reynolds number, Fourier number and heat transfer coefficient. For precise measurement a Talysurf 10 (Taylor-Hobson, Leicester, UK) profilometer was employed prior to bonding. The depth is determined by dragging a stylus across the chip surface. The deflection of the stylus can then be measured to determine channel depth and width.

2.6. Thermal bonding

Once a microfluidic channel has been defined and etched, to create an enclosed channel it is necessary to fix a plate on top of the etched substrate. This can be achieved in a variety of ways including anodic bonding,[117, 118] thermal or fusion bonding,[119] HF bonding[120] and room temperature bonding with either sodium silicate[121] or UV cured epoxy.[122]

Of these methods, anodic bonding and thermal bonding are most regularly used in the fabrication of glass devices. In microfluidic reactors for chemical synthesis, the bonding method must abide by the same requirements as those specified for the substrate material, i.e. chemically resistant, cheap, easily processed and transparent. Anodic bonding is based on the charge migration process performed between a high sodium (3.5 %) borosilicate glass substrate and a glass, polysilicon coated quartz, or most frequently, silicon substrate. At elevated temperatures (773 K), a high negative potential is applied to the glass (450V), attracting and neutralising the sodium ions at the electrode, creating a space charge at the silicon-glass interface. This results in a strong electrostatic

attraction between the two substrates. Oxygen in the glass, attracted by the positive potential applied to the silicon, causes migration to the silicon surface, where it combines with silicon to form silicon dioxide. This has the advantage over UV cured epoxy that the bond formed isn't subject to attack by solvents or chemicals. The special glasses required however are far more costly in comparison to conventional green soda lime glass.

Thermal bonding, as with anodic bonding, is not subject to chemical attack, with the additional advantage that no specialised glass type is required and is applicable to both glass and quartz.[123] The two glass substrates are fused together at high temperature such that the van der Waals repulsion forces are overcome. To successfully fuse two glass substrates, the surface of each must be scrupulously clean and the surface in a hydrophilic state. This is achieved by washing in organic solvents to remove any organic contaminants prior to prolonged immersion in concentrated sulphuric acid before rinsing with ultrapure water and drying with a filtered stream of nitrogen.

Once clean, the substrates are placed between flat, polished ceramic plates. These plates ensure that the upper and lower surfaces remain flat during the bonding process. Polishing prevents any surface imperfections in the plates being transferred to the glass, and any distortions in the plate morphology generating uneven pressure distribution that may result in uneven or incomplete bonding. To ensure a firm contact between glass layers, metal weights are placed onto the top of the ceramic/glass stack. The placement, as opposed to the quantity, of weight is most essential as uneven pressure distribution not only deforms the glass under the weight but reduces pressure in other areas. By employing multiple ceramic plates stacked vertically, several glass devices can be bonded in one cycle without the need for additional weight.

The bonding method used in this thesis heats the glass rapidly beyond the glass transition point. At this temperature the glass morphology becomes less crystalline. Holding the glass at this temperature anneals the glass, removing any

internal stresses that may lead to bonding imperfections. The glass is then heated further, towards the glass softening point, at which it is held for several hours before cooling back to the glass transition point. Holding at the glass transition point allows the crystal morphology to equilibrate and decreases internal stresses that may lead to fracture during the cooling process. The fused chip is cooled slowly over a period of several hours to prevent cracking through thermal shock. If unsatisfactorily bonded, gaps being indicated by the presence of Newton's rings, the bonding cycle is repeated and temperature and pressure distribution adjusted accordingly.

2.7. Chip interface methods

Many microfluidic systems previously demonstrated (see Chapter 1 for a review) are composed of a number of components such as heating modules, pumps, valves and interconnects. The majority of these components are custom made for the microfluidic system in question. When such a large number of components are required for the system to operate, not only does the potential for component failure increase, but also the assembly time and manufacture costs. This is especially true where machining and manufacture tolerances are extremely high, as is in the case of microfluidic reactor assembly. Where unusually toxic or dangerous reactions are being performed on a microfluidic reactor, a component failure or reactor failure during assembly, although small by industrial scales, may cause some concern.[36]

One of the most basic operations involved in the use of microfluidic reactors, and one of the most challenging, is the introduction and removal of samples from the device, the fidelity of which is of crucial importance. For the advancement of microfluidic reactor technology, the method of interface between the outside world (e.g. capillary tubing, sample reservoirs and pumps) and microfluidic systems is one of foremost importance. Any method employed must be easy to implement, be chemically compatible and structurally robust, at low unit cost while permitting a range of analytical methods as well as channel interrogation

by optical microscopy. Despite the large number of publications on the subject of functional microfluidic devices, the methods of fluid introduction are rarely discussed in detail and the number of articles offering a working broadly applicable solution is low.[124-127] In this section, each of the design requirements of microfluidic to macroworld interfaces will be described, and a variety of design prototypes examined and discussed.

2.7.1. Dead volume

In the majority of microfluidic devices, where total system volumes are typically measured in nanolitres, handling small quantities of sample is a relatively simple task. Where these channels interact with the outside world however, interface dead volumes are often several orders of magnitude greater than the volume of the reactor. A dead volume is described as an area in which a fluid may flow, but due to its size or geometry, the average flow rate drops and at the extremities may approach zero. In applications where sample size is crucial, for example precise analytical methods and synthesis or reaction of expensive or dangerous compounds, dead volumes are a critical issue. Samples caught in these dead volumes may become delayed, leading to precipitation, or entirely lost. In the case of unstable or short-lived intermediates, this may result in decomposition and contamination of the system.

2.7.2. Chemicompatibilty

In organic synthesis, the range of harsh reactive and corrosive systems is vast, and larger than in any other area. To achieve total chemical compatibility is an impossible task, but for any interconnect system to be widely applicable to organic synthesis, its chemicompatibilty is of utmost importance. Most synthetically useful systems involve the use of organic solvents, both chlorinated and non-chlorinated, and operate over a wide pH range. This excludes the use of silicon as discussed in section 2.2, and many polymeric materials often used as gaskets for liquid sealing.

2.7.3. Sealing

For an interface to function it must allow for the introduction of liquids and gases over a variety of flow rates, reliably and without leakage. Typical flow rates range from nanolitres per minute to hundreds of microlitres per minute, which in long or narrow channels may generate pressure drops of up to 800 psi (55 bar). The production methods used in microfabrication of chips results in devices that are generally flat, thin, rectangular solids that are featureless except for the presence of inlet and outlet holes. For a connection to function successfully, a seal has to be achieved either around the hole on the surface of the device, or inside the inlet hole itself.

Despite the advanced technology often incorporated within the chip design, the vast majority of fluidic interconnects are based on the use of either macroscale or microfabricated gaskets or O-rings. These are often pierced sheets or circular layers of rubber which when compressed absorb any surface flaws and form a watertight seal. Being cut from thin rubber layers, gaskets are awkward to handle and easily damaged and contaminated. In addition, by relying on an easily deformable seal to maintain fluidic integrity, upper pressure limits are reduced. This is demonstrated by Gonzalez *et al.*[125] who report pressure integrity of 20 psi (1.4 bar) for a polysiloxane O-ring and Grey *et al.*[124] who report a maximum pressure of 60 psi (4.1 bar) for a silicone gasket. Silicone is available in thin films and is widely utilised as a gasket material due to its compressibility. Although silicones are generally inert, exposure to solvents can cause swelling and disfiguration of the silicone thus preventing a tight seal. PTFE has excellent chemical compatibility and is available in many forms but generally it is too hard to form an effective seal. Viton[®] (Dupont, U.S.A.) is a range of fluorinated elastomeric materials used in O-rings and gaskets that are in contact with organic fluids in a range of industrial machinery. Although Viton[®] offers a solution to the problem of achieving a solvent resistant seal, the softer Viton[®] elastomers are not totally inert and are thus prone to attack from ketones, alcohols and strong acids. As the chemical compatibility increases, elasticity and compressibility decrease. Glass or silicon microfabricated devices are not

structurally robust. As a result, forces applied during clamping or gasket sealing must remain low to prevent fracture of the device.

By far the simplest and cheapest approach to introducing reagents to sample inlets is via a glued capillary or tube. This technique has been widely applied, with most leading microfluidics researchers utilising this approach. Advanced examples include a precision-engineered interface for minimum dead volume interconnection presented by Gray *et al.*[124] In this case a three dimensional fluidic network is fabricated using multi-layer, reactive ion etching technology before the tubing is secured to the device by glue. As with polymeric gaskets and O-rings, adhesives are subject to chemical attack, however they have also been shown to withstand extremely high pressures of up to 2000 psi (138 bar)[128] and have the additional advantages of simplicity and reliability. For interconnection of devices to the macroscopic environment of sample vials and syringes in this thesis, the use of adhesives and tubing as well as inlet hole fabrication was examined in some detail, particular attention being paid to strength and chemocompatibility.

2.7.4. Size

Microfluidic devices by their nature and the fabrication techniques used can range in scale from only a few mm to hundreds of mm.[129, 130] By operating at the limits of microfabrication, very small devices can be fabricated. The limiting factor with such devices is manual handling performed by its operators. As the scale of the device decreases, manual handling becomes increasingly difficult and tedious. For ease of operation, mechanical features used for inlets and clamping should ideally measure in tens of mm.

2.7.5. Tolerance

Although microfluidic channels are fabricated to high tolerances and hole placement is also accurately defined, the dicing of a wafer to form individual devices is not a precise operation. This often results in the relationship between

the inlets and the external edges of the chip being quite variable. This variation prevents the external edges from being used as a reference point for positioning and an interface device must allow for variations in external dimensions whilst retaining high tolerance between inlet hole positions.

2.7.6. Adhesives

2.7.6.1. Cyanoacrylate

Initially, a connection was achieved between tubing and the inlet of the microfluidic device using a cyanoacrylate type adhesive (Loctite U.K. Ltd., U.K.). This type of glue has an extremely short curing time and allowed fast interconnection, but alone the tubing is unsupported leading to a structurally weak interface. By incorporating a ferrule into the interface, a structurally supported connection can be achieved. The connection is completed in two phases, firstly adhering the capillary to the ferrule, and once cured adhering the ferrule to the surface of the device. However, if the protruding capillary is too long, the ferrule is prevented from connecting with the surface of the chip and no stability is gained. If the protruding capillary is too short, improper alignment with the inlet hole may result. Additionally, the thin layer of cyanoacrylate is quickly attacked by most organic solvents making this method suitable only for aqueous based reaction systems.

2.7.6.2. Araldite Rapid

As an alternative to cyanoacrylate, a quick setting two-component epoxy resin (Araldite Rapid, Vantico Ltd., U.K.) was employed. Unlike cyanoacrylate, epoxy resins occupy the same amount of space when the adhesive has cured as when initially applied. This property provides not only adhesion but also structural support of the capillary or tube. Despite Araldite Rapid's fast setting characteristics, the cure time is still approximately 15 minutes. During this time, the adhesive can flow into the inlets and block the device.

2.7.6.3. Araldite 2014

Araldite 2014 is chemically resistant variant on Araldite Rapid with an increased viscosity and strong ceramic bonding characteristics. However, the curing time of 2014 is in excess of 12 hours and it remains vulnerable to chlorinated solvents. To render an adhesive inert to chemical attack, the level of crosslinking must be maximised. This can be achieved by heat curing the adhesive (340 – 350 K for 2 – 3 hours). Once heat cured, Araldite 2014 was impervious to attack from all of the solvents commonly available, even during sonication or boiling. Although highly chemically resistant, 2014 is not thermally resistant. By heating the device to 390 K, the adhesive could be removed with a small amount of force, leaving behind a clean glass surface.

2.7.7. Access holes

To gain access to the microfluidic channels, inlet and outlet holes have to be fabricated. In the case of glass there are several methods available including laser ablation, spark erosion,[131] chemical etching,[132] powder blasting[116] and mechanical drilling. For the devices fabricated in this thesis, inlet holes were created by either spark erosion or mechanical drilling.

2.7.7.1. Mechanical drilling

Mechanical drilling is the crudest method of creating holes in a wide range of materials. The attractiveness of mechanical drilling lies in its simplicity and speed. Thanks to the dental industry there is a large range of burrs or drill bits available in small sizes which can be applied to drilling glass and ceramics. The smallest burr commercially available is composed of a tungsten carbide alloy of 0.5 mm diameter. This burr was used for the majority of structures in combination with a Minicraft MB 1012 miniature high precision drill and drill press (Roto Zip U.K. Ltd., U.K.).

Holes were drilled after the substrate had been patterned and etched, but prior to bonding, to prevent the introduction of drilling debris. In cases where

supplementary access holes had to be created after bonding, pressurised water was applied through the existing inlets such that when the drill pierced the top plate, the glass fragments produced were forced back out through the hole that created them.

In all cases, the inlet holes were drilled through the etched substrate, eliminating the need for alignment when the cover plate was applied. Additionally, as the target area was etched, the hole could be precisely located as the tip of the drill settled into the etched channel as opposed to skidding on the surface.

To prevent heat build-up, which leads to premature wearing of the burr and can lead to cracking around the hole, water was used as a coolant. Immersion of an entire device hampers alignment due to decreased visibility created by the glass-contaminated water. Alternatively, a water droplet was placed at the site of drilling and was replenished during drilling as necessary. If the burr was allowed to pass through the glass unimpeded, the layer of glass on the underside of the hole was shattered and flaked as opposed to being ground away. Placing a supporting glass back plate significantly reduced this, especially if the layers were temporarily bonded together using beeswax or similar. To remove the necessity of a glass back plate, the depth of drill was accurately set such that only the sharp tip of the burr broke through. The remaining glass could then be drilled from the back side using the small hole created by the tip as a locator.

2.7.7.2. Spark erosion

Electrical Discharge Machining (EDM), or electrochemical spark erosion is a method for producing holes and slots, or other shapes, by using an electric discharge (spark) to remove unwanted material.[131] EDM can be defined as a removal method which, by means of an opposite pole electrode, activates sparks through a magnetic field. The whole process is carried out under strictly controlled conditions in a liquid medium (called a dielectric oil). The dielectric fluid acts as a coolant and rinses away the particles once sparks have removed them from the substrate. By placing an electrode very close to the work piece, a

spark can be repeatedly produced between the two. As the electrode is eroded as well as the substrate, very small electrodes, i.e. less than 0.5 mm were avoided. In comparison to drilling, EDM is painstakingly slow and intricate, however high quality conical holes can be placed accurately in close proximity to one another without substrate fracture.

To drill holes using EDM, the substrate is placed in a shallow bath of 50% sodium hydroxide. Platinum tipped electrodes were employed to reduce corrosion and increase lifetime. The cathode was secured into the dielectric fluid and the anode placed over the desired location of the hole. To ensure a spark was generated, the resistance must be kept as high as possible. To reduce conduction from the anode, an insulating jacket was positioned such that approximately 0.5 mm of the electrode length was exposed. If the insulation failed or too much electrode was exposed no spark was generated. For thin glass substrates (<1 mm), only 80 V was necessary to generate a spark. As the hole was formed, the insulation jacket was displaced exposing a greater electrode area and higher voltages were required to create a spark. For thicker glass (>1 mm) voltages of 115 V were found to be optimal. Prolonged operation at 115 V however, led to melting of the insulating jacket and thus a shorter operational lifetime.

2.7.8. Tubing

2.7.8.1. Fused silica capillary

Fused silica capillaries (FSC) are frequently used for introducing liquid to chips due to their small internal diameters, which are comparable to that of the channels in microfluidic devices. This not only reduces the total volume required to fill the device, but also reduces dead volumes and pressure differentials at the tubing/channel interface.

Glass is traditionally a fragile material, especially when capillary wall thicknesses are measured in microns. To strengthen the glass capillary, a

polyimide coating typically 15 – 20 microns thick is used as a protective coating or stress buffer. Polyimides are a range of incredibly strong, thermally and chemically resistant polymers. Coated with polyimide, fused silica capillary can withstand pressures up to 4350 psi (300 bar), have high tensile strength, a high fracture toughness and are highly flexible while maintaining optical transparency above 180 nm.[133] As with conventional glass, fused silica is chemically inert having excellent compatibility with organic solvents and most acids, but is vulnerable to hydrofluoric and phosphoric acids, and alkalis, which cause devitrification of the glass surface.

2.7.8.2. Fluorinated Ethylene Propylene

In situations where strong alkalis are required, Teflon[®] based tubing can be used in the place of FSC. FEP (fluorinated ethylene propylene resin) tubing (Anachem, U.K.) is a melt-processed variant of poly(tetrafluoroethylene) (PTFE) that was used throughout this thesis. FEP is chemically inert to strong acids, bases and organic and chlorinated solvents, can withstand pressures of up to 4000 psi (275 bar) and has greatly improved optical clarity over PTFE.[134] Available in a large range of internal diameters comparable to those of fused silica capillary, FEP can also be used to join two pieces of capillary quickly and reversibly. This is achieved by choosing an inner diameter FEP tubing slightly smaller than that of the outer diameter of the fused silica capillary. By using a short length of FEP, the two pieces of capillary can be inserted from each end to butt against one another in the middle creating a zero dead volume connection. Unlike FSC however, due to the inert nature of FEP tubing, adhesives such as cyanoacrylate or Araldite 2014 are unable to make a secure bond between the tubing and the surface of a microfluidic device.

2.8. Reversible interfacing

Despite the advantages of simplicity, cost and ease of implementation, the use of adhesives to secure connections is far from ideal. The process itself is ill-defined in reproducibility and is a potential source of contamination. Once set in place,

the interface is only alterable by destruction of the adhesive and reinstallation, a time-consuming process. In situations where the tubing interface is not mechanically secure prior to the use of adhesive, solvents may be allowed to come into contact with the adhesive presenting chemocompatibility issues.

Ideally, a method of connecting a sample inlet tube or capillary is required that meets or exceeds the performance of an adhesive based interface system. The device should allow rapid, reversible, leak-free connection and assembly in a seconds-to-minutes time frame and require no specialist knowledge or training for its operation. In addition to previously specified criteria of chemical compatibility, low sample dead volume and high pressure integrity, additional properties such as low unit cost, high functionality and high reproducibility are desirable while providing adequate visibility of the device for observation of the mixing process.

2.9. In-line connectors

An additional disadvantage of securing tubing to a device by adhesive is the finality of the connection. Although capillaries can be removed, the process is neither rapid nor convenient. To prevent tubing blockages from disabling a whole device, a method was required to hold the capillary or tubing to the surface of the device. For the reversible connection of capillary and tubing to syringes and other macro devices, in-line poly(etheretherketone) (PEEKTM) connectors or 'fingertights' (Upchurch Scientific, U.S.A.) are frequently used. These employ a mismatched male/female cone, such that when screwed together, the female part applies pressure to the male cone, which in turn squeezes the tubing running through the middle of the device. The application of such a device allows two pieces of mismatched tubing to be connected in a leak proof, reversible manner. By use of adhesive, one half of an in-line connector was attached to the surface of a chip, thus allowing this technology to reversibly connect tubing with the surface of microfluidic devices (Figure 2.3).

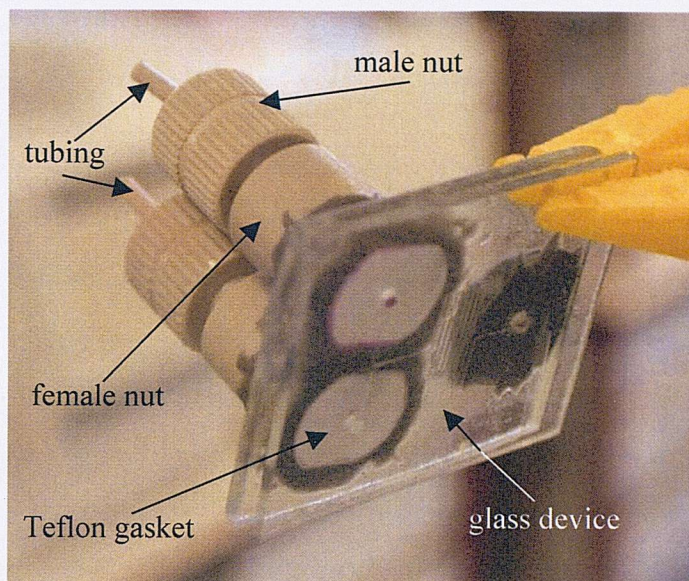


Figure 2.3 PEEK™ in-line connectors incorporating PTFE gaskets for the reversible connection of capillary and tubing to syringes and other macro devices.

To prevent solvents interacting with the adhesive, a Teflon® film was incorporated between the interconnect and the surface of the device. Due to the cost of each connector, this method of microfluidic interconnection proved prohibitively expensive for experimental designs, however a commercial product is now available through Upchurch Scientific.

2.10. A reversible high-pressure interface

Initial work on a re-useable clamped interface focussed on the interconnection of standard glass capillary tubing to a glass-silicon-glass microfluidic device designed by Bessoth *et al.*[129] The microfluidic reactor used in this section operate on the distributive mixing principle previously discussed in Chapter 1. From an interface perspective, the significant design features of a device are no longer the fluidic design and dimension of the channels, but the external dimensions of the device, the inlet hole size and the hole spacing or pitch. Due to the fabrication methods of most glass and silicon microfluidic devices, the resultant shape of the device is frequently a thin rectangular solid of low

structural strength, and consequently it cannot be relied upon as a structural component of the assembly (Figure 2.4).

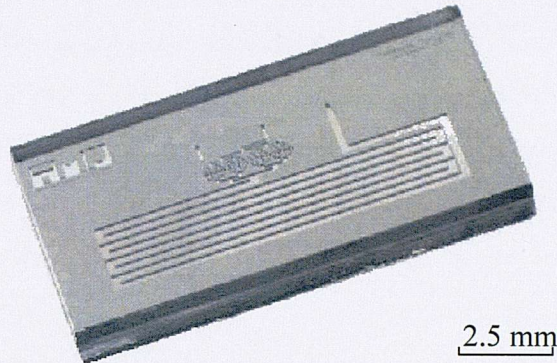


Figure 2.4 A glass-silicon-glass distributive microfluidic mixer by Bessoth *et al.* measuring 5 x 10 x 1.3 mm and inlet pitch of 1.4 mm. Initial flows are split into two sets of 16 parallel flows before recombination resulting in a 32 alternating laminae.[129]

In addition, the smooth shape provides no purchase for the addition of external components or modules. Since clamping of the device is unavoidable, high tolerances were required for fabrication of the interface. For those components in contact with the micromixer, typical tolerances of $\pm 5 \mu\text{m}$ were consequently used.

The micromixer featured in Figure 2.4 measures 5 x 10 x 1.3 mm. These small external dimensions make manual handling of the device difficult and tedious. In addition to the small external dimensions of the device, the inlet hole spacing provides additional design complications. The inlets on this particular device measure 500 μm in diameter and have a distance between hole centres or pitch of 1.4 mm. This spacing, although arbitrary, significantly effects the overall design of the device more than any other feature. The pitch distance is considerably smaller than any commercially produced, off-the-shelf parts such as fingertight connectors. Should custom-fabricated fingertights be produced, for a linear path between connectors and inlets to be employed the fingertight would have to

measure less than 1.4 mm in diameter. A component of such small dimensions would be prohibitively weak and due to its reduced dimensions and its manual handling would be near impossible. The dicing of glass or silicon wafers produced during the fabrication process to form individual chips is an inexact process. The location of the inlet holes in relation to the chip edges varied by $\pm 30\text{ }\mu\text{m}$. This meant that the chip edges could not be used as a reference for positioning of the chip within the interface. As the inlet holes were the only precisely located features of the chip, these had to be used as locators for interface assembly.

2.10.1. Interface design

The final design of interface for the glass-silicon-glass micromixer was decided in collaboration with Dr Mike Mitchell of Imperial College London and Mr Vas Nittis from the Applied Technology group at GlaxoSmithKline and produced by AZTEC Precision Engineering, U.K. For production of the main body of the interface PEEK[™] was used. PEEK[™] is a chemically resistant polymer with excellent machining characteristics, good thermal stability and functions over a wide pH range. A schematic of the assembled device is shown in Figure 2.5.[135]

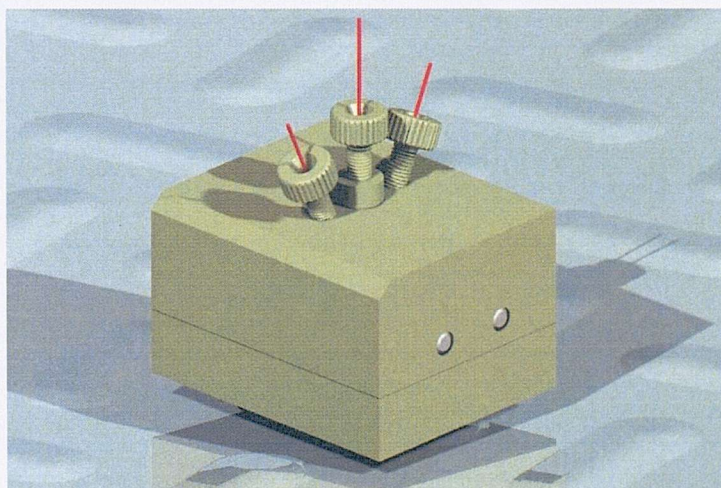


Figure 2.5 A schematic of the assembled PEEK[™] interface for reversible interconnection of a fluidic micromixer.

The main body incorporates tapered threaded holes for the use of fingertights to secure the capillary in place. As previously mentioned in section 2.10, the narrow pitch distance prevented the use of a linear path between the fingertights and the inlets. To overcome this, the main body included three channels each measuring 400 μm in diameter. The two outer channels were curved to artificially increase the pitch distance. The fingertights were then angled to provide increased clearance and a user-friendly fingertight head size of 7 mm. The channels were machined into two halves of the main body. The two halves of the body, when brought together created a circular cross section channel and allowed the inserted capillary to be accurately guided to its target location (Figure 2.6). The two sections of the main body were then secured by structural rods and screws.

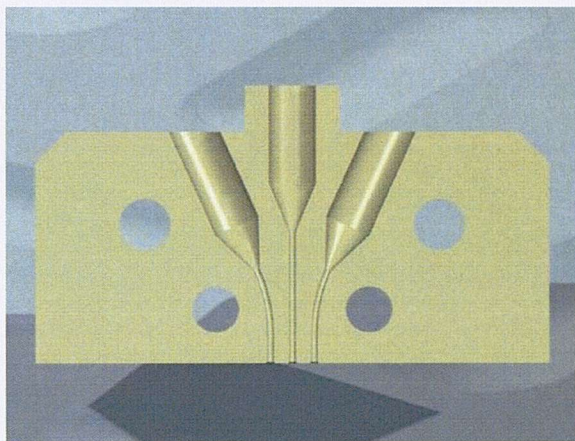


Figure 2.6 Machined channels and angled fingertights in the main body artificially increase the pitch distance. The two halves were joined by use of structural rods and screws.

As the lower flat surface of the main body applied pressure to the surface of the micromixer directly, once the two halves of the main body were fixed, the surface was machined to a high tolerance ($\pm 5 \mu\text{m}$). Although the two components were not permanently fixed, if taken apart re-machining of the lower surface would be required.

A silicone gasket was employed to provide a seal between the capillaries and the glass inlets. As previously mentioned in section 2.7.3, compression of a

polymeric gasket is one of the most popular ways of creating a seal between a chip and the outside world. Not only does compression of the 100 μm silicone elastomer provide a seal between the capillaries and the edges of the inlet holes preventing sample leakage, its flexibility absorbs any machining tolerances in the assembly. The gasket was cut from a sheet of silicone elastomer to cover the same surface area as the chip and included three pre-cut holes. These holes were created using a jig consisting of two plates of aluminium, each with the same footprint as the micromixer and incorporated three holes in the same location as the inlets. Holes were then transferred to the silicone elastomer by use of a fine needle or a 300 μm drill bit. The second surface in contact with the micromixer consisted of a glass back-plate held in place by a PEEKTM base unit machined to incorporate the glass as a window and screws to clamp the lower assembly and the main body together. To maintain horizontal alignment between the glass, the chip and the base unit an aluminium precision spacer was included, an exploded view of the interface is given in Figure 2.7.

2.10.2. Assembly

Although no specialist knowledge or training was required to operate the interface, as with any precision apparatus, correct assembly methodology and order was crucial to ultimate performance and reproducibility. Due to the fragility of the membrane and the potential for damage in re-introducing the capillaries through the preformed holes, the gasket holes were instead formed *in situ*. The pre-cut elastomer (7) was placed over the entrance holes in the main body (4/5) by using the precision spacer (9) to aid location. The jig was then placed over the elastomer while the capillaries (1) were introduced to the assembly through the fingertight connectors (2).

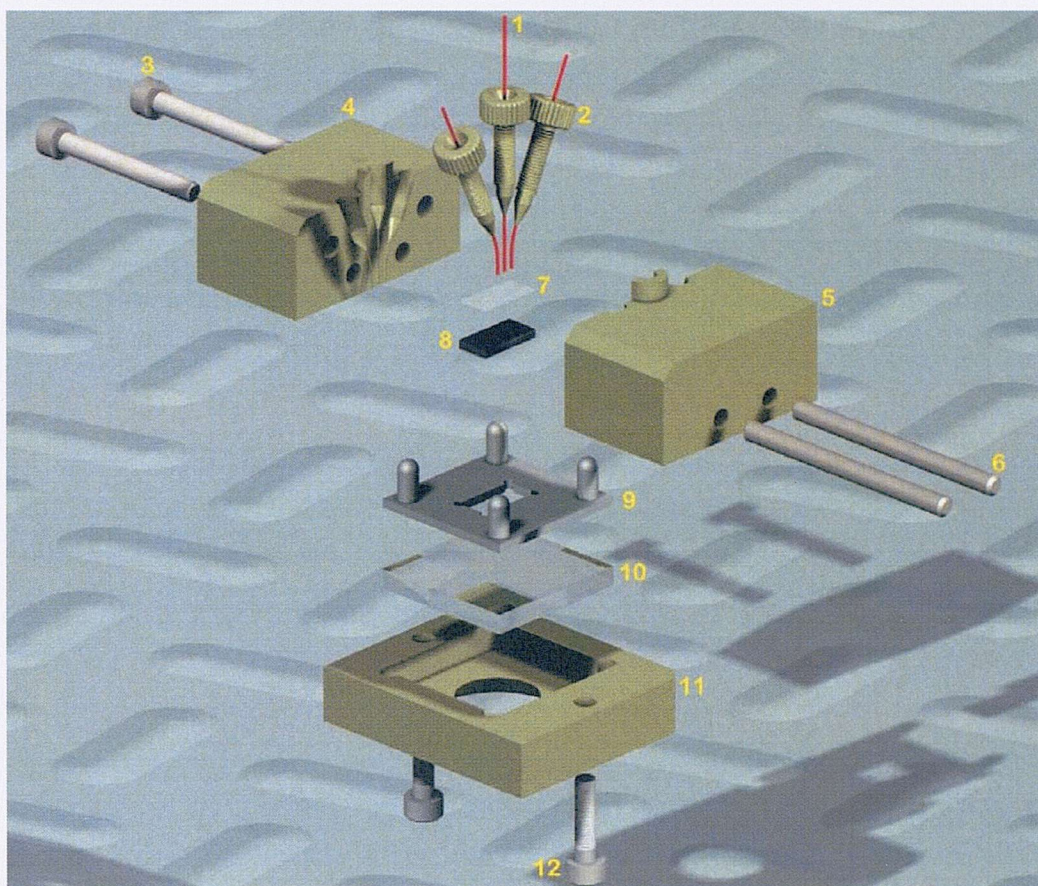


Figure 2.7 An exploded view of the PEEK™ interface. 1. Capillaries, 2. fingertight connectors, 3. structural screws, 4/5. main body, 6. structural rods, 7. silicone gasket, 8. micromixer, 9. precision spacer, 10. glass window, 11. lower assembly, 12. M3 screws.

As the capillaries exit the guide channels they pierce the gasket in the exact location. Once the capillaries protrude approximately 2 mm from the bottom of the main body, the jig can safely be removed leaving the gasket in place.

The micromixer chip (8) can then be placed onto the protruding capillaries forcing them into the holes. Once flush against the main body/gasket the fingertights can be lightly tightened to secure the capillaries in place. The lower PEEK™/glass assembly (10/11) can then be safely placed onto the main body and screwed into place. Tightening of the plastic M3 screws (12) forces the lower and upper assemblies together causing compression of the micromixer against the gasket. This deformed the gasket creating a seal and ensured that the

capillaries were located fully in the inlet holes before the fingertights were fully tightened.

2.10.3. Discussion

Using the above procedure, the chip/interface assembly could be completed in less than 5 minutes. One of the most difficult stages of the assembly process was the passing of the capillaries through the guide channels. For the machining of PEEK[™], the ideal angle between the cutter and the work surface, known as the rake angle, is 0-10°. As the cut glass capillaries have sharp defined corners cut perpendicular to the long axis, the corner angle is ideal for the scoring and cutting of the PEEK[™] guide channels. As the capillaries were passed through the curved guide channels, the capillaries frequently caught on the channel wall. Once passed through the channel, the capillary ends were re-cut to ensure that the end of the capillary was clean and flush with no debris capable of causing a blockage of the device.

An additional problem area was the silicone gasket. Due to its size (5 mm x 10 mm x 0.15 mm), handling the gasket was extremely tedious. During the hole formation process, the capillary could cause irreversible plastic deformation of the elastomer as opposed to piercing it. This rendered the gasket unusable and a replacement gasket would have to be made. During use, leakage of the interface/chip assembly would occasionally occur. This was attributed to inefficient sealing by the gasket. The inlet holes of the micromixer were fabricated to measure 500 µm in diameter. The standard fused silica capillary used for fluid introduction for this and other devices measured 375 µm in diameter. This left a gap between the chip and the FSC of over 60 µm for the gasket to seal. If any imperfection was introduced during the formation of the gasket inlet holes, the gasket compression would be insufficient to close the gap and a leak would occur. By using multiple gasket layers, the potential for defective gasket holes was diminished and sealing reliability improved.

An additional issue for the interface is chemocompatibility. As mentioned, PEEK[™] is chemically resistant to nearly all organic compounds, alkalis and resistant to dilute acids. The silicone gasket however offered no such compatibility. Although the structure of the silicone is not altered by immersion in solvents, swelling and deformation does occur rendering the device unsuitable for a wide range of chemistries.

2.10.4. Summary

The fabrication of a reversible interconnect for the introduction and removal of solutions from a glass-silicon-glass micromixer was achieved. Despite the difficulties raised by the size and inlet placing on the microfluidic device, the interconnect was extremely successful. However, the device suffered from two major drawbacks. Firstly, the inclusion of an elastomeric gasket material proved most detrimental to the performance of the device. The silicone sealing material, although relatively inert, was prone to swelling in organic solvents causing leakage. Secondly, was the cost of the interface. Two channels incorporated into the main body of the interface to artificially increase the pitch distance greatly increased the complexity and manufacturing cost of the interface (Figure 2.8).

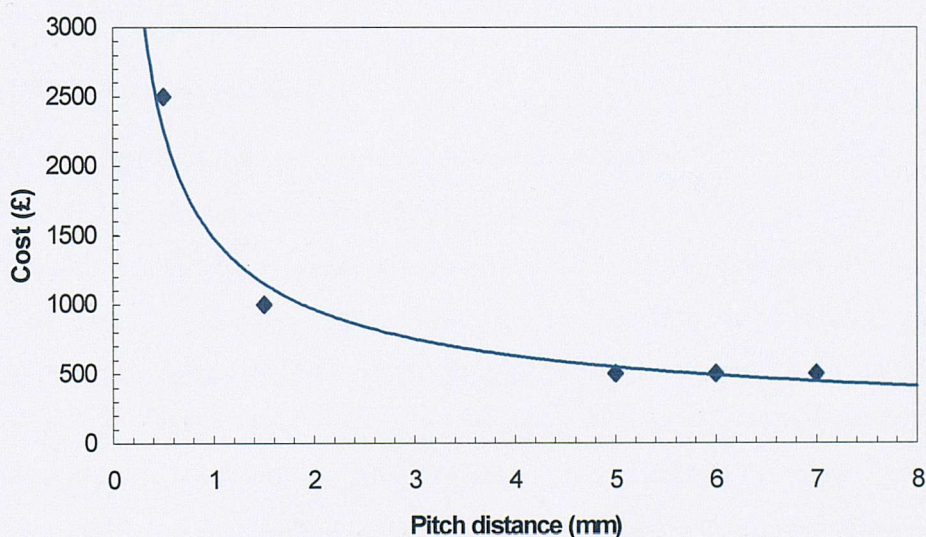


Figure 2.8 The relationship between pitch distance and manufacturing cost for a high-pressure interconnection device.[136]

It is estimated that a three-fold increase in pitch distance from 1.5 mm to 4.5 mm would decrease the cost of the device by half.[136] An alternative source of expense was the high machining tolerances required to maintain a seal between the gasket and the micromixer. To produce a reliable interface for alternative microfluidic devices, the issues of sealing and cost must be resolved.

2.11. A flanged tubing interface

In the previous example, the size of the microfluidic reactor and the pitch distance were fixed. For the production of reactors in this laboratory, the DWL method for channel patterning was utilised (section 2.3). By employment of a DWL, there were no restrictions on the pitch distance or on the size of the micromixer other than that imposed by the glass wafer. With the exception of the screws that secured the main body and the lower assembly together, each component of the previous design was custom produced. Accidental damage was not only expensive, but time consuming as replacement parts had to be specially fabricated. For everyday use in a practical research laboratory, an interface device should be robust and incorporate as many commercially produced, 'off-the-peg' components as possible.

2.11.1. Interface design

As with the microfluidic mixer described in section 2.10, the fabrication of microfluidic devices by photolithography and wet etching in this laboratory resulted in a thin rectangular solid of low structural strength and an absence of other external features. To eliminate the problem of inlet hole spacing previously encountered, the pitch distance for future microfluidic reactors was set to 10.0 mm. This large pitch distance allows the use of commercially produced components for the introduction of fluids. These off-the-peg components are designed to be manually manipulated and are structurally robust.

In contrast to the previous micromixer, inlet holes were drilled manually using 500 μm dental burr (as described in section 2.7.7.1). By incorporating a 10.0 mm pitch distance, the potential for fracture of the glass due to applied stress during drilling is reduced. Although the inlet position was defined accurately during the design process and was marked by the DWL, the production of the hole was performed manually and therefore the location tolerance of each hole was approximately $\pm 125 \mu\text{m}$. Although for a simple 1 + 1 mixing process only 3 inlets were required (two for sample introduction and one for collection), to allow for maximum flexibility for future designs incorporating multiple inlet and outlet processes, 8 inlets for fluid introduction and removal were specified (Figure 2.9).

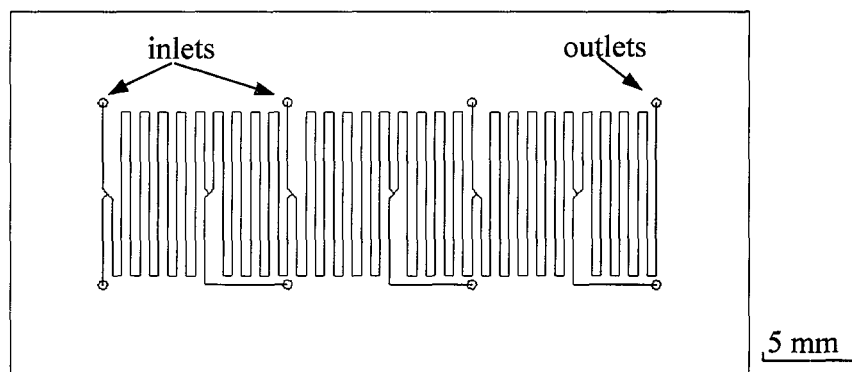


Figure 2.9 A glass microfluidic reactor measuring 20 x 40 mm incorporating 8 inlets and a 10 mm pitch distance.

By placing each inlet 10 mm apart a design 10 mm x 30 mm was produced. To minimise the potential for fracture during the dicing process, a clearance distance of approximately 5 mm was allowed between the inlet holes and the substrate edge. This resulted in a total microfluidic reactor size of approx. 20 mm x 40 mm and consequently facile handling.

To minimise costs, commercially available fixtures and fittings were examined for their applicability. Flanged end tube fittings consist of PTFE type tubing, a plastic nut and a stainless steel washer. To produce a seal between the tubing

and a flat surface the tubing was flanged or flattened using a flanging tool that applied either heat or pressure in a localised manner. The nut then forced the flange against the device surface when tightened. The washer was included to prevent binding or twisting of the tubing when the fitting was tightened. Being fabricated from Derilin[®] or Tefzel[®], chemical and biological compatibility of the nut was excellent should a leak occur. However, in normal operation, fluid samples came only in contact with the tubing and the chip.

To allow use of flanged tubing with the glass devices described above, an interface body was machined from aluminium. The body was composed of an upper section machined to accept standard 1/4-28 fittings and a lower section, consisting of a recess and observation window (Figure 2.10).

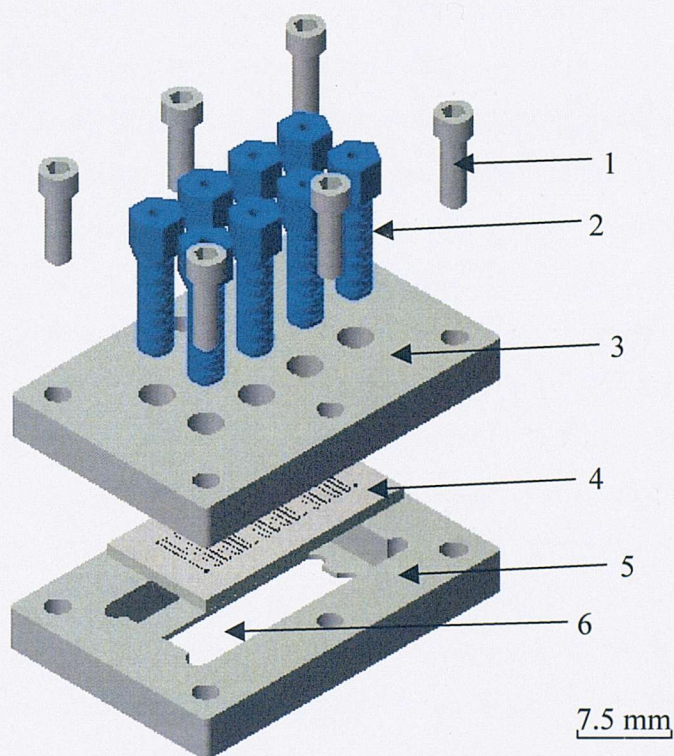


Figure 2.10 An aluminium interface incorporating flanged tubing connectors for the introduction and removal of fluids from a microfluidic device. 1. M3 bolts, 2. flanged tubing nut, 3. upper aluminium section, 4. glass microfluidic reactor, 5. lower aluminium section, 6. observation window.

As both the inlet holes and the dicing of the wafer were performed manually, the tolerance of the recess edges and the threaded flanged fitting holes was low, incurring no additional machining costs. Once a microfluidic reactor had been placed in the recess, the two halves were brought together and secured by six M3 bolts around the perimeter. At this point no pressure is applied to the microfluidic reactor. Once secured, the flanged fittings were introduced to the desired locations. To allow for the inaccuracies in inlet hole placement, 350 μm ID PTFE tubing was employed. Once flanged, the area covered by the opening of the end of the tubing approached 1000 μm , sufficient to enclose the entire area possibly inhabited by the glass inlet.

2.11.2. Discussion

For a satisfactorily tight seal to be formed the nut must be closed sufficiently such that the PTFE flange is pressed evenly around its circumference. To achieve this, finger pressure was insufficient due to the restricted access dictated by the layout of the inlet holes. Employing a spanner to better grip the flanged fittings allowed a greater force to be applied, however overly aggressive use resulted in a force sufficient to cause fracture of the glass device. This was attributed to uneven application of pressure to the device, the machining of the interface being performed to lower tolerances (± 0.1 mm) than previously to reduce costs. With a small number of parts and a simplistic design, assembly of the device could take place in under one and a half minutes. To reduce the chance of chip fracture, only minimal force was applied to each flange nut before testing. Only if a leak was observed was additional force applied. Due to this precautionary measure, the initial success rate of device interfacing was only 50%. Once the final tightening of the flange nuts had been performed, the reliability of the device was 95%, the remaining failures due to fracture of the glass device.

2.11.3. Summary

The combination of in-house fabricated glass microfluidic reactors with commercially produced fixtures and fittings for a reversible interconnection was

extremely successful. This is despite the alignment problems associated with manual inlet location and low tolerance machining. The design incorporated the reversible connection for a variety of microfluidic devices in a robust metal housing. Each component of the device was cheap to manufacture or purchase and easily replaceable. A range of flow rates was achievable well within those typical for microfluidic devices with minimal dead volume. However, the flanged tubing interface suffered from two major drawbacks. First, the increased sample volume required to fill a length of 350 μm ID PTFE tubing over that of 150 μm ID FSC. Where small samples of dangerous or expensive chemicals are required, this sample volume increase is undesirable. Second was the reliability of the interface. Due to the direct application of force to the micromixer to create the seal, several micromixer devices were fractured.

2.12. Tapered glass inlets

In situations where the formation of a seal is required, but the application of adhesives undesirable, pressure is universally employed. As has been previously demonstrated in sections 2.10 and 2.11, to successfully perform pressure based sealing on a small scale requires the precise and careful application of force.

Capillary column connectors are cylindrical glass tubes with a tapered internal diameter used routinely in gas chromatography (GC) (Figure 2.11). These are used to quickly connect lengths of fused silica tubing, for example, the fitting of a guard column or the repair of a broken column. As the capillary is forced into the taper, the polyimide coating on the outside of the capillary is compressed, creating a seal between the glass connector and the capillary. Due to the small area over which the force is applied, only a very low force is necessary to form a tight seal. This removes any need for an additional gasket material. Once compressed between the glass capillary and the tapered column connector, the seal is capable of withstanding pressures of 1725 psi (119 bar), and is such that the polyimide may have to be separated from the capillary to break the seal.

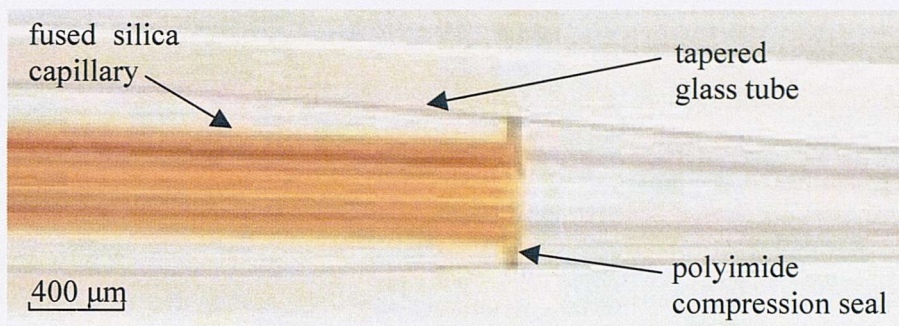


Figure 2.11 A tapered glass device for the reversible connection of fused silica gas chromatography columns and guard columns.

By the incorporation of tapered inlets into microfluidic devices, the capillary column connector technology may be directly transferred to microfluidic reactor devices. As the seal is formed by the polyimide coating of the capillary, the mechanism of compression of the ‘gasket’ is removed from the clamp and instead occurs on the microfluidic device. Microfluidic devices are by their nature, manufactured to very high tolerances, thus the need for high tolerance machining of the interface is removed; the only requirement of the interface being to reliably align the capillary with the inlet hole. In this way the problems associated with reproducible clamp production or assembly are removed, and in addition, polyimide has extremely high chemical resistance.

2.12.1. Interface design

The microfluidic reactor layout chosen for this interface design was identical to that of the flanged tubing interface (section 2.11). As the manufacture of accurately tapered inlet holes were beyond the production capabilities of our laboratory, microfluidic devices incorporating a tapered inlet were produced by Micronit Microfluidics B.V. (Enschede, The Netherlands) (Figure 2.12). Powder blasting or abrasive jet machining (AJM) was used for channel and inlet hole fabrication.

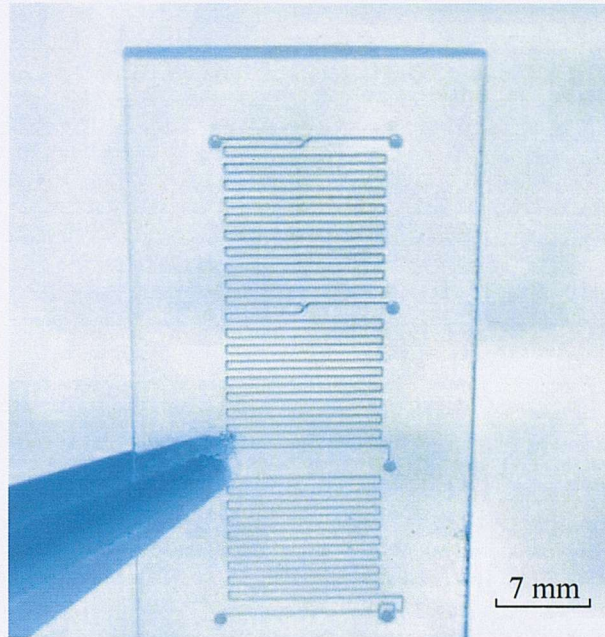


Figure 2.12 A glass microfluidic reactor fabricated by powder blasted measuring 20 mm x 40 mm incorporating up to 8 tapered inlets and a 10 mm pitch distance. Device manufacture by Micronit Microfluidics.

This method was chosen due to the nature of the channels produced in the AJM process. When the glass is targeted with particles, the particle trajectory and the masking effect of the copper combine to form a channel with slightly tapered walls. The walls then act to guide subsequent particles towards the middle of the channel which eventually narrows to a point (Figure 2.13).

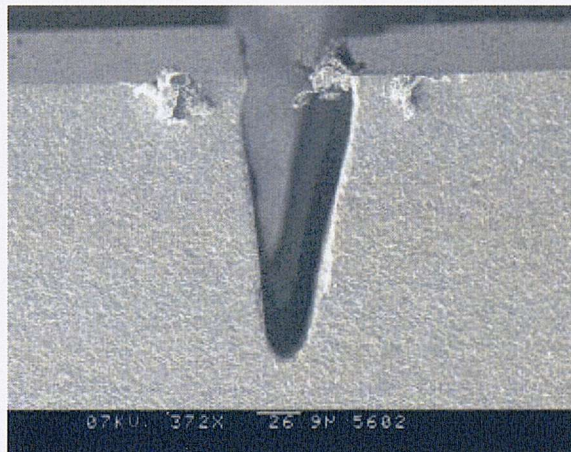


Figure 2.13 A channel fabricated by AJM by Micronit Microfluidics incorporating steep tapered sidewalls.

However, reduction of the inlet angle results in the reduction of the seal length and therefore a decrease in ultimate leak pressure. This is illustrated in (Figure 2.14). The fabrication of an inlet incorporating an angle of 85° results in a seal length of approximately $135\text{ }\mu\text{m}$. This can be compared to an inlet hole of 45° , which would give rise to a seal length of only $17\text{ }\mu\text{m}$.

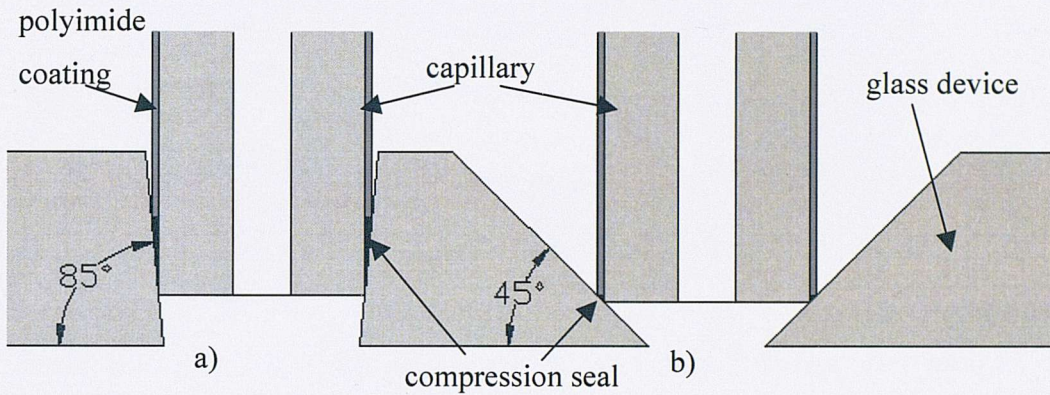


Figure 2.14 A comparison of compression seals formed by a) steep and b) shallow tapered inlets.

The final design of the interface for the AJM machined glass microfluidic reactor was produced in consultation with Mr Vas Nittis from the Applied Technology group at GlaxoSmithKline. A schematic of the assembled device is illustrated in Figure 2.15; an exploded view of the interface is given in Figure 2.16. The unit consisted of three main components. First, the aluminium base or lower section of the device incorporated a 20 mm by 40 mm recess to accommodate the glass microfluidic reactor and a 10 mm by 30 mm window for channel observation (8). The middle section of the assembly consisted of a custom PEEKTM spacer (6). This was machined to accept eight, commercially available, flangeless PEEKTM fingertight type connectors (1,3,5), to present a flat surface to the upper surface of the microfluidic reactor. To allow sufficient force to be applied to the fingertights, two dimples were incorporated into the top cap (1) for use with a peg spanner.

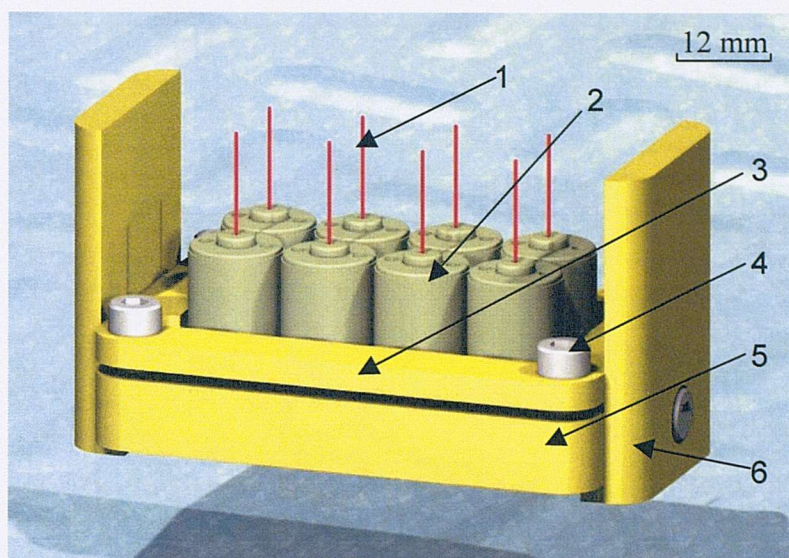


Figure 2.15 A 3D CAD model of an assembled interface for reversible interconnection of a fluidic microfluidic reactor incorporating tapered inlets. 1. Capillaries, 2. finger tight union, 3. upper aluminium section, 4. M3 bolts, 5. lower aluminium section, 6. inversion legs.

The top cap (1) and base (5) of each fingertight unit were threaded to allow compression of the cone (3). The use of a peg spanner as opposed to a conventional hexagonal head allows a completely unrestricted rotation of the union top caps, despite their close proximity. To complete the device, an upper aluminium plate (4) was employed which was also drilled to accommodate the eight fingertight connectors. Once a microfluidic reactor (7) had been placed in the recess, and the PEEK[™] middle section placed on top, the two aluminium pieces were brought together and secured by six M3 bolts (2) around the perimeter of the interface. Although pressure is applied to the microfluidic device by the clamp, it is not to compress the microfluidic reactor, but to securely hold the various sections together and did not cause device fracture at any time.

2.12.2. Assembly

Due to the variation in inlet hole-to-edge distance created by the wafer dicing process, the chip edges could not be used as a reference point for positioning the

device underneath the inlet holes in the PEEKTM spacer and thus the correct assembly methodology was crucial to final interface performance. To ensure a good seal between the polyimide coating on the capillary and the tapered inlet of the glass microfluidic device, a clean flat end to the capillary and the polyimide coating was essential. Before introduction of the capillaries, to decrease the number of actions required after their introduction, the upper aluminium section (4) is placed onto the PEEKTM spacer (6) incorporating the base of each fingertight union (5). The cone (3) and top cap (1) of the union were then lightly screwed down to form one half of the unit.

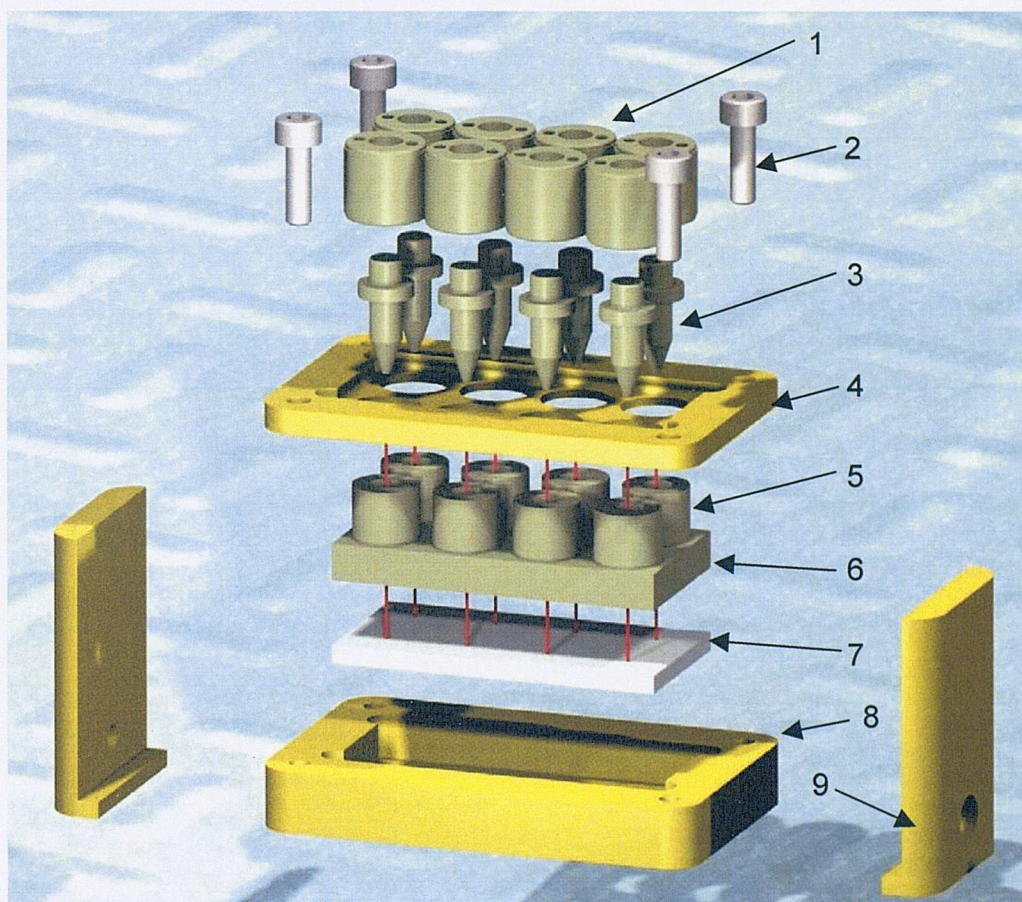


Figure 2.16 A 3D exploded view of the tapered inlet interface for reversible interconnection of a fluidic microfluidic reactor incorporating tapered inlets. 1. top cap, 2. M3 bolts, 3. cone, 4. upper aluminium section, 5. finger tight union base, 6. machined PEEK spacer, 7. microfluidic device, 8. lower aluminium section, 9. inversion legs.

Clean, flat capillaries were then introduced into the device through the fingertight unions on the upper section to protrude by approximately 2 mm from the flat surface of the PEEK[™] spacer (5/6). The micromixer chip (7) can then be placed onto the protruding capillaries. Once flush against the PEEK[™] spacer, additional pressure is applied to the capillaries to form a seal. Once a secure seal was formed, the fingertight unions were fully tightened to secure the capillaries in place. The lower aluminium section (8) was then placed onto the chip/upper section and screwed into place.

2.12.3. Discussion

By the combination of the upper section with the PEEK[™] unions and spacer, the number of actions for assembly is reduced, allowing rapid interfacing in under one and a half minutes. Initially a ceramic glass cutting tile was employed for cutting capillary ends. A light score is made in the polyimide coating which causes surface fracture of the glass. Once weakened the glass can be easily snapped at the point of fracture. This technique, if carelessly performed, can create an uneven end with polyimide debris. If polyimide is not present uniformly around the circumference of the capillary for the first 100 microns of the capillary's length no seal could be formed. To ensure a clean flush end to the capillary a specialist diamond capillary cleaving tool (Shortix, Supelco, U.K.) can be used leading to a perfectly uniform, flush end with no debris or overhang (Figure 2.17).

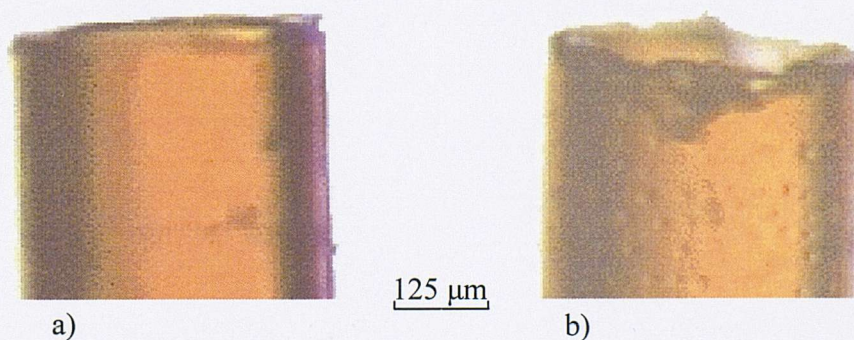


Figure 2.17 Comparison of a) a clean capillary end cut using a specialist cleaving tool and b) a damaged capillary end created using a ceramic tile cutter.

The reproducibility of the seal was extremely poor. It was observed that while some inlets sealed consistently, others failed to seal regardless of the number of attempts. However, when the inlets of the glass microfluidic reactor were examined more closely, it became apparent that the cone shaped inlet was often irregular and poorly fabricated. As opposed to the 80-85° angle originally specified, the inlet shape was totally irregular. Although towards the bottom of the inlet, the desired angle was approached, the inlet had a curved, unsymmetrical profile (Figure 2.18).



Figure 2.18 Curved, unsymmetrical inlets produced by AJM.

This resulted in an asymmetric force being applied to the polyimide and therefore no seal was formed. Although the reproducibility of the inlets was poor, where a microfluidic structure did incorporate an inlet fabricated to the original specifications, the reliability of the seal was good.

2.12.4. Summary

The microfluidic interface described herein successfully removed the need for high tolerance machining often required for microfluidic device interfacing. In addition, the use of an elastomeric sealing gasket was also removed and the clamping force reduced. The use of a commercially available connecting union

provided facile replacement of components and the low tolerance aluminium clamping components reduced costs. By direct introduction of the capillary into the inlet and reduction of the sealing area, the force required to form a watertight seal was minimised and the dead volumes associated with the device were extremely low. For the interconnection of more delicate microfluidic devices, incorporating additional features such as micropumps and valves, this feature is of significance. More importantly for use with chemical microfluidic reactors, is the issue of chemical resistance. Although polyimide does not have the chemical resistance of PTFE, it can be used safely with chlorinated and non-chlorinated organic solvents as well as most acids and bases. This places few restrictions on the range of chemistry compatible with the interface.

2.13. Experimental

To assess the performance of each interface, an aqueous solution was passed through the microfluidic device at a variety of flow rates. The fluid exiting the chip after a set time interval was then collected in a sealed container and its volume calculated according to the weight of the sample collected. Figure 2.19 illustrates the performance of the high pressure interface described in section 2.10 between 0 and $1100 \mu\text{Lmin}^{-1}$.

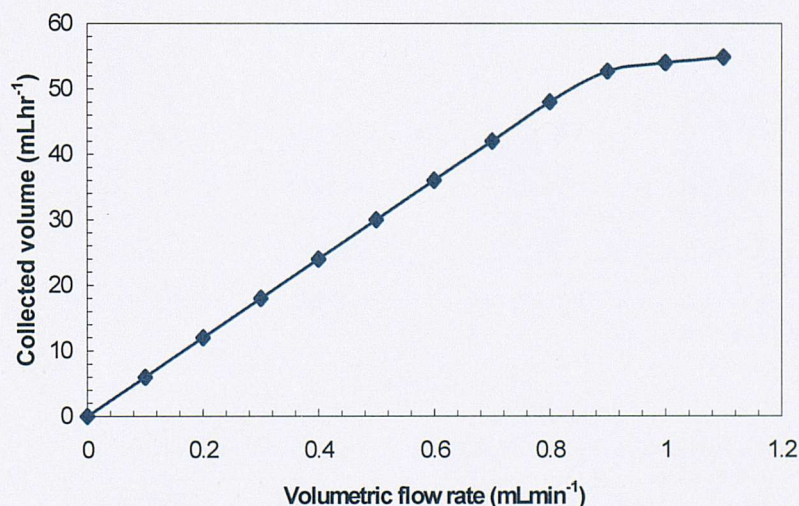


Figure 2.19 The variation of the volumetric flow with the volume collected upon exiting the high-pressure interface after a set time interval.

As can be seen in Figure 2.19, the variation of the volumetric flow with the volume collected is essentially linear whilst the seal remains intact. Once the relationship ceases to be linear, the collected volume begins to decrease in relation to the volumetric flow. This signifies that although the majority of the solution is flowing through the device, a small proportion is leaking into the area surrounding the device. As the flow rate increases, little additional flow throughput is achieved with significant leakage into the device, and no additional increase in flow throughput.

To allow for variation in channel dimensions and the friction factor of the different materials used for the microfluidic reactors detailed in sections 2.10, 2.11 and 2.12, the maximum pressure before failure was determined for each interconnection method (Figure 2.20). An aqueous solution was passed through each interface at a variety of flow rates by means of pneumatic motivation. The gas pressure was set by a precision pressure gauge and the flow rate determined according to the weight of the collected sample over a 1 hour period.

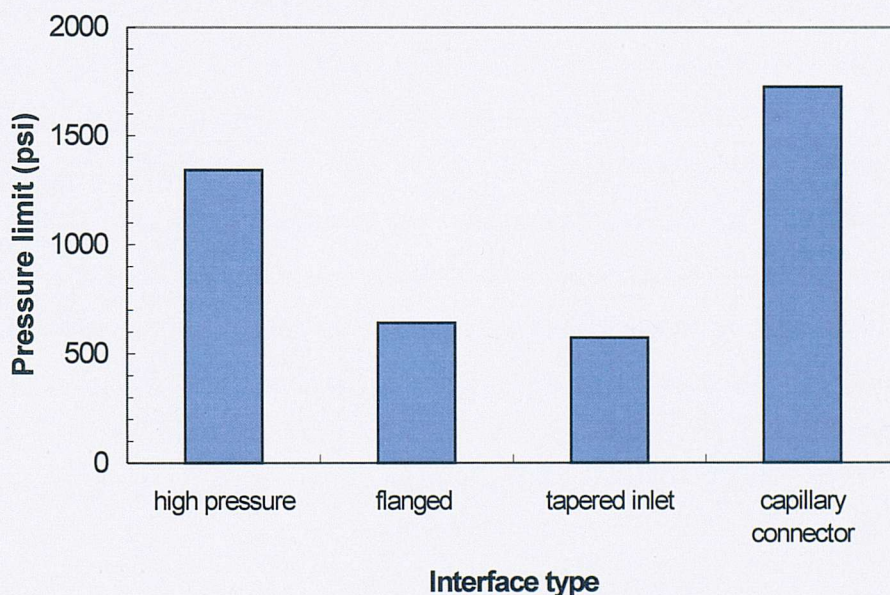


Figure 2.20 The maximum pressure attained prior to failure or leakage for three types of microfluidic interconnection and a commercially available capillary connector.

From the results depicted in Figure 2.20, it can be seen that despite the incorporation of a polymeric gasket, the PEEKTM interface could withhold a considerable amount of pressure 1300 psi (90 bar) before the seal was compromised, the performance of the device being attributed to the high tolerance manufacture. The relatively low ultimate pressure before failure of the flanged tubing interface could be assigned to the moderate sealing pressure applied to the sealing flange. With the production of a significantly flatter and more uniform clamp surface, the ultimate pressure performance of the device should improve considerably at additional manufacturing expense.

As previously mentioned, the tapered inlets fabricated by AJM were misshaped in appearance and their uneven surface led to frequent interface failure and consequently a very low-pressure capability. In addition to the three interfaces discussed here, a conventional capillary column connector was also tested. This represents the theoretical maximum that should be obtainable using the tapered inlet design, should the inlets be fabricated to the required specifications. The ultimate pressure prior to failure of the seal of the capillary column connector was determined to be 1740 psi (120 bar), three times greater than that observed for the tapered interface connector and significantly greater than the PEEKTM interface. At pressures above 1740 psi, the polyimide coated capillary became detached from the capillary column connector resulting in the immediate and catastrophic failure of the compression seal holding the capillary in place. By the combination of the fingertights included in the design described in section 2.12, with accurately fabricated tapered inlets, ultimate pressures should exceed that of the capillary connector (1740 psi) and failure should occur in a more gradual manner, as demonstrated for the high pressure interface (Figure 2.19).

2.14. Interface dead volumes

As previously discussed, the minimisation of dead volume is a key design requirement in microfluidic interfaces. For simplicity, the dead volume calculation is based on the assumption that a dead volume is defined as any area

where the localised flow rate is lower than that of the mean flow rate of the bulk solution. This is in comparison to the definition used previously where a dead volume is described as any volume where the local flow rate approaches zero. For this reason, the dead volumes detailed here may appear higher than those quoted in the literature for similar devices. Figure 2.21, Figure 2.22 and Figure 2.23 illustrate each of the three interconnection devices, the dead volume in each case is indicated by a solid filled area.

In the case of the high-pressure interface, since the seal between the capillary and the inlet is formed at the surface of the chip and not at the capillary end, liquid is able to flow up around the outside of the capillary due to a mismatch in the chip inlet hole and the capillary. The volume between the capillary and the chip inlet walls gives rise to a dead volume of 129 nL per inlet.

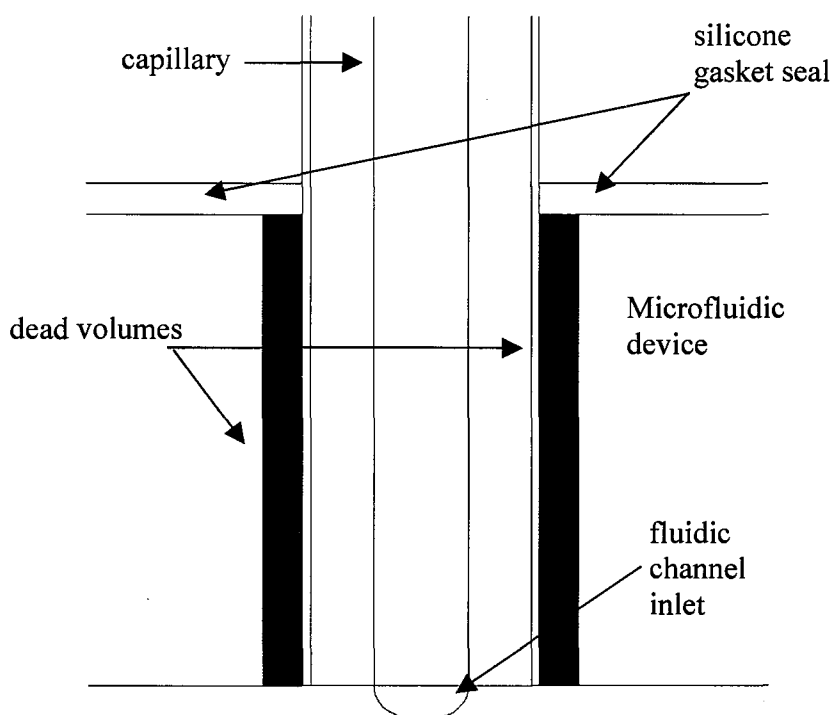


Figure 2.21 Sealing of the high-pressure interface occurs away from the chip inlet leading to a large dead volume due to the inlet hole/capillary mismatch.

In comparison to the high-pressure interface, the flanged tubing interface also forms the seal between the tubing and the chip at the surface of the glass

microfluidic reactor. In this instance, the tubing does not enter the inlet and the solution fills the entire inlet hole. However, since the tubing internal diameter approaches the diameter of the inlet hole, the transition of flow from the tube to the hole is gradual and over the length of the inlet hole the average flow rate does not appreciably decrease. However, where the tube entrance is enlarged to form the flange and encompasses the inlet hole, the overlap of the tube creates a volume in which the solution may become trapped. In addition to the trapped volume at the seal, a second dead volume may occur due to the mismatch between the inlet hole size and the channel size. Here, the sudden size change from the 500 μm hole to the 150 μm channel creates a zone of decreased flow around the channel as indicated in Figure 2.22. These two areas give this design a total dead volume of 41 nL per fluidic inlet.

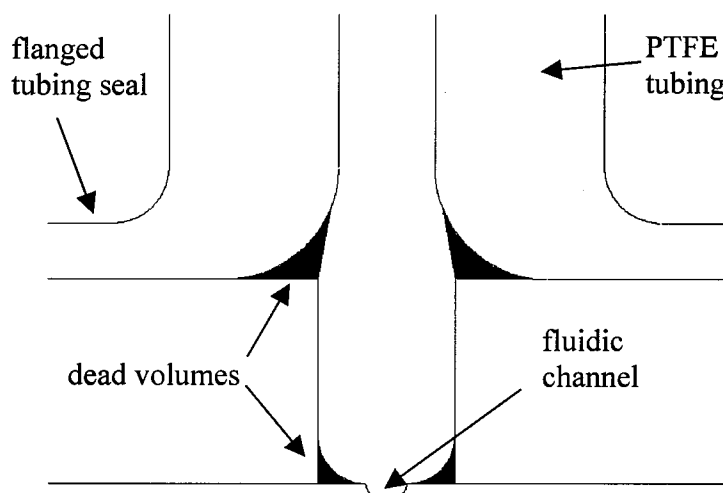


Figure 2.22 Mismatches in the PTFE tube flange and the inlet hole lead and the inlet hole and the fluidic channel lead to the creation of small dead volumes.

The insertion of a capillary into an inlet hole of a microfluidic device and the creation of a seal at the capillary end in the tapered inlet design combine to give an extremely low dead volume (Figure 2.23). The dead volume associated with this interface also originates from a mismatch in capillary internal diameter and the inlet hole size. As the fluid expands rapidly to fill the enlarged section the velocity decreases appreciably. The second area of dead volume, occurs due to

the mismatch between the inlet hole and the fluidic channel. In this case due to the reduction in tube size and the decrease in inlet hole size the dead volume is only 2.7 nL per inlet hole.

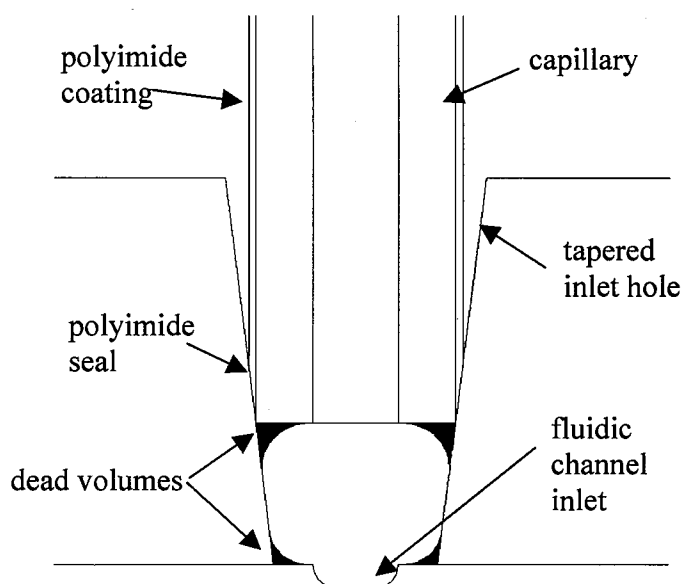


Figure 2.23 Creation of a seal within the inlet hole reduces dead volumes to small mismatches in capillary and fluidic inlet hole sizes.

2.15. Conclusion

To the layman, the most basic operation of sample introduction and removal from microfluidic devices may, at first, appear trivial. In contrast, the interfacing of microfluidic systems to the ‘macroworld’ is one of the most challenging tasks faced by the microfluidic scientist. To this end, a series of interface methods have been examined, resulting in a clearer understanding of the requirements and design constraints imposed by the size and form of most microfluidic devices. For the reversible connection of such devices, a series of clamped interfaces were designed and fabricated. The merits and drawbacks of each were discussed, culminating in a low cost, gasket free interface of good chemocompatibility and low dead volume. With adequate refinement of the inlet fabrication procedure, the tapered inlet interfaces described herein could become the standard method of microfluidic interfacing for glass and silicon devices.

3.

TWO-PHASE FLOWS IN A MICROFLUIDIC REACTOR

Mixing in two-component, biphasic flows such as oil and water or gas and liquid poses significant problems in comparison to mixing in single-phase systems. High interfacial forces between phases cause the coalescence of segregated streams and a reversal of the mechanical splitting process. This chapter presents a study on the effect of expansion/contraction ducts on mixing efficiency in biphasic flows on microfluidic devices. The mixing efficiency for a range of duct geometries and volumes is compared with mixing in both single and biphasic flows in a plain featureless microfluidic channel by fluorescent labelling and emission spectra.

3.1. Mixing in microfluidic reactors

For fluidic channel dimensions measured in the micron range, Reynolds numbers are typically very low and the flow regime is laminar, (see Chapter 1). With the facility for turbulence removed, alternative mixing mechanisms become increasingly important. This can be confirmed by the plethora of papers in the literature describing new mixing methods within microfluidic devices.[1-14] One of the most successful of these methods is mixing by multilamination.[21, 137-142] This has been described previously in Chapter 1, and refers to the physical splitting of parent streams into multiple parallel streams and their subsequent interweaving and recombination. This method not only decreases diffusion distances, but also increases the number of diffusion dimensions. By incorporating n parallel streams, the rate of diffusion is increased by n^2 . [21] This approach however has several drawbacks, most importantly reliability. If an equal pressure drop across each channel is not maintained by the inclusion of additional pressure regulation devices, an uneven pressure drop across the channel can result.[77]

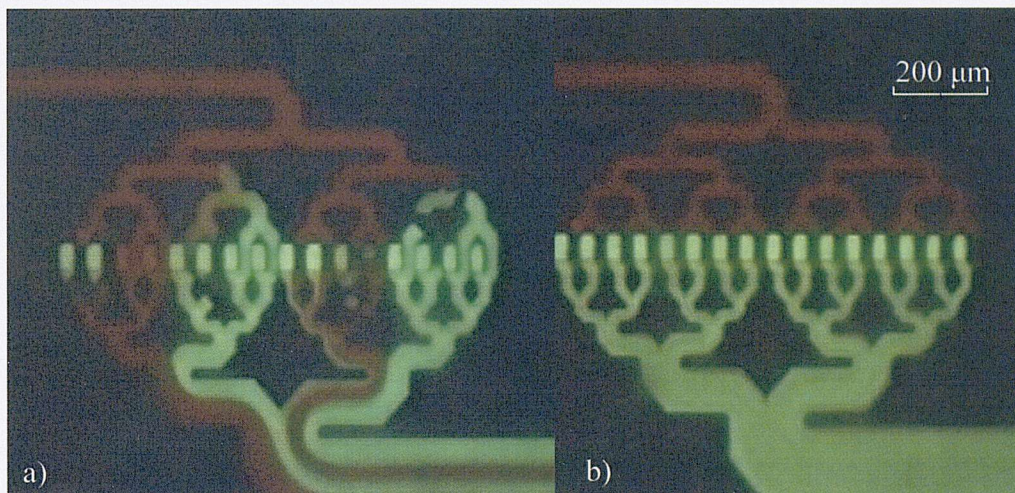


Figure 3.1 Solutions of 40 μM fluorescein in 10 mM borate buffer (pH 9.4) and 100 μM rhodamine B. Comparison of a) Uneven recombination of fluid streams in a multilamination device and b) even recombination of fluid streams.[143]

This leads to an uneven recombination of streams, a decrease in the number of diffusion dimensions and an increase in the diffusion distance (Figure 3.1). In addition to these operational problems, a device for the physical splitting and recombination of streams requires expensive manufacturing techniques to fabricate the multilayer designs normally required.

As an alternative approach, several groups have investigated the use of chaotic advection. Chaotic advection is a flow regime in which fluid streams can adopt long range chaotic trajectories initiated by small, local flow effects.[144-146] Chaotic advection rapidly distorts and elongates fluid elements, significantly increasing the area across which diffusion can occur, and thus facilitating rapid mixing. A transverse component is introduced to the flow by using a steady axial pressure gradient. This pressure gradient is generated by a series of ridges incorporated into the bottom of the channel causing circulation and three-dimensional twisting. This significantly increases mixing rates by increasing the number of diffusion dimensions and decreasing diffusion distances (Figure 3.2).

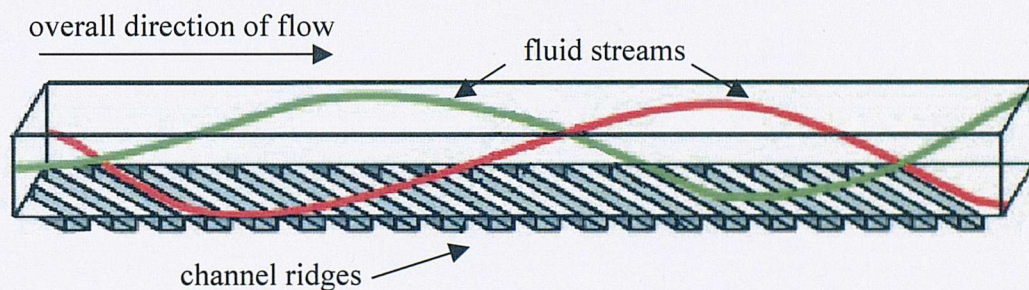


Figure 3.2 Transverse motion induced by ridges at an oblique angle with respect to the long axis. Red and green streams are twisted three-dimensionally and mixing is enhanced.[144]

As previously discussed in Chapter 1, mixing efficiency in a monophasic reaction system can have a profound effect on the extent of reaction and product distribution. In two-component, biphasic flows such as oil and water or gas and liquid, mixing is significantly more problematic. In single-phase systems, diffusion normally occurs between initially highly segregated miscible streams,

until the mixture is considered homogeneous at the molecular level. In two-component flow systems, high interfacial forces cause the segregated streams of individual phases to coalesce. The coalescence of the phases causes a reversal of the mechanical splitting performed by most microfluidic reactors and maintaining discrete, dispersed fluid streams is considerably more difficult. Efficient mixing between oil and water systems has been performed by the formation of emulsions in a microfabricated mixer.[147] By generation of oil and water emulsions, large surface areas are available for diffusion but separation of the two phases become problematic (Figure 3.3). However, it is generally recognised that mixing by diffusion is achievable by contacting liquid-liquid streams without the formation of emulsions, as described by Harper[148] and Harston *et al.*[149]

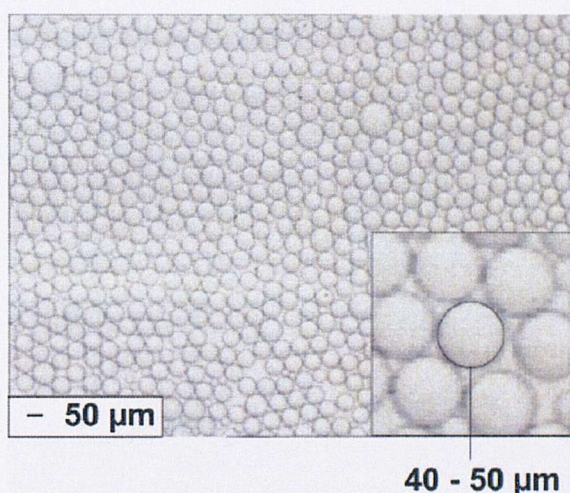


Figure 3.3 Droplets of oil in a dominant continuous aqueous phase. By generation of oil in water emulsions, large surface areas are available for diffusion but separation of the two phases becomes problematic.[140]

Biphasic systems can exhibit a variety of flow regimes due to forces at the liquid-liquid or liquid-gas interface. These interfacial forces are brought about by differences in physical properties of the two phases such as viscosity and surface tension. At lower flow rates ($2 - 20 \mu\text{Lmin}^{-1}$) in an organic/aqueous two component flow through a hydrophilic microchannel, the aqueous phase is dominant and the oil travels as slugs along the channel. Slug flow is defined as

bubbles of one phase in another where the diameter of the bubble is greater than the channel width. As the flow rate increases, ($>20 \mu\text{Lmin}^{-1}$) the pressure increases and the force required to overcome the interfacial tension is approached. This results in a destabilisation of the flow and slugs become smaller and form more rapidly as the interfacial tension is overcome. This unstable, 'bubble flow' then deteriorates further into either annular flow or stabilises as a slug flow. Annular flow describes the formation of a stream of oil occupying the centre of the channel as the hydrophilic channel sidewalls are wetted by the aqueous phase. Further increase in fluid flow rate leads to a rapid dispersion of both phases. It is this dispersed flow regime that has the highest interfacial area and leads to the formation of stable emulsions.

The employment of slug flow to increase mixing in biphasic micromixers was first suggested by Burns and Ramshaw.[57, 150] As previously described in Chapter 1, a velocity gradient exists across the width of the channel due to the friction experienced at the channel walls under hydrodynamic flow conditions. Unlike single-phase flows where a parabolic flow profile exists, friction experienced at the channel walls cause slugs of solvent to undergo internal circulation (Figure 3.4).

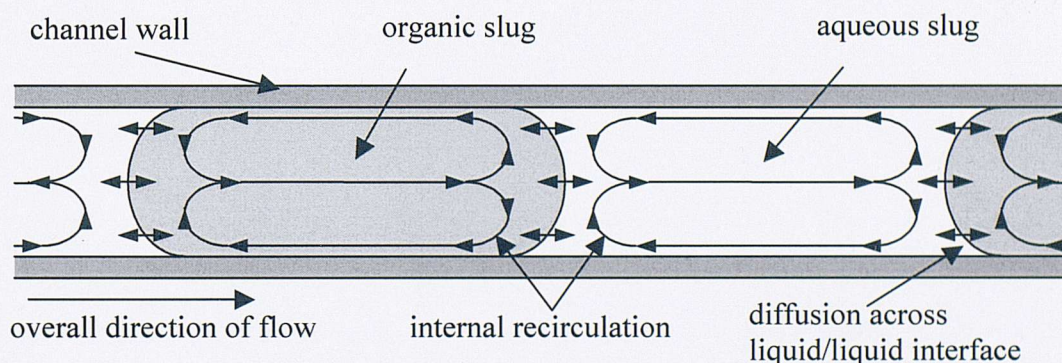


Figure 3.4 Internal circulation refreshes the liquid/liquid interface allowing more rapid mixing within immiscible slug flow.

This circulation continually refreshes the liquid/liquid interface allowing more rapid mixing than offered by simple interlaminae diffusion. In the slug flow

systems described by Burns and Ramshaw, the interdiffusion area between slugs in biphasic systems is greatly reduced in comparison to that experienced in single-component flows. For two laminae, the surface area available for diffusion between the two streams over a channel 10 mm in length, 380 μm deep is 3.8 mm^2 . For slug flow over the same channel length, the diffusion area for each phase is reduced from the length of the channel to the width of the channel multiplied by the number of slugs. For the above system, depending on the slug length, this can equate to a surface area available for diffusion of 0.95 mm^2 . This results in a reduction in surface area of 75% over laminar flow. In addition, mixing in the system described is dependent on the internal circulation of the slugs.

3.2. Expansion-contraction ducts

When a macroscopic pipe undergoes a steady enlargement the fluid experiences a gradual pressure drop as the sloping walls guide the fluid during its expansion and deceleration. If the fluid experiences a sudden pressure drop through an enlargement in the pipe diameter, the velocity abruptly decreases. The sudden decrease in velocity and pressure leads to the generation of turbulence and fluid circulation (Figure 3.5), which in turn leads to minor energy losses (Equation 3.1).[151]

Equation 3.1

$$h_L = K_e \left(\frac{u^2}{2g} \right)$$

Where h_L is the head loss (m), u the linear velocity (ms^{-1}) and g the acceleration due to gravity (ms^{-2}). K_e is the expansion loss coefficient, a unitless representation of proportionality between the energy loss and the velocity head. The magnitude of K_e depends on the geometry of the device that causes energy losses in the fluid flow, (Equation 3.2).[151]

Equation 3.2

$$K_e = \left[1 - \left(\frac{D_1}{D_2} \right)^2 \right]^2$$

Where D_1 (m) and D_2 (m) are the smaller and larger diameters respectively.

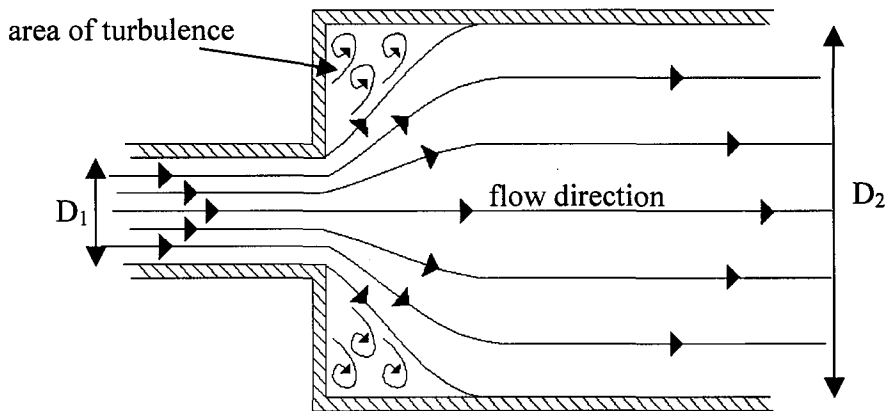


Figure 3.5 A fluid flow through a sudden enlargement in the pipe diameter leads to an abrupt velocity and pressure decrease and the generation of turbulence. Adapted from [151].

Similarly, the energy losses created by a sudden contraction can be calculated in the same way from the ratio of the two pipes and on the velocity of the flow (Equation 3.3).[152] In addition to losses due to turbulence, a contractor has an additional point of energy loss known as the *vena contracta*. As the laminae approach a contraction duct they assume a curved path. Once a curved path has been brought about by the contraction, it continues beyond the narrowest point of the contractor. This leads to a decrease of the stream width to a diameter less than the narrowest point of the pipe (Figure 3.6). Once beyond the *vena contracta* the flow stream undergoes deceleration and expansion again to fill the pipe generating additional turbulence.

Equation 3.3

$$K_c = \left[\frac{D_2}{D_c} - 1 \right]^2$$

Where K_c is the contraction loss coefficient, D_2 the smaller pipe diameter and D_c the diameter of the *vena contracta*.

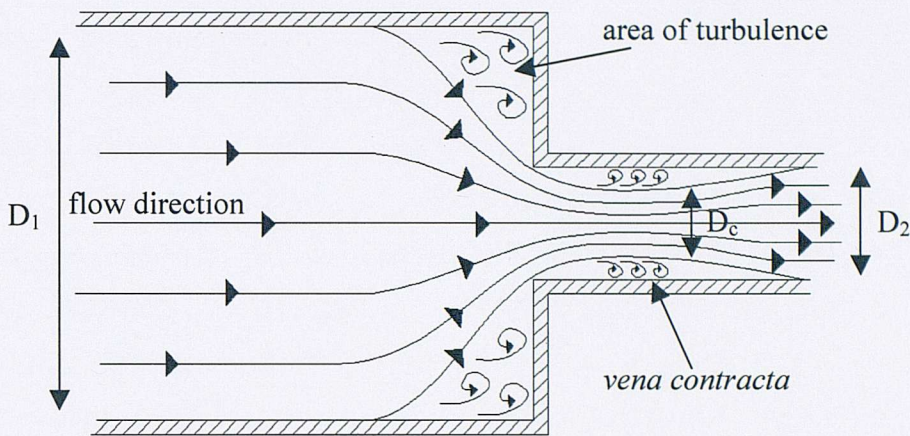


Figure 3.6 Minor losses due to turbulence in an area of sudden contraction. Necking beyond the contraction leads to a second area of turbulence, the *vena contracta*. Adapted from [151].

As K is proportional to the amount of turbulence generated, the above equations can be used to optimise the size ratio of the two pipes in the channel design for maximum mixing efficiency by sudden contraction and expansion (Figure 3.7).

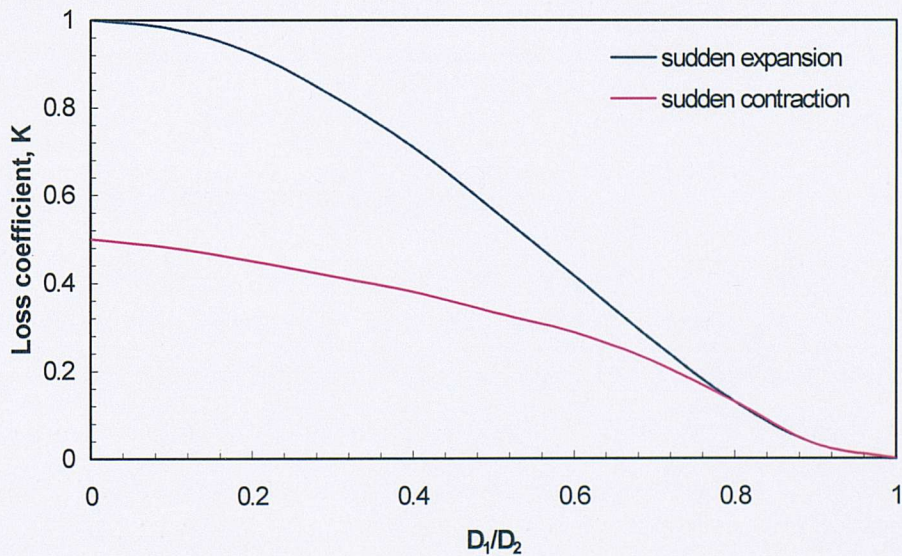


Figure 3.7 The effect of the diameter ratio of two pipes on the resistance coefficient (K) for sudden expansion or contraction in pipe diameter (D).[153]

In macroscale pipes, valves and fittings, areas of sudden expansion and contraction are generally avoided. In large pipe networks these add to the overall pressure drop of the channel and can cause cavitation and other unwanted mixing effects. In the microscale however, such areas of sudden expansion and contraction may lead to increased fluid recirculation and therefore increased mixing in laminar flow regimes. In two component flows, this may be of special importance, where increased mass transfer may lead to increased yields and selectivity. The effect of the channel shape on flow profiles has recently been investigated by Kitamura *et al.*[154] and Mengeaud *et al.*[155] They reported that at high flow rates, laminar recirculations, localised at the corners and walls of a zigzag microchannel, induce transversal components to the velocity gradient. In addition, Verpoorte *et al.* have presented a planar microfluidic device incorporating planar diverging and converging channel elements connected by a narrow microchannel.[156] By combination of these expansion/contraction zones with opposing electroosmotic and pressure-driven flows, the authors were able to trap and manipulate free moving beads in the recirculation zones for heterogeneous assays on microfluidic devices. It is the aim of this chapter to continue these investigations into the effect of channel shape on flow profile. With precise control of diverging and converging channels, the recirculation of fluid streams, in the absence of an opposing electroosmotic flow, may lead to a significant increase in the rate of mixing in microfluidic biphasic systems.

3.3. Microfluidic reactor design

To investigate the effect of sudden expansions and contractions on mixing in biphasic systems, microfluidic channels were fabricated from glass as previously described in Chapter 2. Microfluidic chip structures consisted of two inlets (X and Y – see Figure 3.8), a serpentine channel 120 mm long and an exhaust (Z) at which samples may be collected. Along the first 100 mm of the serpentine channel, expansion/contraction mixing stations were placed periodically. The remaining 20 mm of the serpentine channel consisted of a plain featureless channel for flow observation in an area without mixing stations. The etched

channels have an average depth of 50 μm , an average width of 150 μm and a total volume of 2.2 μL .

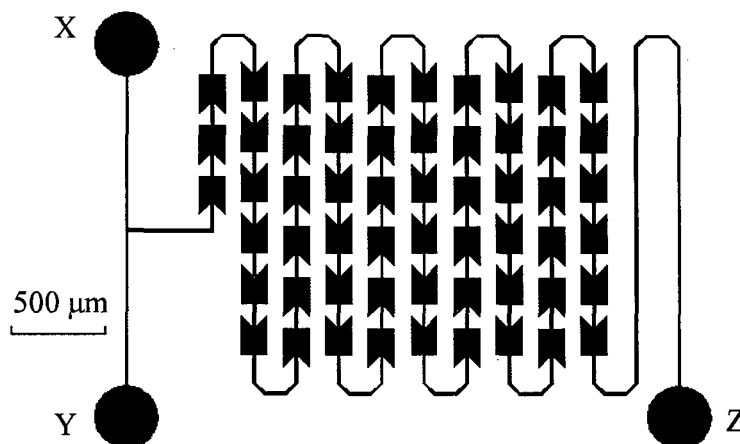


Figure 3.8 Schematic of an example glass expansion/contraction microreactor, consisting of two inlets (X and Y), a serpentine channel 100 mm long with periodically placed mixing stations, a featureless observation channel and an exhaust (Z).

3.3.1. Initial filling

From Figure 3.7 it can be seen that K increases with a reduction in diameter ratio and reaches a maximum when D_1/D_2 equals zero. When a fluid enters an empty, large expansion, for example with a diameter ratio of 0.01, the fluid flow rate is insufficient to overcome surface tension effect and is unable to uniformly fill the expansion region. As the incoming fluid fails to efficiently expel the air from the expansion region, air bubbles are frequently trapped preventing the use of very large expansion zones. To ensure the proper filling of the expansion zones by excluding regions of trapped air, a diameter ratio of 0.25 was employed for all devices giving loss coefficients of expansion and contraction of 0.88 and 0.50 respectively.

For the process of flow pattern characterisation, flow visualisation was used as a tool to gain qualitative information about flow and mixing behaviour. Fluorescein in pH 9 borate buffer was quenched by acetic acid in toluene. The

two solutions were introduced into the serpentine mixing area incorporating a number of expansion/compression mixing stations through a T shaped mixer. In a conventional serpentine mixing channel, introducing both aqueous and organic phases into a channel through a T mixer leads to slug flow, assuming that the T mixer is a simple combination of the two inlet streams with no additional geometrical features. These slugs will be of sufficient volume to fill the channel, their length being dependant on the interfacial tension and fluidic pressure.

When both aqueous and organic phases were introduced simultaneously into the expansion/contraction mixing devices at low flow rates, ($1-2 \mu\text{Lmin}^{-1}$) uneven filling occurred. Once a sudden enlargement section was encountered, the volume of either phase slug was insufficient to completely fill the enlarged section. This led to anisotropic filling and uneven flow with stagnant areas of both aqueous and organic solutions trapped in the enlarged sections. To overcome this, the devices were pre-filled with aqueous phase. The hydrophilic nature of the glass ensured complete filling due to capillary action at the channel wall (Figure 3.9), and prevented stagnant areas of trapped organic phase.

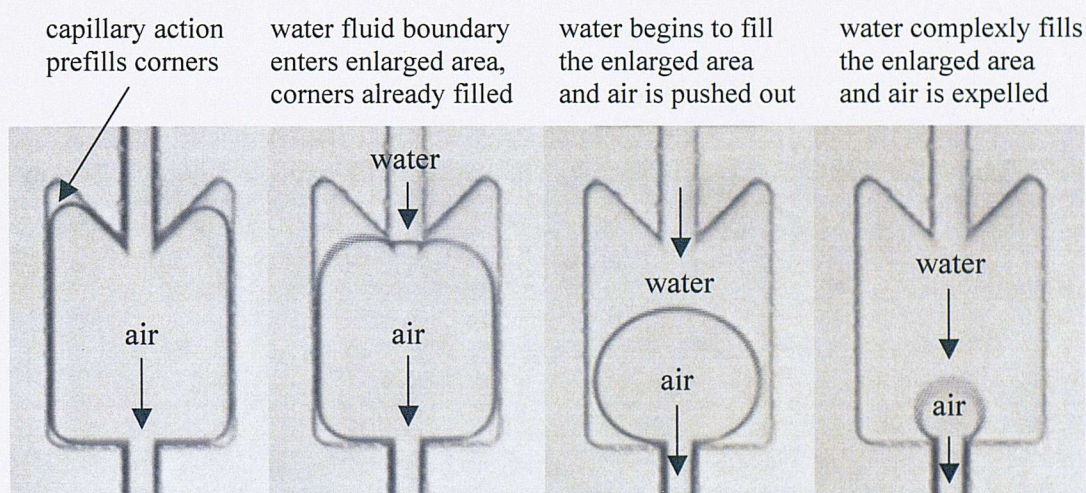


Figure 3.9 Initial filling of an enlarged expansion section only by aqueous solution ensured complete filling and prevented stagnant areas of trapped organic phase.

To ensure symmetrical filling at higher flow rates, ($5\text{--}20\ \mu\text{Lmin}^{-1}$) the channel layout was modified to incorporate a ‘split and mix’ configuration (Figure 3.10). The first inlet channel (X) was divided into two separate streams. The second fluid channel (Y) was then introduced between these streams before their recombination, which created a layered stream (XYX). By introducing the aqueous phase into the first inlet (X), this arrangement ensured that only the aqueous phase interacted with the hydrophilic channel edges throughout the device, essential to symmetrical slug formation.

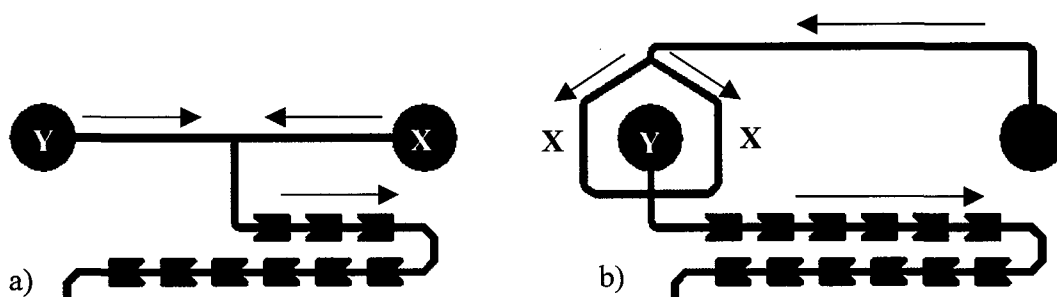


Figure 3.10 a) Use of a traditional T shaped mixer led to uneven filling of expansion/contraction mixing stations. b) A modified split and recombine channel layout for symmetrical filling at high flow rates.

3.3.2. Fluid circulation volumes

As described in section 3.2, once a fluid has undergone expansion in macroscale systems, turbulence can occur in the corners of the enlarged section, the volume of which provides the critical area required for fluid circulation. To examine the effect of corner size and geometry, initial experiments focused on the effect of these corners on the filling and flow of the device. Devices were designed and fabricated, with each incorporating an increasingly large volume for fluid circulation, (Figure 3.11). To maintain experimental continuity, channel depths and widths were fixed to give an aspect ratio of 0.30 and an expansion ratio of 0.25 for all experiments. During injection of fluorescein and toluene into the design of smallest circulation volume at a rate of $5\ \mu\text{Lmin}^{-1}$, the volume of each individual slug of either phase was sufficient to entirely fill the mixing stations as each fluid slug passed through. During the passage of the toluene however, the

hydrophilic channel wall remains wetted by an extremely thin layer of fluorescein throughout the device.

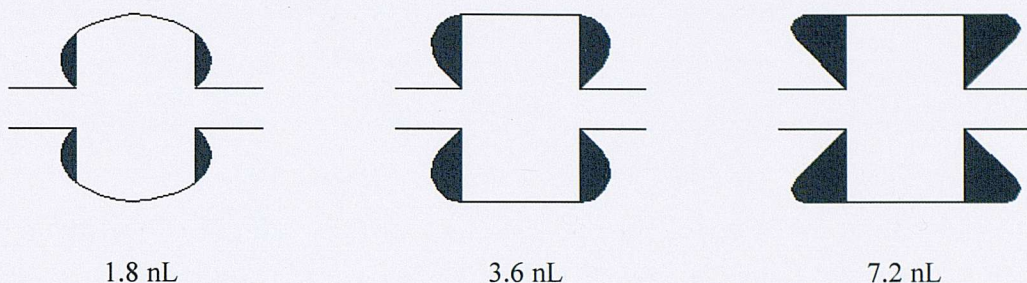


Figure 3.11 Expansion/contraction ducts of increasing circulation volumes (indicated as solid areas).

In addition, as the fluorescein slug exited the mixing station, two small volumes of fluorescein appeared to become trapped by the channel features and the incoming flow of toluene (Figure 3.12). Upon entry of the following slug of aqueous phase, the trapped fluorescein is reabsorbed by the bulk flow. This cycle is repeated at each mixing station throughout the device

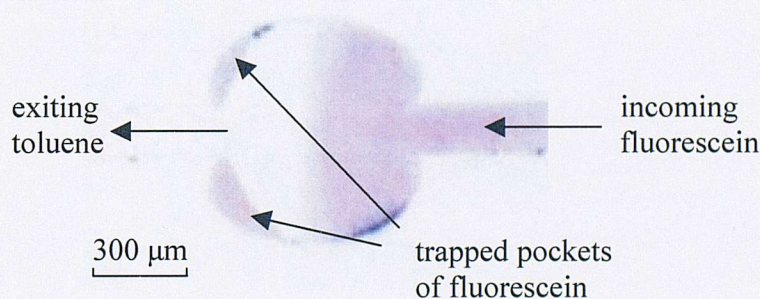


Figure 3.12 Fluorescein trapped by the geometry and the incoming flow of toluene in a 1.8 nL circulation volume.

Once the corner volume of the mixing stations is increased to 3.6 nL, not only is there an increased volume available for circulation but also an alteration in the way the device fundamentally operates. As the organic plug enters the enlarged section it undergoes rapid expansion as predicted by basic fluid theory (section

3.2). However, with an increase in corner size, the volume of a single fluid slug was no longer sufficient to completely fill the enlarged section. This resulted in a 'bubble flow' regime occurring in the enlarged sections where the remaining volume occupied by fluorescein. Upon entry of an organic slug, the aqueous phase in all four corners and at the edges became trapped and was prevented from flowing out of the mixer station, as was previously observed in the front corners of the 1.8 nL device (Figure 3.2). This resulted in an organic bubble occupying the centre of the device surrounded by aqueous phase. As the organic slug is forced out of the enlarged section by an incoming aqueous slug, the trapped aqueous volumes in the corners are replenished by the incoming aqueous slug. Observations of the quenching of fluorescein by acidic toluene did not demonstrate the recirculation of fluid streams in the corners of the expansion zones. Alternatively, it appeared that increased mixing was achieved by the stretching and folding of the fluid elements (Figure 3.13). Firstly the fluorescein rapidly expands to fill the corners of the mixing zone, the corner elements are then compressed and stretched as the following slug of toluene enters the expanded region. These corner elements are then flushed out and replaced by the next incoming fluorescein slug.

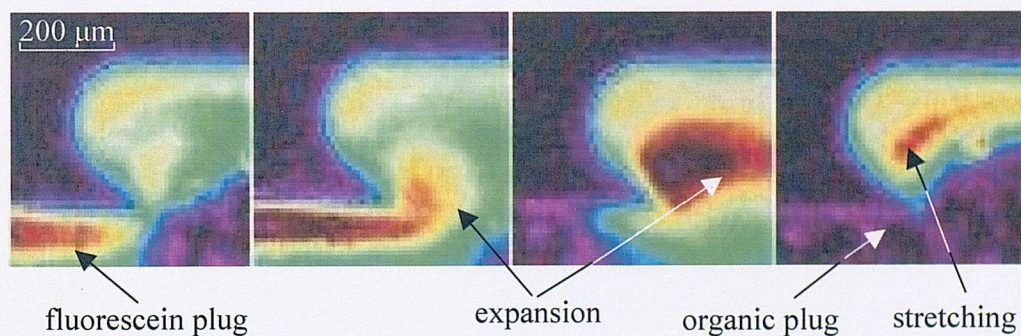


Figure 3.13 The expansion of a fluorescein slug is rapidly followed by deformation and stretching caused by the expansion of the following organic plug (in false colours).

As previously observed in the 1.8 nL volume device, the forward corners of the 3.6 nL device still appear to trap the flowing solutions more effectively than the backward facing corners. Increasing the corner size to 7.2 nL appeared to

increase the stability of these trapped pockets of fluorescein, the organic phase slug was thus surrounded equally on all sides by aqueous phase resulting in a device that operated in the ‘bubble flow’ regime as previously discussed (Figure 3.14).

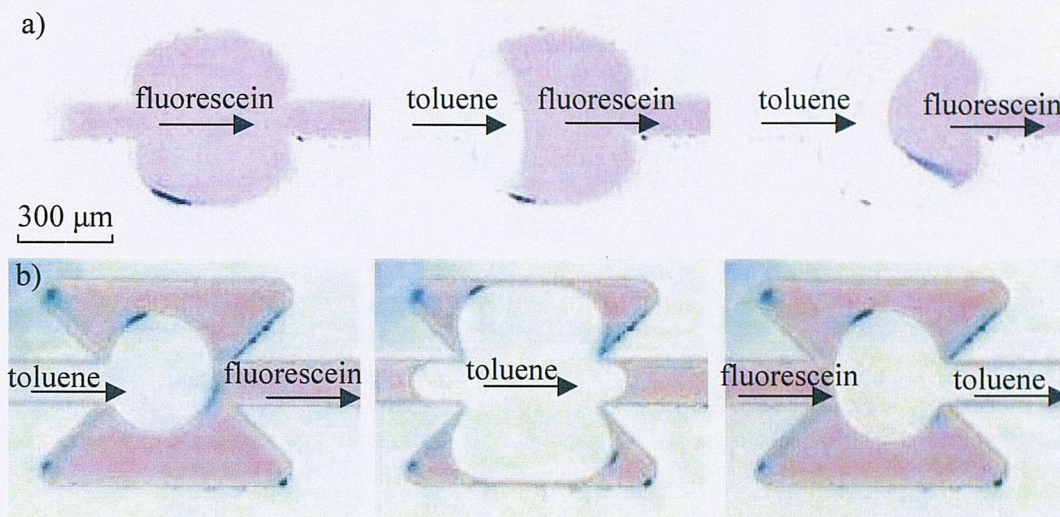


Figure 3.14 a) Complete filling of a 1.8 nL mixing station by toluene at $5 \mu\text{Lmin}^{-1}$.
b) Increased corner volume allowed the complete encirculation of the organic bubble by fluorescein in the 7.2 nL mixing station at $5 \mu\text{Lmin}^{-1}$.

3.3.3. Angle of abruptness

In addition to the size ratio of the two pipes, the resistance coefficient is also effected by the angle or abruptness of the pipe diameter change. The variation of resistance coefficient with cone angle has been investigated and determined experimentally by Crane.[157] Figure 3.15 represents a series of contraction ducts where the transition from the larger to the smaller diameter (a to d) is increasingly severe. With a gradual transition from a large to a small diameter, an angled or curved duct guides the fluid through the contraction gradually, limiting the amount of turbulence generated. In Figure 3.15c. the transition is sudden, resulting in a greater amount of friction and therefore turbulence, as suggested by a K value of 0.5. In Figure 3.15d the smaller diameter pipe intrudes into the larger pipe, this increases the fluid path length and leads to the greatest

amount of turbulence generated from a sudden contraction. However, it must be noted that the values of K detailed here are those determined using friction factors for clean commercial steel pipe with flow in a zone of complete turbulence.

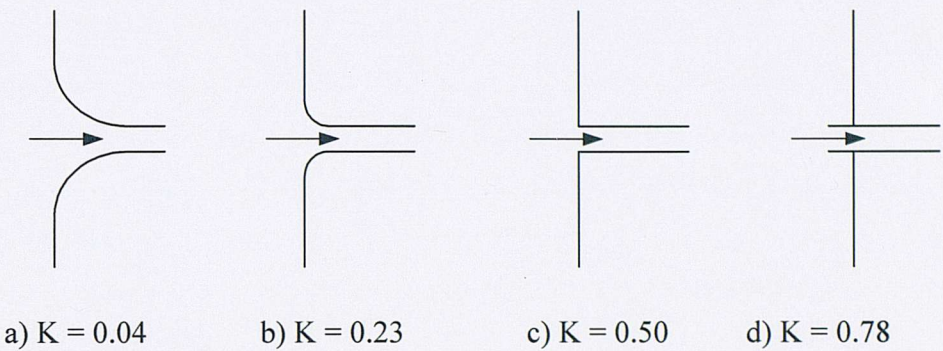


Figure 3.15 Values of K for varying geometrical pipe contractions and expansions.[157]

In experimental studies,[153] the value of the resistance coefficient tends to be lower than the theoretical values when fluid velocity is increased, (Figure 3.16).

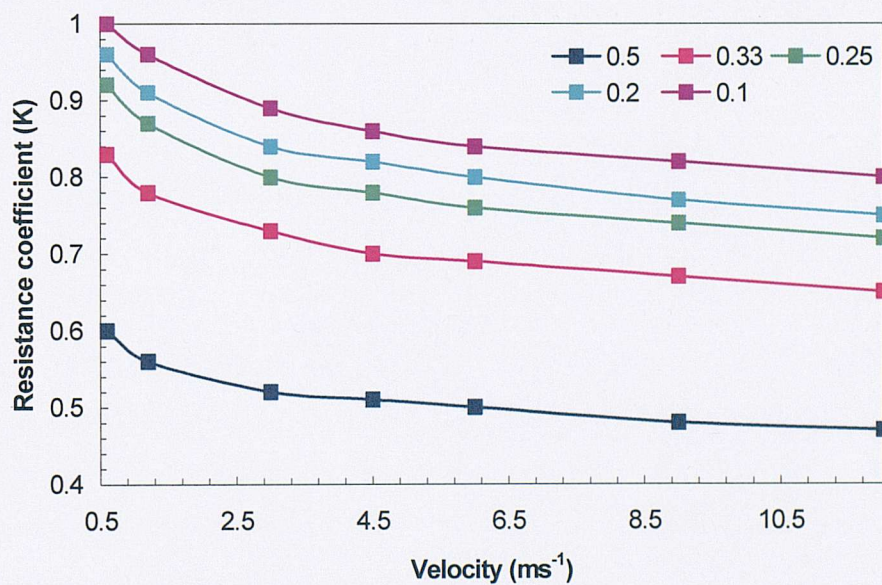


Figure 3.16 Change in resistance coefficient with increasing velocity for a range of dimension ratios.[153]

This has significant implications for microfluidic devices where flow velocities are considerably lower than those experienced in the majority of macrofluidic pipes, valves and fittings.

To investigate the effect of sudden expansion and contraction on mixing efficiency, the structures depicted in Figure 3.17 were fabricated. Here, each design incorporates the 7.2 nL rearward facing corner size previously investigated. This sudden expansion is followed by a contraction, the angle of contraction being reduced from 270 to 33 degrees over a series of designs. As previously stated, each mixer design incorporates an expansion ratio of 0.25 and an aspect ratio of 0.30.

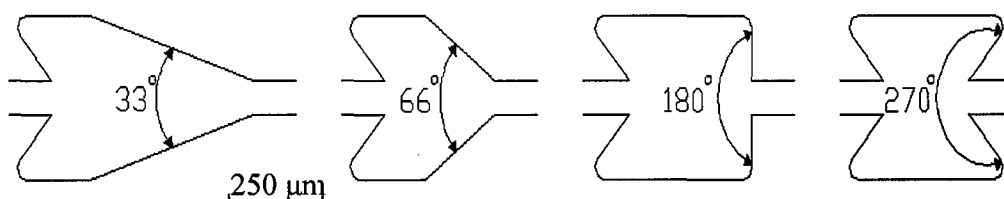


Figure 3.17 Potential geometric configurations of mixing stations for the investigation of the effect of increasing angle of contraction on mixing.

3.4. Two component flow

3.4.1. Results

Mixing stations incorporating an angle of contraction of 270 degrees were excluded from quantitative measurements. Due to the large corners, air pockets frequently became trapped in the direction of flow, (Figure 3.18). With the irregular removal of the circulation areas, the mixing efficiency of the device was decreased leading to a decrease in mixing efficiency.

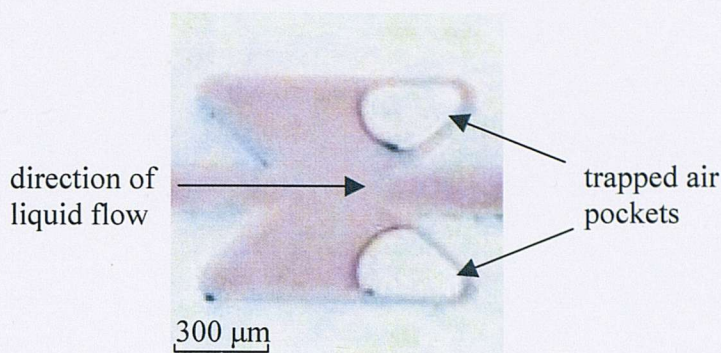


Figure 3.18 The reduced recirculation of fluorescein due to trapped air pockets in a 270° mixing station (in false colours).

The measurement of the mixing efficiency of each contraction/expansion device was performed by the fluorescamine labelling of oleylamine in hexane and compared to that performed in a featureless glass microfluidic channel of comparable dimensions (150 μm wide, 50 μm deep and 120 mm long). Quantitative data were obtained and recorded by the measurement of emission using a photomultiplier tube (PMT) at regular intervals along each device. Figure 3.19 provides an example of raw fluorescence data acquired for the fluorescent labelling in two-component flow on an expansion/contraction micromixer at 5 μLmin^{-1} and demonstrates the slug flow nature of the mixture.

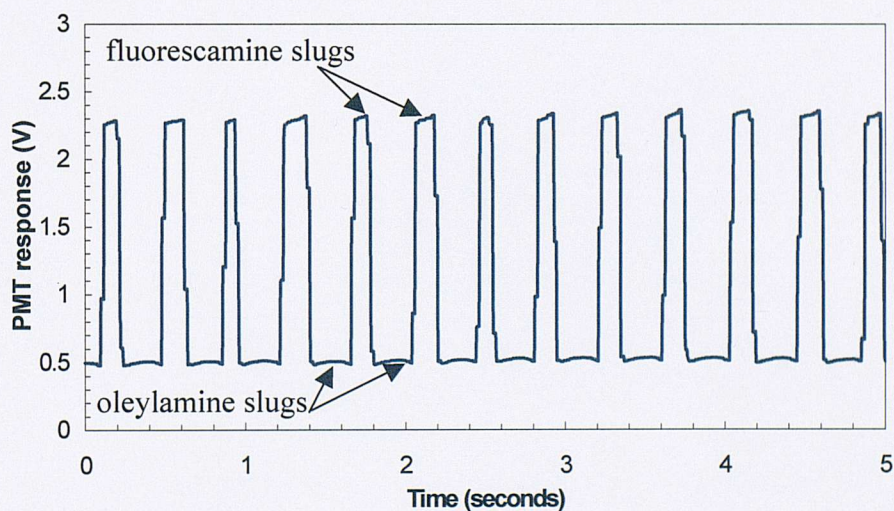


Figure 3.19 PMT signal obtained for fluorescent labelling of oleylamine by fluorescamine (3.5 mM) in two-component flow on a micromixer at 5 μLmin^{-1} , $\lambda_{\text{exc}} = 355 - 425 \text{ nm}$, $\lambda_{\text{em}} 460 \text{ nm}$.

To ensure only fluorescence from a desired point was recorded, a detection window covering a volume of about 1 nL was defined using a 150 μm aperture. The selected area covered the width of the channel and a well-defined length such that the location could be reproduced easily.

Slugs of acetonitrile containing derivatised fluorecamine are clearly visible as peaks in the PMT signal whilst slugs of hexane are represented by troughs in the PMT signal. Fluorescence measurements for each design were collected at 200 samples per second for 30 seconds from each of the five sample areas. Data were then averaged, normalised and plotted as a function of distance in Figure 3.20.

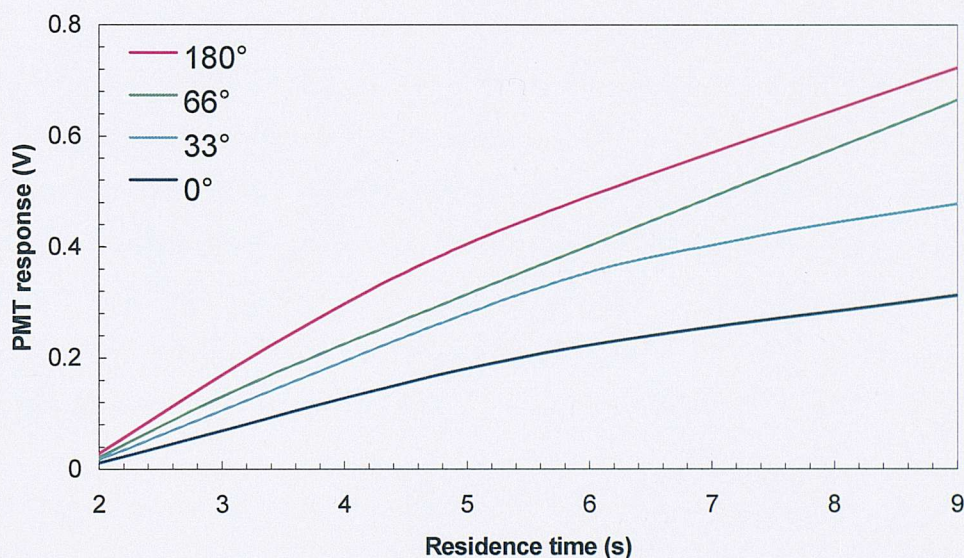


Figure 3.20 The degree of mixing as represented by fluorescence intensity as a function of residence time expansion/contraction mixers of varying contraction angle in a two-component flow system at $5 \mu\text{Lmin}^{-1}$.

Figure 3.20 represents the degree of reaction at set points of increasing distance along the reaction channel converted to a residence time based on the assumption that the volume of each channel is the same. In each case it can be seen that the reaction does not go to completion. This concurs with Frei *et al.* who reported a

reaction time of about 50 seconds for the complete reaction of fluorescamine with oxytocin.[158]

When represented in this form, the use of expansion/contraction mixing stations is demonstrated to lead to an increase in the efficiency of mixing biphasic systems over that of a straight featureless channel and that an increase in fluorescence signal, which is proportional to the degree of mixing, is observed with increasing contraction angle as predicted by Crane.[157] When compared with a straight featureless channel, the amount of fluorescence after 5 cm of channel (equating to 4.5 seconds residence time), is approximately 2.5 times greater in the case of a 180-degree compression angle and 1.5 times greater in the case of a 33 degree compression angle.

An alternative and possibly more accurate representation of the efficiency of mixing for each design is based on the residence time calculated from the volume of each device and the volumetric flow rate. The volume of each device varies due to the changes of geometry. Whilst each of the parameters of the mixing station that may effect mixing efficiency remained the same between each device, i.e. length, width and expansion ratio, it was not possible to vary the angle of contraction and maintain an identical volume for each design.

When the data is represented in this form, it appears that varying the geometry of the mixing stations has little impact on the efficiency of mixing within the microreactors, and additionally that those reactors incorporating expansion/contraction mixing stations are less efficient than a straight featureless channel. However, it should also be noted that the average width and therefore diffusion dimension for the devices incorporating mixing stations is substantially greater than that of the featureless channel. The analysis of this data is further complicated by the complexity of the channel shape and the confining effects of the organic slug on the aqueous phase as it enters the expanded regions. Examination of the currently available data is therefore inconclusive due to the

inability of either model to accurately express all of the various parameters which may positively or adversely effect the comparison of each mixing device.

3.5. Single-component flow

Once the effectiveness of expansion/compression mixers had been examined for biphasic, two-component flows, as a control experiment, the mixing of miscible solvents was also performed. In this case, the water-soluble amino acid norvaline was substituted for oleylamine for binding by fluorescamine. Norvaline was dissolved in a pH 9 borate buffer at 2 mg per 1 mL of buffer and the concentration of fluorescamine adjusted accordingly to 2 mg per 1 mL of acetonitrile. As noted previously, the reaction did not go to completion over the distance examined and, as observed with the acetonitrile/hexane system, an increase in fluorescence signal was observed with increasing angle of expansion (Figure 3.21).

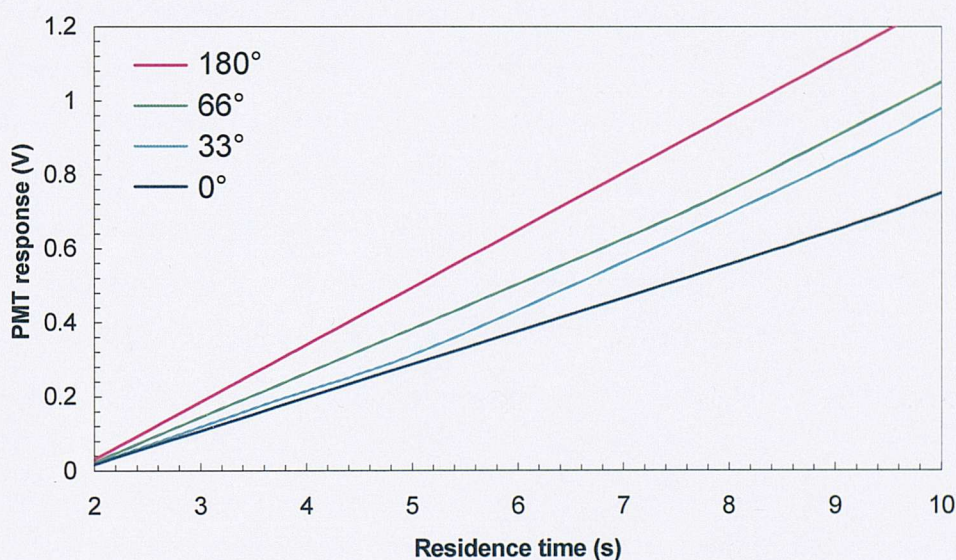


Figure 3.21 The degree of mixing as represented by fluorescence intensity as a function of residence time expansion/contraction mixers of varying contraction angle in a single component flow system at $5 \mu\text{Lmin}^{-1}$.

However, when compared with a straight featureless channel, the increase in the amount of fluorescence after 5 cm of channel, in the case of a 180 degree

expansion, is only 1.6 times greater compared with 2.5 times greater in the case of the biphasic system. Assuming that in both the single and two-component flow systems, the sudden expansion and compression causes circulation of the fluids in the enlarged areas, the increase in mixing efficiency over the featureless channel should be comparable in each case. However, in a single-phase reaction system, the area available is initially restricted to the diffusion interfaces between streams. Due to the miscibility of the two reagent streams this interface area is not fixed and simultaneous diffusion of the two solvents as well as the reagents may occur, increasing the area for reaction to the entire volume of the reaction channel. This miscibility is present in both the straight channel and the expansion/compression designs and therefore no significant difference in the reaction area is encountered.

In the biphasic mixture reaction where the reaction solvents are immiscible, reaction may occur only at the liquid-liquid interface. In a slug flow regime, as encountered in both the straight and expansion devices, the bubbles of organic phase and aqueous phase are so large as to entirely fill the channel for some length. This reduces the surface area available for reaction to the width of the channel at each end of the slug, which is independent of the slug volume. When a slug passes through an enlarged section of an expansion/compression mixer, the slug is able to expand due to the decrease in pressure and reform a circular bubble shape. This expansion is believed to be responsible for the compression, folding and stretching of the aqueous fluid elements within the corners of the device as illustrated in Figure 3.13 leading to more efficient mixing. In addition, the expansion of the organic from a slug to a bubble results in an effective surface area increase of over 2.5 times; a phenomenon not present in the single-component reaction.

3.6. Effect of Reynolds number

In addition to the resistance coefficient, the energy lost due to turbulence generated by a fluid flowing through a sudden expansion is proportional to the

flow rate (Figure 3.22). As previously stated in section 3.3.3, an increasing flow rate leads to a decrease in the resistance coefficient. The variation of Reynolds number as a function of velocity within expansion/contraction mixers was therefore investigated over a range of velocities representative of those encountered on a microfluidic reactor.

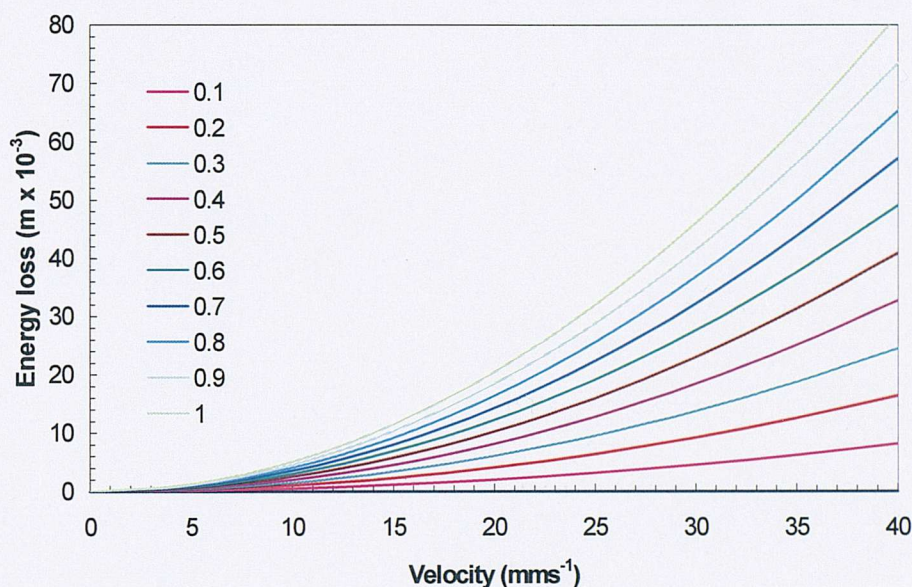


Figure 3.22 Energy loss with increasing velocity for differing values of (K).[153]

In low Reynolds number regimes in a macroscale system, when a flow passes through a single expansion region, in an otherwise featureless channel, recirculating flow occurs on each side of the duct. Previously it has been observed by Cherdron *et al.* that as Reynolds number increase, the length of the regions of recirculating flow on either side of the duct also tend to increase.[159] In addition, their investigation revealed that the disturbances generated at the edge of the expansion may be amplified in the shear layers and that the eddy structure also decreases less rapidly than for low Reynolds numbers. In the case of the microfluidic expansion/compression devices detailed here, the distance available for any recirculating flow to occur was restricted by the distance between the two duct areas. Consequently any additional mixing potential gained from more intense, longer, or more stable eddies may be negated. To

investigate the effect of increasing Reynolds number in the systems detailed above, quantitative fluorescence data were obtained as a function of flow rate ($2.5 - 10 \mu\text{Lmin}^{-1}$) at a fixed point in each device for the single phase labelling of fluorescamine (Figure 3.23).

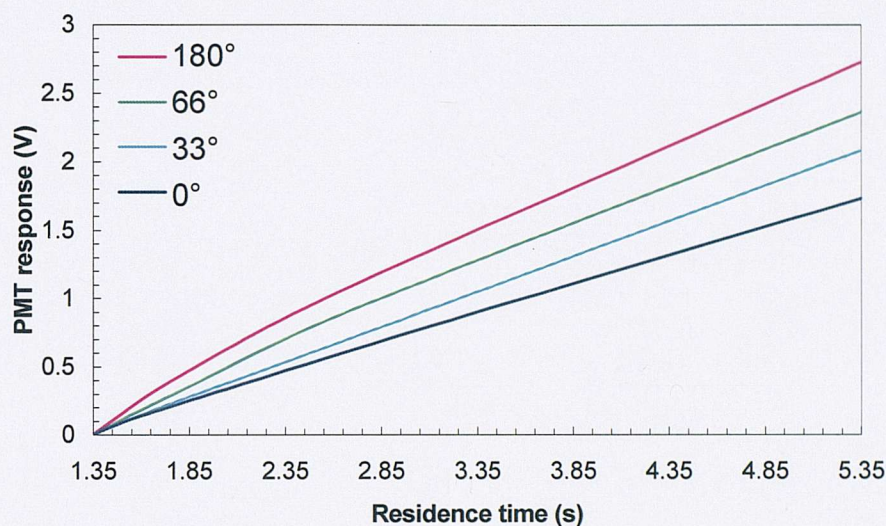
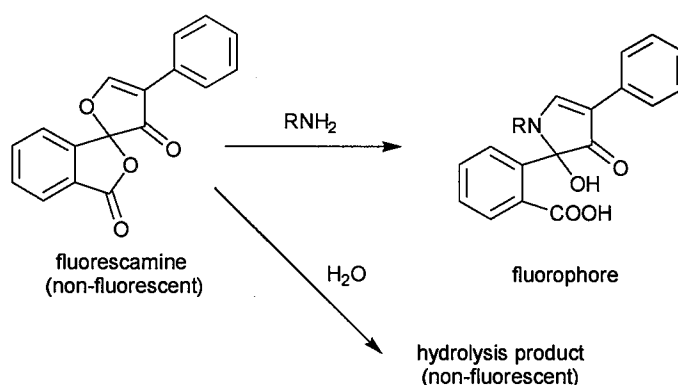


Figure 3.23 Effect of flow rate on the extent of mixing in single component flow with a change in contraction angle.

As demonstrated in section 3.4.1, for a given flow rate, an increase in PMT response was observed for an increasing angle of contraction. However, over the range of flow rates examined, for a constant contractor angle, no significant increase in mixing was observed with increasing flow rate. As the residence time begins to approach 2000 ms, corresponding to a velocity of 25 mms^{-1} a slight increase in gradient is observed. This steepening is representative of a decrease in mixing efficiency with an increase in velocity. It is also apparent that the increase in gradient is most significant for a higher angle of contraction and therefore may be a consequence of a decreasing resistance coefficient. Velocities above 40 mms^{-1} were not investigated in this study. Beyond 40 mms^{-1} the slug flow regime becomes unstable and irregularly deteriorates into an annular type flow. Further investigation into the effect of velocity on mixing is required before any firm conclusions can be drawn on this subject.

3.7. Experimental

For quantitative measurements of the extent of mixing, a fluorescent labelling reaction was chosen. Fluorescamine (4-phenylspiro[furan-2(3H), 1 -phthalan]-3,3 -dione is a commercially available fluorophore precursor that binds with primary amines, usually under mild alkaline conditions,[160, 161] (Scheme 3.1). Fluorescamine has a high fluorescence quantum yield and due to its high rate of reactivity with primary amines, is often used for the quantification of amino acids, peptides and proteins.



Scheme 3.1 Fluorescent binding of fluorescamine for the quantitative analysis of primary amines and its competitive hydrolysis.

Although fluorescamine undergoes a competitive hydrolysis in aqueous conditions, both fluorescamine and its hydrolysis products are non-fluorescent, enabling measurements against a low background.[162] Udenfried *et al.*, who initially developed the reagent, reported reaction half-times in the range of 200 – 1000 milliseconds, depending on the amount of amine used (the experimental conditions were not given), whereas the hydrolysis occurred in approximately 5 to 20 seconds.[161] Due to the hydrolysis of fluorescamine, a non-hydroxilic solvent should be used. For this series of experiments acetonitrile was used due to fluorescamines higher fluorescence signal in acetonitrile over alternative solvents.[158] To maintain a biphasic solvent system, the primary amine (oleylamine) was dissolved in hexane. Concentrations of 1 mg fluorescamine per

1 mL acetonitrile and 1 mg oleylamine per 1 mL of hexane were used for all experiments. Fluorescamine-labelled products absorb light at approximately 390 nm and emit at 460 nm. For excitation, a 75W Xenon short arc lamp (Ushio Inc, Japan) fitted inside an Optosource lamphousing (Cairn Research Ltd, U.K.) was used in combination with a UV+ violet excitation filter ($\lambda_{\text{exc}} = 355 - 425 \text{ nm}$, $\lambda_{\text{em}} = 455 - 470 \text{ nm}$). Fluorescence emission was collected by an “Integra” dual mode photodetection system (Cairn Research Ltd, U.K.). This includes a PMT unit fitted on the head of the microscope, and a separate photomultiplier controller. The PMT unit consists of a PMT, an adjustable diaphragm and a dichroic mirror fitted for simultaneous viewing. The signal was integrated over a period of 20 ms and communicated to a PC via a parallel port.

3.8. Conclusion

The feasibility of increased mixing due to the stretching and folding of laminae induced by sudden expansion and contraction on a microfluidic device has been successfully demonstrated. A series of microfluidic devices incorporating areas of sudden expansion and contraction of varying resistance coefficients were designed and fabricated. Fluorescent derivatisation reactions were employed as a sensitive method to detect and quantify the extent of mixing on the devices for both single and twin-phase systems over a range of flow rates. The relationship between the geometry and resistance coefficients however has not been clearly established where alternative methods of data analysis gave conflicting results. Mixing in two-component systems was established to be significantly improved over that of single phase systems for a given geometry, the increased efficiency being attributed to the stretching and folding of the fluidic elements of the dominant phase in the corners of the expanded mixing stations. Although further work is required for firm conclusions to be drawn, initial experiments suggest that incorporating zones of sudden expansion/contraction where aspect ratio, expansion ratio and the degree of abruptness have been optimised may lead to increasing reaction efficiency in the field of biphasic reactions on microfluidic devices.

4.

DIAZONIUM CHEMISTRY

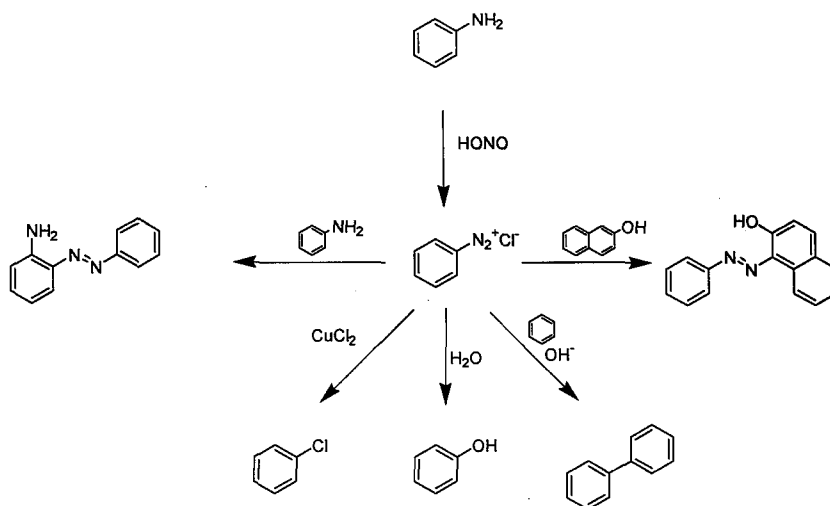
Microfluidic reactors offer many potential advantages to the synthetic chemist including increased product purity and yield. More significant however, is the possibility of new synthetic pathways offered by the high heat and mass transfer properties of microfluidic devices. In this chapter these concepts are explored by the diazotisation of aromatic amines and their *in situ* transformation to azo dyes, and the production of chloroarenes in anhydrous conditions by the Sandmeyer reaction. Furthermore, the use of Raman spectroscopy is demonstrated for dynamic feedback control and online optimisation during synthesis on a microfluidic reactor for the first time.

4.1. Diazonium chemistry

As discussed in Chapter 1, almost any chemical reaction is a mixed reaction/diffusion system. In this situation, product distribution depends on both chemical factors, (e.g. rate constants) and also on the efficiency of mixing. In fast reaction systems where the reagents are initially present in separate feed streams, the reaction seldom occurs throughout the volume available. Alternatively, a mixture is formed that is inhomogeneous at the molecular level leading to a restriction of the reaction. In single product reactions, the rate of reaction is simply reduced to the rate of mixing. However, in systems resulting in two or more products, inhomogeneous mixing may effect the product distribution. In very fast reactions, for example those involving reactive intermediates and competitive-consecutive reactions as described in Chapter 1, the rate of consumption of the reagents is such that steep concentration gradients may be introduced. The increase in segregation, resulting from these steep concentration gradients, may in turn result in an alteration in the product distribution.

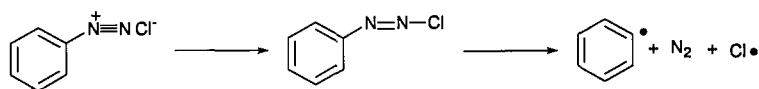
In order to study the application of microfluidic reactor technology to multi-stage synthesis involving reactive intermediates, the formation of diazonium salts, and their *in situ* transformation on microfluidic reactors, was studied. The diazotisation of aromatic amines is an industrial process of vast importance,[163] being useful for the synthesis of a wide range of species including azo compounds, biphenyls, hydroxyarenes and chloroarenes (Scheme 4.1).

The difficulties involved in large-scale diazonium chemistry are numerous however, diazonium salts being sensitive to physical agents such as heat, light, shock, static electricity and dehydration that can lead to rapid, uncontrollable decompositions and explosions.[164]



Scheme 4.1 Some example de-diazonation reactions.

Conventionally, diazonium chemistry is performed on the bulk scale in aqueous conditions. In the absence of water, diazonium salts undergo molecular rearrangement. This is followed by rapid decomposition by either the explosive spontaneous elimination of nitrogen or the production of the free neutral phenyl radical and atomic chlorine (Scheme 4.2). Diazonium chemistry can be performed on the bulk scale in anhydrous conditions, but only by using diazonium tetrafluoroborates, certain arenediazonium sulfonates or in the presence of complex anions e.g. zinc double chlorides and hexafluorophosphates.[165] Despite these precautions however, incidents of explosive decomposition of diazonium salts[166] and vapour phase reaction[167] have been reported and subsequently the use of diazonium intermediates in industry is normally subject to stringent safety procedures.



Scheme 4.2 The molecular rearrangement and decomposition of benzene diazonium chloride.

4.2. Diazonium coupling - the formation of azo dyes

For processes such as the formation and coupling of diazonium salts, the high degree of reaction control and increased safety make microfluidic reactors extremely attractive for reaction optimisation and operation in a continuous-flow format. Synthesis of coloured compounds and intermediates is a well-established technique for following the progress of a reaction and the characterisation of mixing in both a macro format and in microfluidic reactors. For example, the synthesis of azo dyes by the reaction of pre-formed diazonium salts under EOF has been performed previously in single-function reactor chips.[68], [168] In these investigations, the rapid formation of the azo dye from the diazonium salt was utilised for the characterisation of electro-osmotic flow for reagent motivation. In addition, a fast-flowing microfluidic reactor utilizing mixer vanes and heat exchangers has been demonstrated by the production of azo dyes by Clariant.[169]

It is the aim of this chapter to investigate the application of microfluidic reactors to the initial mixing of the nitrite and an amine for the production of potentially explosive diazonium salts *in situ* followed by reactive quenching for the synthesis of azo dyes and chloroarenes. In addition to the potential safety advantages to be gained from operating in a continuous flow microfluidic format, increased mixing efficiency and isothermal operation may lead to increased yields and decreased by-product formation.

4.2.1. Microfluidic reactor design

As previously discussed, the formation of azo dyes is a two-step process. First is the treatment of a primary aromatic amine with nitrous acid to form the diazonium salt, aliphatic diazo compounds being too unstable to be isolable. Resonance stabilised aromatic diazonium ions prepared in aqueous conditions are stable at temperatures below 5°C and can be used without isolation. The second step involves treatment of the diazonium salt with a phenolic substrate leading to the formation of an azo dye. To accommodate both the formation of

the diazonium salt and its *in situ* conversion to the azo dye, initial experiments were performed on a modular design device comprising two microfluidic reactors coupled via a short length of capillary (Figure 4.1).

The glass microfluidic reactors were fabricated in-house using direct-write laser lithography, wet chemical etching and bonding techniques as described in Chapter 2. Firstly, the fluids were mixed diffusively in a simple T-junction mixer before being transported via capillary to a second serpentine delay module. Operating in a modular format allows greater operational flexibility. For example, should either process prove to require an increased, (or reduced) mixing time, each section could be replaced independently.

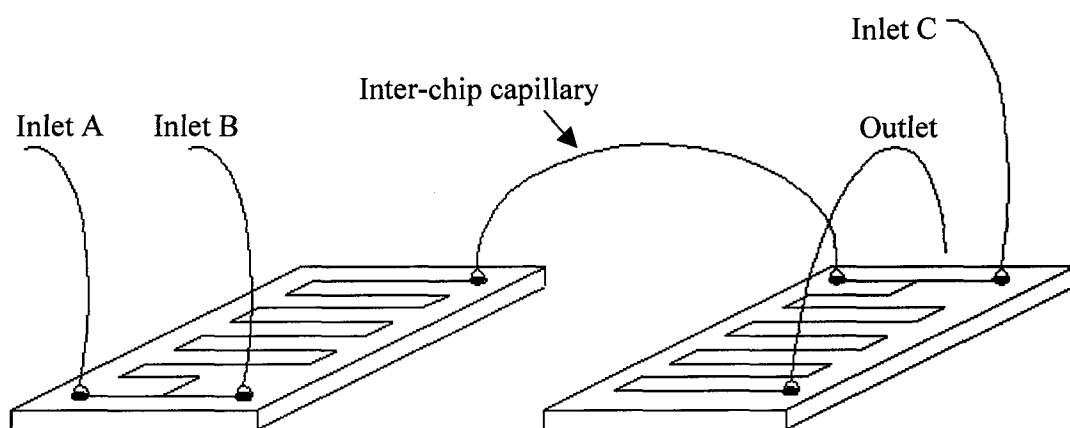


Figure 4.1 A modular approach to multistep synthesis of azo dyes.

However, the advantages of operating on a modular format were counterbalanced by experimental factors. Of particular concern was the increase in dead volumes associated with the coupling of reactor units. In dead volumes, where flow rates may approach zero, reagent precipitation can lead to blockages and fouling of the reactor. In systems involving unstable compounds, or those which require expeditious quenching, salt deposition has been shown to lead to explosive decomposition of reactive intermediates.[166] In addition, the increased transportation distances associated with a modular design leads to an increase in experimental complexity. Whilst stabilised diazo compounds can be handled

with relative ease, transport of more reactive diazo intermediates must be carried out at low temperatures to avoid thermal decomposition of the intermediate leading to unnecessarily elaborate support equipment. Consequently, to avoid blockages and fouling of the reactor an alternative approach to diazonium salt generation and quenching was necessary. A simpler and more elegant approach is to minimise transport distances by incorporating both the initial mixing section for the generation of the unstable intermediate and the reactive quenching on one monolithic chip design (Figure 4.2).

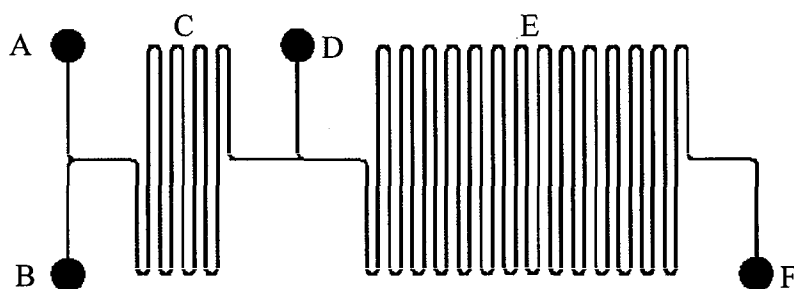
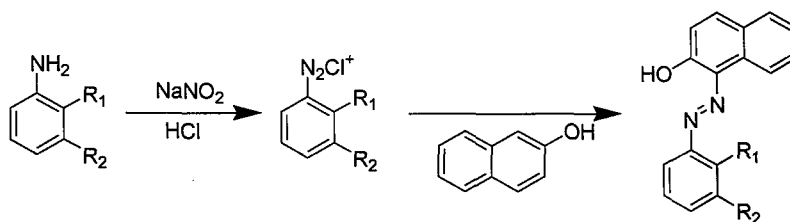


Figure 4.2 Schematic of a monolithic microfluidic reactor channel pattern.

By incorporating reagent mixing and quench addition onto a single device, transport distances and dead volumes are minimised preventing premature decomposition and salt precipitation. The combination of the two operations is realised in the device layout represented in Figure 4.2. The chip consists of a 'T' shaped inlet (A and B), a serpentine primary reaction sector 80 mm in length (C), a second inlet channel (D), a 260 mm long serpentine reaction sector (E), and an exhaust (F) at which samples may be collected for analysis. The etched channels have an average depth of 50 μm and an average width of 150 μm and a total volume of 2.55 μL .

To study the utility of a monolithic microfluidic reactor for diazonium chemistry, the synthesis of Sudan 1 and its analogues was performed (Scheme 4.3).



Scheme 4.3 The synthesis of Sudan dyes from benzene diazonium chloride.

The Sudan series of azo dyes, typified by Sudan 1 (Figure 4.3, 3), are commonly used as microbial stains.[170] The highly coloured nature of the products renders detection facile and the reaction is rapid enough to avoid excessive delay in dye formation. Azo dyes came to prominence in medicinal chemistry after the discovery that sulphanilamide (Figure 4.3, 1), the active metabolite of the azo dye Prontosil Rubrum, had *in vivo* antibacterial properties. Despite being superseded by the penicillin antibiotics in most antimicrobial applications, sulpha drugs such as sulphasalazine (Figure 4.3, 2) still find application in the treatment of Crohns disease and ulcerative colitis.[171]

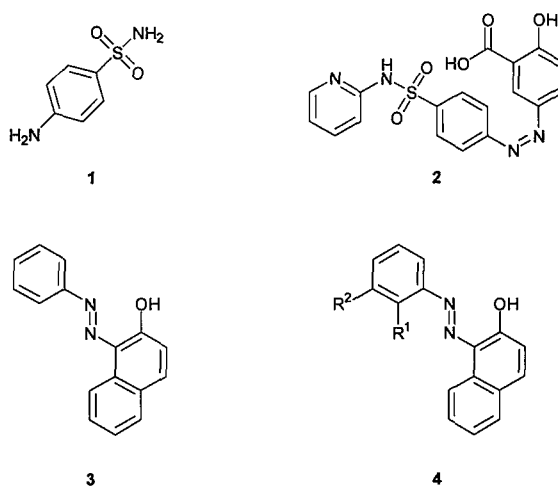


Figure 4.3 Sulphanilamide (1), and some typical azo dyes, (2) Sulphasalazine, (3) Sudan 1 and (4) 1-(2-methylphenylazo)-2-naphthol where $R^1 = \text{Me}$ and $R^2 = \text{H}$ and 1-(3-methylphenylazo)-2-naphthol where $R^1 = \text{H}$ and $R^2 = \text{Me}$.

Using the fluidic network as shown in Figure 4.2, solutions of amine and sodium nitrite were introduced into the first mixer segment, each at a rate of $3.2 \mu\text{l/min}$ using a Harvard Apparatus PHD 2000 syringe pump. The flow rates used in

these experiments were chosen to ensure a good degree of mixing (Fourier number = 0.25), while minimising the time required for a sufficient sample volume to be collected for analysis. A solution of β -naphthol was then introduced into the second mixer segment at a balancing rate of 6.4 $\mu\text{l}/\text{min}$ (Fourier number = 0.4, Reynolds number = 2). Azo dye formation was evident by the immediate formation of a persistent red coloration in the efferent streams of the second serpentine section (Figure 4.4). After visual detection of the products, the reactors were run for extended periods to collect samples for analysis.

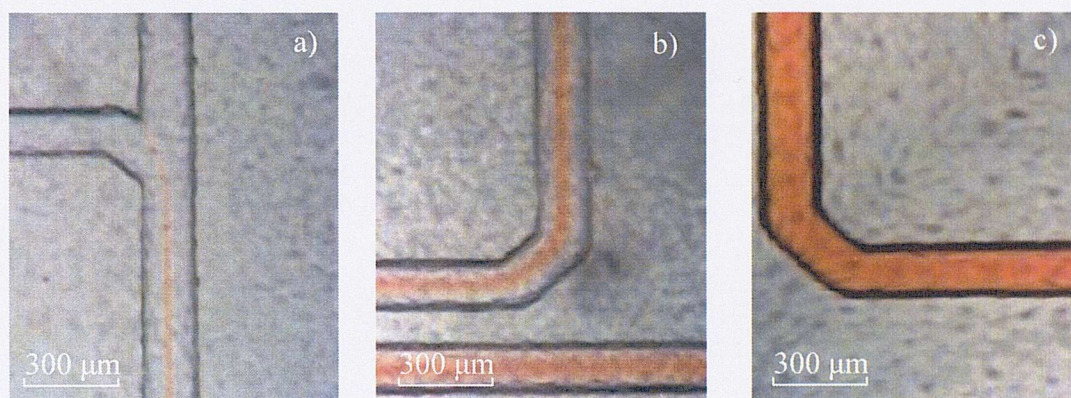


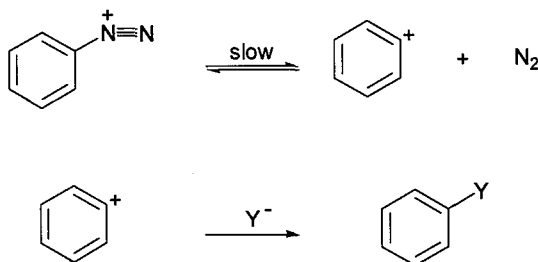
Figure 4.4 Azo dye formation on a moolithic microfluidic reactor (see Figure 4.2). a) Addition of reactive quench from inlet (D). b) Formation of the dye at the beginning of section (E) as diffusive mixing occurs. c) Complete mixing occurs prior to collection at the outlet (F).

4.2.2. Results and discussion

The formation of dye in the secondary section of the microfluidic reactor represented in Figure 4.2 (E) conclusively demonstrates the successful synthesis of the diazonium salt intermediate in the first mixing section (C). The formation of the coloured azo dye also allows rapid characterisation and optimisation of the mixing process by examination of the colour intensity and diffusion pattern (Figure 4.4). For example, the formation of dye some distance after the introduction of β -naphthol (inlet D) would indicate that an increase in residence time is required for the formation of the diazonium salt. In contrast, a striped

pattern (as demonstrated in Figure 4.4 b) at the exit (F) would suggest that an increase in residence time during section E is required to achieve a maximum yield of the dye.

Product analysis was performed by ^1H and ^{13}C NMR and infrared spectra which showed close agreement with data given in the literature.[172, 173] In these initial experiments the diazotisation reaction was not optimised. Conversions ranging between 9 and 52% were obtained, calculated from the parent amine by extinction coefficient, the variation in yield being dependent on both flow rate and the substrate diazotised. The reaction of diazonium salts is thought to proceed by the rare unimolecular aromatic $\text{S}_{\text{N}}1$ mechanism (Scheme 4.4) in water and with most nucleophiles.[174]

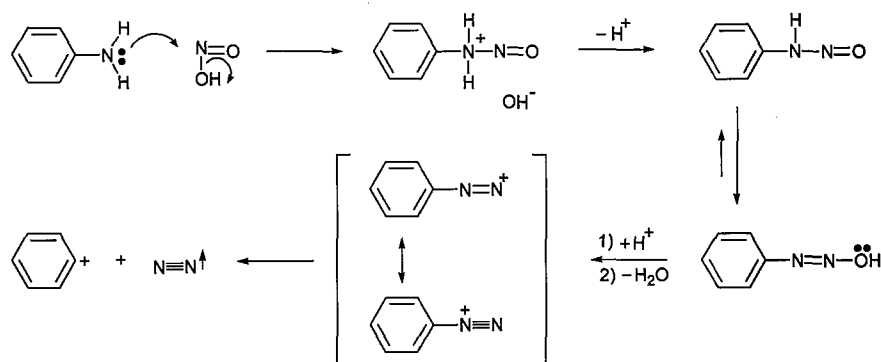


Scheme 4.4 The aromatic $\text{S}_{\text{N}}1$ mechanism with an aryl cation intermediate.

In this situation, the reaction rate is first order with respect to the diazonium salt and independent of the concentration of the nucleophile. From close examination of GC-MS data obtained for the formation of Sudan 1 and its analogues, a concordance between the extent of diazotisation of the aminoarenes and the yields given above was observed. This was ascertained from the presence of unreacted amine and insignificant amounts of by-products from cross-coupling reactions. The relationship between yield and formation of the diazonium salt concurs with the assumption of an $\text{S}_{\text{N}}1$ mechanism and suggests that optimisation of the diazotisation step would lead to enhanced reaction yields. In an enclosed, continuous flow microfluidic reactor, this is additionally noteworthy. During the de-diazonation step, nitrogen evolution has a considerable effect on the residence time. However, since the more significant

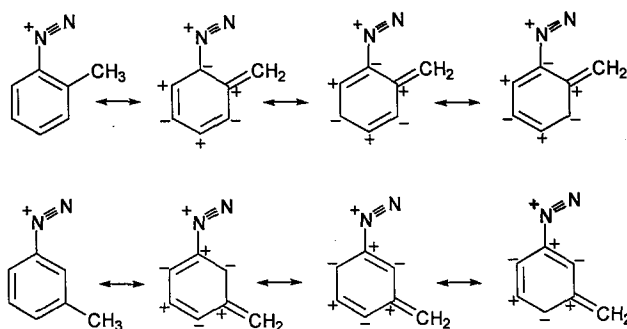
rate determining diazotisation step occurs prior to the substitution, the reduced residence time caused by the nitrogen evolution should have little impact on the yield.

As previously mentioned, the yield was also affected by the substrate diazotised. Substrates containing alkyl groups such as the toluidines used in these experiments often result in disappointing yields.[175] By examination of the effect of steric hindrance alone on the ultimate yield, it would be reasonable to expect that aniline would demonstrate the greatest yield, followed by meta toluidine and ortho toluidine respectively. Alternatively, the relative positions of ortho and meta substitution in terms of yield are reversed, while the diazotisation of aniline remains most efficient. The influence of the methyl substituent on yield can be explained by an examination of a proposed mechanism for formation of the diazonium salt (Scheme 4.5).



Scheme 4.5 A proposed mechanism for the formation of the diazonium salt.[174]

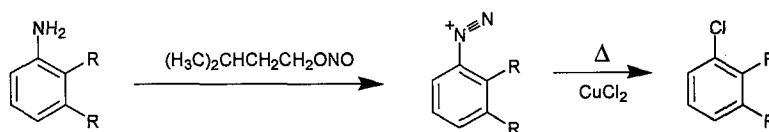
The relative stability of the aryl diazonium salts compared to alkyl diazonium salts is largely due to the stabilising effect of the π orbital system. Consequently, by the addition of electron donating group such as CH₃, further stabilisation and greater yield may be expected. However, by examination of the inductive effect of the methyl substitution on the diazonium salt (Scheme 4.6), it becomes clear that increased stabilisation and therefore yield would be expected by ortho over meta substitution.



Scheme 4.6 The inductive effect of ortho and meta substituted diazonium salts.

4.3. Chloro de-diazonation - the Sandmeyer reaction

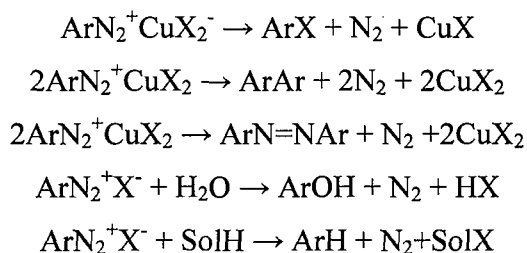
In order to further demonstrate the utility of microfluidic reactors for the formation and reactive quenching of diazonium salts, the microfluidic reactors were utilised in anhydrous conditions (Scheme 4.7).



Scheme 4.7 The Sandmeyer chloro de-diazonations of diazonium salts.

The Sandmeyer de-diazonation of diazonium salts is one of the best ways of introducing a bromine or chlorine into an aromatic ring. Although ineffectual for the production of fluorides or iodides, the Sandmeyer reaction is widely applicable and the yields are usually high. However, the aqueous conditions usually employed in diazonium synthesis limits the applicability of the Sandmeyer reaction to some substrates. In addition, on the bulk scale, precise temperature control of process operations is needed. At temperatures above 283 K, hydroxy de-diazonation is promoted in aqueous media leading to the formation of phenols.[176] In the case of some diazonium salts, higher temperatures can lead to reaction of the parent arene in an explosive manner,

even under normally quenching aqueous conditions.[177] However, at low temperatures the de-diazonation reaction is often slow. Excessive delay leads to an increased likelihood of intermediate decomposition and cross-coupling reactions resulting in increase by-product formation (Scheme 4.8). Although satisfactory yields of aryl halides are usually obtained, the Sandmeyer reaction is complicated by several competing reactions. In addition to the reaction stoichiometry and temperature, the mode of mixing is a significant determinate of the yield of the aryl halide as previously mentioned.



Scheme 4.8 Competitive reactions during the chloro de-diazonation of a diazonium salt.

By performing the Sandmeyer reaction in anhydrous conditions on a microfluidic reactor, a far wider range of previously restricted substrates are made available, while eliminating the competitive hydroxy de-diazonation reaction. Utilising the monolithic microreactor described above, the formation of diazonium salts in anhydrous conditions was studied. To effect diazotisation in anhydrous conditions, isoamyl nitrite was chosen since it is often used on a laboratory scale to perform diazotisations on water sensitive substrates.[178] However, alkyl nitrites are thermally unstable and prone to decomposition or explosion on heating,[172] lower alkyl nitrites have even been known to decompose and burst the container in refrigerated storage. This has limited their use in large-scale synthesis. By using microfluidic reactor components however, such materials can be utilised for industrial processes in relative safety.

In contrast to the formation of azo dyes, the Sandmeyer reaction is performed at elevated temperatures.[175] Using the modular approach allowed the two steps

of room temperature generation and sequential quenching of the diazonium salt to occur independently of one another. By utilisation of a monolithic microfluidic device, although the two serpentine mixing sections are removed from one another by only 10 mm, a steep thermal gradient exists establishing two separate temperature environments. This is due to the low thermal conductivity of glass ($0.937 \text{ Wm}^{-1}\text{K}^{-1}$), and the large surface area for heat dissipation. Depending on the velocity of the solution and the geometry of the microfluidic reactor, a given fluid element can experience either a gradual or rapid temperature variation.

4.3.1. Results and discussion

The optimisation of the rate determining diazotisation step was studied by the variation in concentration of isoamyl nitrite (1-15 mM) and amine (2-10 mM), followed by thermal chloro de-diazonation to the aryl substrate (Figure 4.5 and Figure 4.6).

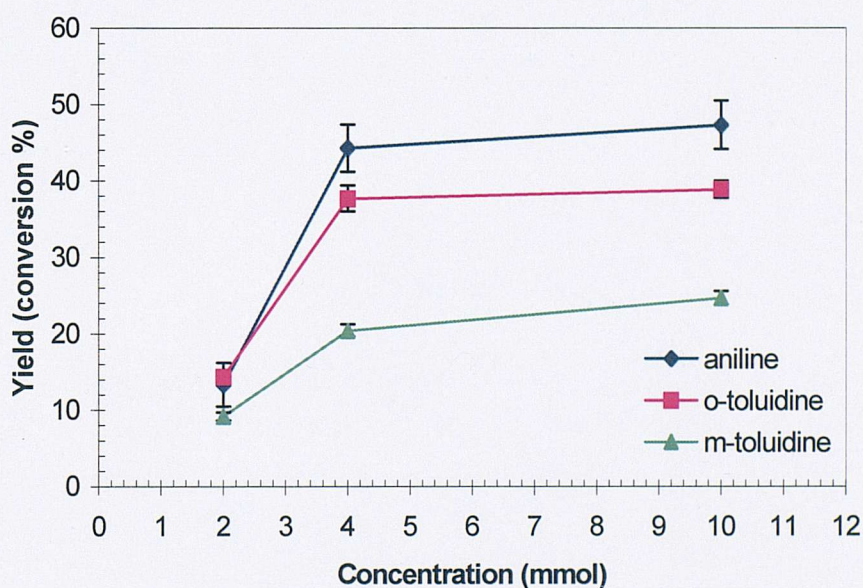


Figure 4.5 The effect of amine concentration on product yield for the aromatic diazotisation/chloro de-diazonation reactions of aniline, *o*-toluidine and *m*-toluidine. Error bars are standard deviations where $n = 2-3$.

Inspection of Figure 4.5 and Figure 4.6 shows that the extent of diazotisation is heavily dependent on both substrate concentration and isoamyl nitrite concentration. Utilising optimised concentration conditions as determined for the diazotisation, chloro de-diazonation was subsequently performed for three separate aryl amines each being diazotised by isoamyl nitrite under identical conditions. Crude material from the microchip effluent was submitted to GC-MS analysis and demonstrated chlorobenzene and chlorotoluene as the majority products. As previously observed in the synthesis of azo dyes, analysis of GC-MS data showed the reduction of the diazonium ion to the aryl radical and its halogen abstraction from cupric chloride to be extremely efficient as established from the presence of unreacted amine and insignificant amounts of by-products from cross-coupling reactions (Figure 4.7).

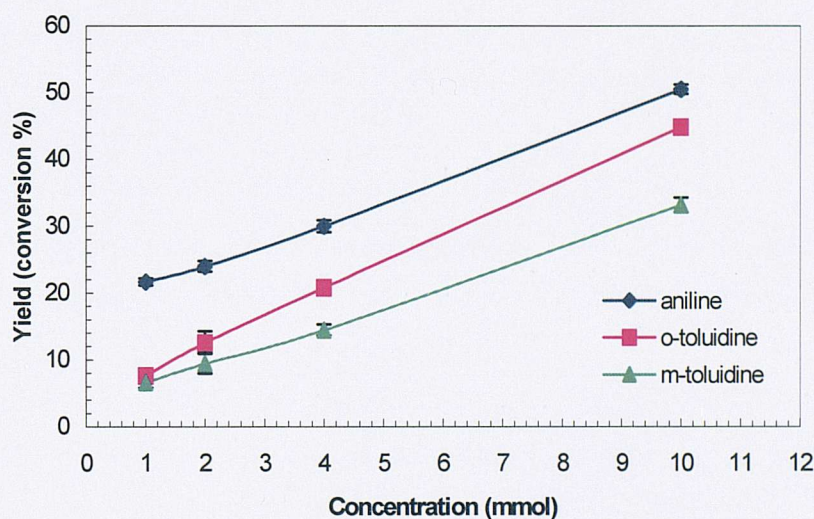
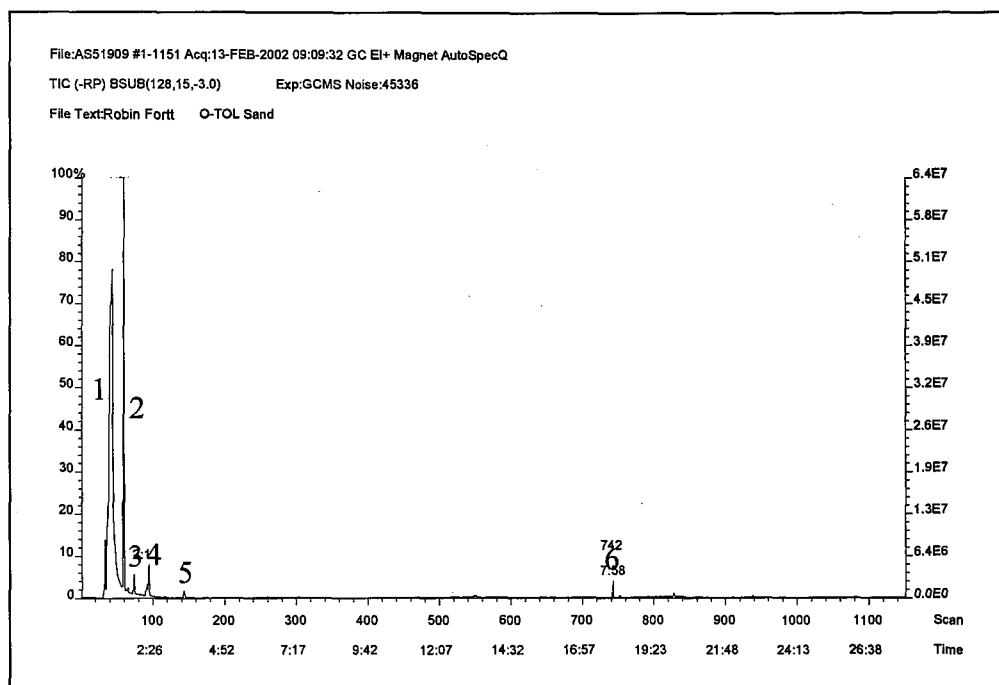


Figure 4.6 The effect of isoamyl nitrite concentration on product yield for the aromatic diazotisation reactions of aniline, *o*-toluidine and *m*-toluidine. Error bars are standard deviations where $n = 2-3$.



Key: 1) $m/z = 73$ DMF (solvent), 2) $m/z = 126$ chlorotoluene, 3) $m/z = 121$ 1-methyl-2-nitrosobenzene, 4) $m/z = 172$ methylbutylester, 5) $m/z = 137$ 1-methyl-2-nitrobenzene, 6) $m/z = 210$ 2,2 -azotoluene.

Figure 4.7 A GC-MS spectra of the resulting reaction mixture following the formation of toluene diazonium chloride and its halogen abstraction from CuCl_2 .

To compare and contrast reaction efficiency in a microfluidic reactor with a traditional laboratory method, a small-scale reaction flask of appropriate dimensions was employed utilising the same solutions and reaction conditions.[175] Utilising the microfluidic reactor described above at flow rates of $3.2 \mu\text{Lmin}^{-1}$ and $6.4 \mu\text{Lmin}^{-1}$ a Reynolds number of 1 and 2 respectively can be calculated for the two sections of the monolithic microfluidic mixer. These values of Reynolds number firmly establish the device as operating in the laminar flow regime with a parabolic concentration profile. In comparison to the bulk scale reaction flask, in each case significant enhancement in yields on a microfluidic reactor (15 – 20%) were obtained in contrast to bulk solutions (Figure 4.8). This yield enhancement may be attributed to several factors, including the excellent heat and mass transfer properties generally observed for microfluidic reactors. By application of Nusselt's number (see Chapter 1) to the

monolithic microreactor illustrated in Figure 4.2, a high conductive heat transfer coefficient of $60,000 \text{ Wm}^{-2}\text{K}^{-1}$ and an overall heat transfer coefficient (U) of $740 \text{ Wm}^{-2}\text{K}^{-1}$ can be calculated. Although the value of U is not significantly greater than that of a conventional macroscale reactor due to the relatively thick reactor walls, it is the conductive heat transfer which is mechanistically important. In macroscale reactions, inefficient mixing leads to the concentration of reagents in localized zones. This leads to the formation of hotspots, which may alter the course of the reaction. Efficient conduction of heat away from these localised zones within the channel act to reduce the formation of undesirable by-products leading to an increased yield.

As previously observed in the synthesis of azo dyes, a range of yields was obtained depending on the substrate where the presence and position of electron donating groups on the diazonium salt has a negative impact on yield.[175] It should be noted that Sandmeyer syntheses employing alkyl nitrites can result in yields inferior to those utilising sodium nitrite. In addition, a full process optimisation of the Sandmeyer reaction was not performed. Furthermore, no attempt to optimise temperature conditions was made during this study nor was an acid catalyst employed.

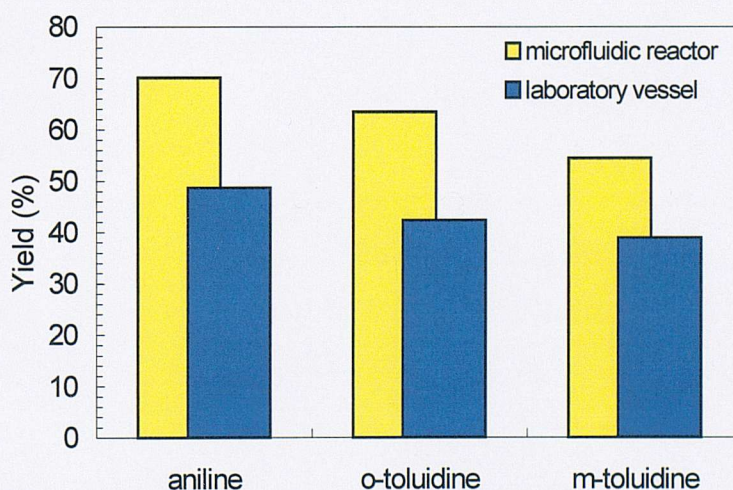
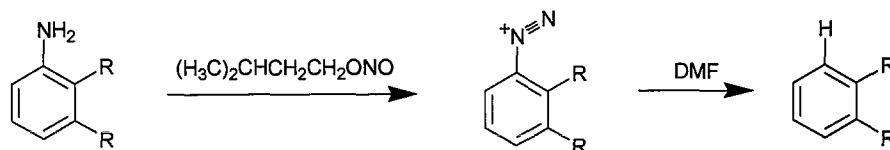


Figure 4.8 Comparative product yields for the aromatic diazotisation/hydro de-diazonation reactions of aniline, *o*-toluidine and *m*-toluidine using the microfluidic reactor and conventional bulk syntheses.

4.4. On-line optimisation

Although attractive as a detection method, GC is an indirect and off-line approach to product analysis. Results are typically obtained after transfer from the reaction vessel with analysis times typically in excess of 30 minutes. Direct monitoring techniques such as UV-Vis absorption and FT-IR could be employed for monitoring the extent of diazotisation as demonstrated by Jensen[86, 105] and Lendl.[179] However, as glass is the favoured medium for developmental organic chemistry, these methods are impractical due to high extinction coefficients of glass in the UV and IR regions of the electromagnetic spectrum.

Raman spectroscopy utilises monochromatic light usually in the visible region of the spectrum and is an established real-time, on-line fingerprinting tool for chemical identification.[180] The combination of glass microfluidic reactors and Raman spectroscopy allows for the direct on-line detection of analytes *in situ*, without the need for reaction quenching. Indeed, Raman spectroscopy has previously been used in microfluidic systems as a detection technique for electrophoretic separations in both capillaries and microfabricated channels.[181, 182] It should be noted that, due to the curved nature of conventional capillary walls, scattered photons are increasingly dispersed generally leading to a weakened Raman signal. However, the flat, uniform nature of microchannels and substrate surfaces in chip-based systems reduces scatter dispersion and ensures that scattered photons are collected only from the solution and not from the glass substrate.[182] To demonstrate the use of Raman spectroscopy for the direct detection and dynamic optimisation of reaction conditions in microfluidic structures, the reductive deamination of aniline in anhydrous conditions by isoamyl nitrite in N,N-dimethylformamide was performed (Scheme 4.9).



Scheme 4.9 The reductive deamination of arylamines by isoamyl nitrite in DMF.

The deamination reaction occurs rapidly in DMF by the replacement of the primary amino group by hydrogen. Competing reactions such as the formation of phenyl and biaryls are minor processes in de-diazonation reactions performed in DMF, consequently the hydro de-diazonation reaction is ideal for the optimisation of reaction conditions. Solutions of aniline and isoamyl nitrite in N,N-dimethylformamide were infused into the microfluidic reactor at a variety of flow rates. Both aniline and isoamyl nitrite have strong characteristic Raman bands between 200 – 1700 cm⁻¹ providing ample information for sample identification (Table 4.1).[183]

Raman shift (cm ⁻¹)	Molecular origin
1662	solvent (DMF)
1630	solvent (DMF)
1622	aniline (NH ₂ bend a ^I)
1603	aniline (ring stretch a ^I)
1501	aniline (ring stretch a ^I)
1468	aniline (ring stretch a ^{II})
1443	solvent (DMF)
1408	solvent (DMF)
1279	aniline (x-sens a ^I)
1176	aniline (CH bend ip a ^{II})
1155	aniline (CH bend ip a ^{II})
1095	solvent (DMF)
Key: a ^I = in plane of symmetry, a ^{II} = normal to plane of symmetry, sens = sensitive, ip = in plane.	

Table 4.1 Vibrational exciton spectrum of aniline in DMF.

However, for the reaction between aniline and amyl nitrite, only inspection of the spectral region between 1050 and 1750 cm⁻¹ is necessary (Figure 4.9). As previously stated above, the hydro de-diazonation reaction is near quantitative with respect to parent diazonium salt. By monitoring for the extent of diazotization by the disappearance of aniline bands as an alternative to the formation of the product, the rate determining diazotisation step (Scheme 4.10, 1) is monitored as opposed a sum of the reaction processes (Scheme 4.10, 1-4). In

addition, process monitoring in the forward mixing section, prior to the elimination of nitrogen, prevents disruption of the Raman signal by the passing nitrogen bubbles (Scheme 4.10, 3).

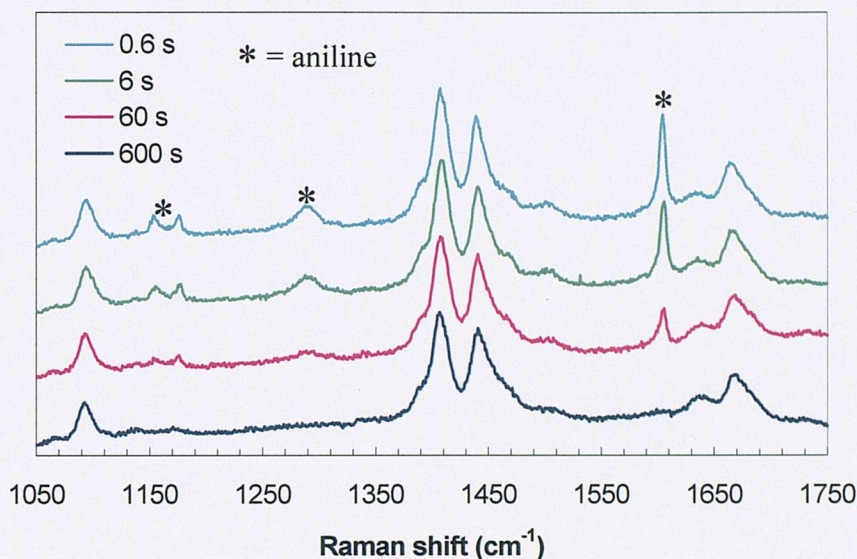
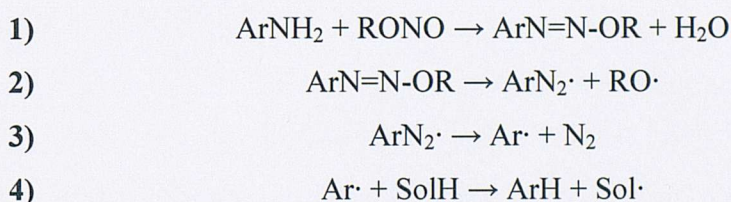


Figure 4.9 Raman spectra of in-channel diazotisation reaction mixtures at various times after initial mixing.



Scheme 4.10 The reductive deamination of arylamines by alkyl nitrite and a hydrogen donating solvent.

In particular, analysis of the in-plane ring-stretch band at 1603 cm^{-1} can be used to directly assess the extent of hydro de-diazonation as a function of residence time (Figure 4.10). Interestingly, the disappearance of the in-plane ring-stretch band is shown to be complete after a residence time of 600 seconds. As discussed in section 4.2.1, the flow rates used in these experiments were chosen

to ensure a satisfactory degree of mixing (Fourier number = 0.25) with a corresponding residence time of 5.6 seconds. The required residence time, as demonstrated by the complete disappearance of aniline Raman signals in 600 seconds equates to a Fourier number of 26. This indicates that the reaction is occurring in the chemical domain, rather than the diffusive domain (see Chapter 1).

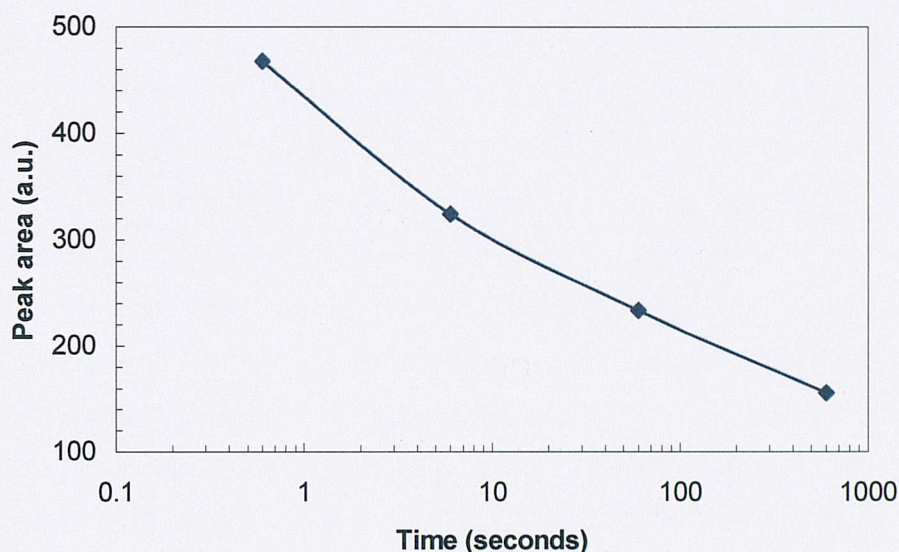


Figure 4.10 The variation of the integrated area of the 1603 cm^{-1} peak as a function of reaction time.

The utility of Raman spectroscopy for direct detection is further demonstrated by the speed of optimisation. The work required to establish the optimum residence time for the chloro de-diazonation on-chip using Raman spectroscopy involves a single reaction being monitored over 600 seconds. Spectral acquisition times are minimal, and data can be fed back to the pump very rapidly for the next run. In comparison, residence time optimisation by GC requires a series of reactions using significantly more reagents, each monitored in series over a range of reaction times taking up to 3 hours to complete.

To allow the comparison of alternative reactor designs in the scale-up of reaction processes, the space-time yield is used to express the efficiency of the reactors in

terms of the quantity of product produced per unit volume of space occupied by the reaction vessel per hour. In this way a small volume continuous flow reaction system with the additional peripheral support equipment required for fluid mobilisation and reagent/product storage can be compared with a batch reactor with a larger reaction volume and a smaller quantity of support equipment. However, in the research environment, this method of reactor comparison is not necessarily a fair one. In this situation, the space occupied by a microfluidic reactor is not determined by its volume, but by the substrate in which it is etched whilst the space required for a bulk scale (50 mL – 1 L) reaction vessel is determined not by the reaction vessel but by the fume hood that it occupies. By removal of the peripheral support equipment from the calculation, and taking into account only the volume that the reaction vessel holds, the microfluidic reactor described herein demonstrates a space-time yield of $13,500 \text{ molm}^{-3}\text{h}^{-1}$ due to the extremely small volume of the reactor. This figure can be compared to a space-time yield of only $128 \text{ molm}^{-3}\text{h}^{-1}$ for a 1 L scale synthesis.[184] It is believed that similar assumptions have been made by Jähnisch *et al.* where increases in space-time yield of between two and three orders of magnitude were obtained by the transfer of photochemical chlorination and direct fluorination from a bulk to a microfluidic format.[39, 85]

4.5. Conclusion

The application of microfluidic reactor technology to multistage reactions involving reactive intermediates has been demonstrated. Utilising a continuous flow methodology the advantages of microscale reactors were illustrated by the intrinsically safe anhydrous diazotisation, chloro- and hydro- dediazonation of a variety of simple arenes, resulting in increased yields over similar reactions undertaken on a traditional laboratory format. Although relatively small amounts of product are produced from this single reactor, a space-time yield of $13,500 \text{ molm}^{-3}\text{h}^{-1}$ is obtained due to the extremely small volume of the reactor. This figure can be compared to a space-time yield of only $128 \text{ molm}^{-3}\text{h}^{-1}$ for a 1 L scale synthesis,[184] indicating that parallelisation may prove to be a viable route

to large-scale production of diazonium salts in anhydrous conditions in complete safety.

On-line optical detection using Raman spectroscopy has also been demonstrated for the first time in microfluidic reactor systems. Reaction monitoring was undertaken in real time with data acquisition rates shown to be ideally suited for on-line reaction feedback and control.

4.6. Experimental

4.6.1. General method for the laboratory synthesis of the sudan series of azo dyes[185]

4.6.1.1. Preparation of benzenediazonium chloride

To a cooled solution of amine (1 mL, 11 mmole) in conc. HCl (3.5 mL) was added approx. 8 g of crushed ice. To this 90% of a cooled solution of sodium nitrite (0.75 g, 11 mmole) in water (4 mL) was added portionwise. Between each addition the solution was stirred well, and for approx. 3 minutes following the final addition. An excess of nitrous acid was indicated by spotting on starch iodide paper. In the absence of an excess, the remaining 10th of the nitrite solution was added dropwise until an immediate blue colouration was obtained. The benzenediazonium chloride was then stored in an ice water bath until required.

4.6.1.2. Synthesis of 1-(phenylazo)-2-naphthol (Sudan I, Figure 4.3, 3)

1.5 g (11 mmole) 2-naphthol was dissolved in 3 M sodium hydroxide (9 mL) by gentle heating. Cooled to room temperature, approx. 20g of crushed ice was added. With vigorous but careful stirring the pre-prepared Benzenediazonium chloride solution was added dropwise. When the addition was complete, the solution was stirred for an additional 10 minutes. The solution was filtered and recrystallised from ethanol:toluene (9:1) to give a red solid.

4.6.1.3. General method for the continuous-flow synthesis of 1-(phenylazo)-2-naphthol (Sudan I, Figure 4.3, 3)

Solutions of aniline (0.07 mL, 0.77 mmol), conc. HCl (0.24 mL) and water (0.7 mL) in *N,N*-dimethylformamide (5 mL) and sodium nitrite (0.1875 g, 2.7 mmol), and water (1 mL) in *N,N*-dimethylformamide (5 mL) were prepared. These solutions were introduced into inlets A and B of the reactor chip at a flow rate of $3.2 \mu\text{Lmin}^{-1}$ using a PHD 2000 syringe pump (Harvard Apparatus, Kent, U.K.). A solution of 2-naphthol (0.15g, 1.0 mmole) and 3% sodium hydroxide (2.9 mL) in *N,N*-dimethylformamide (2 mL) was introduced into the second inlet (D) of the reactor chip at a flow rate of $6.4 \mu\text{Lmin}^{-1}$ and the secondary serpentine section of the device was heated to 338 K. Heating of the secondary section was achieved by contacting the microfluidic reactor to a heated metal block. A continually moving stream of air cooled the first section of the device over both upper and lower surfaces.

4.6.1.4. General method for the laboratory synthesis of chloroarenes[175]

To a solution of isoamyl nitrite (3.5 mL, 26 mmol) and anhydrous DMF (5 mL) heated at 338 K was added the aromatic amine (1 g, 11 mmol) dissolved in 5 mL DMF. The reaction was performed in a 50 mL three necked round bottomed flask equipped with a reflux condenser, addition funnel, a gas outlet tube and a magnetic follower. The vessel was heated by immersion in an oil bath in turn heated on a IKA magnetic stirrer hot plate equipped with a PT-100 probe with Fuzzy logic for self optimising temperature control. Anhydrous copper (II) chloride (1.7 g, 13 mmol) in *N,N*-dimethylformamide (20 mL) was then added. Upon addition, the reaction solution turned from the initial green copper (II) chloride colour completely black as nitrogen was evolved. Generally, gas evolution was complete within 10 min following the addition of the amine. After complete gas evolution the product was analysed by GC giving a conversion by area analysis of 40-49%. GC-MS spectra were also obtained and showed concordance.

4.6.1.5. General method for the continuous-flow synthesis of chloroarenes

Solutions of amine (0.1 mL, 1.1 mmol) in N,N-dimethylformamide (2 mL) and 97% isoamyl nitrite (0.35 mL, 2.6 mmol) in N,N-dimethylformamide (2 mL) were prepared. These solutions were introduced into inlets A and B of the reactor chip at a flow rate of $3.2 \mu\text{Lmin}^{-1}$ using a PHD 2000 syringe pump (Harvard Apparatus, Kent, U.K.). A solution of anhydrous copper (II) chloride (0.17g, 1.3 mmol) in N,N-dimethylformamide (4 mL) was introduced into the second inlet (D) of the reactor chip at a flow rate of $6.4 \mu\text{Lmin}^{-1}$ and the secondary serpentine section of the device was heated to 338 K. Heating of the secondary section was achieved by contacting the microfluidic reactor to a heated metal block. A continually moving stream of air cooled the first section of the device over both upper and lower surfaces. Product analysis was performed by GC without further purification by comparison to spiked standardised solutions giving a conversion by area analysis of 55-71%. GC-MS spectra were also obtained and showed concordance.

4.6.1.6. Reaction monitoring

Optical monitoring was performed on an inverted microscope (Leica DMIL: Leica Microsystems Ltd, Milton Keynes, U.K.) with a CCD camera (SONY, CCD XC-999P) mounted onto the microscope and connected to a TV/VCR (Samsung/Panasonic). Transformation was monitored using a Hewlett Packard HP6890 capillary gas chromatograph (GC), utilising a HP Innnowax stationary phase. Samples were introduced using an HP 6890 autosampler and autoinjector array to minimise operator errors. Samples were analysed without further purification by comparison to spiked standardised solutions (Lancaster Synthesis Ltd, Morecambe, U.K.). Traces were accumulated over a 42 minute period and displayed using HP Chemstation software.

The LabRam Infinity Raman microscope (Jobin Yvon Ltd, Middlesex U.K.) consisted of a 632 nm excitation laser, an inverted microscope and a 50x/0.5 numerical aperture objective. The microfluidic reactor was secured to an XY

translation stage and aligned with the excitation beam. Spectra were accumulated over a period of 30 seconds and displayed using Labspec V 4-02 software (Jobin Yvon Ltd. Middlesex U.K.) running on a Pentium computer.

Proton and carbon NMR spectra were recorded on a Bruker AC-250 spectrometer with a QMP probe, in CDCl_3 and reported in ppm relative to TMS. One dimensional ^1H and ^{13}C spectra utilized 11 ppm and 210 ppm spectroscopic windows respectively.

5.

THE APPLICATION OF MICROFLUIDIC REACTORS TO SYNTHETIC CHEMISTRY

The scale-up of processes from the laboratory to the bulk and fine chemical industries is often a complex and expensive operation where precautions must be taken to safeguard against reactive hazards and loss of reaction specificity. This chapter illustrates the advantages of microfluidic reactors when applied to exothermic organic reactions. Particular attention is paid to those which involve dangerous delayed exotherms, where the potential for thermal runaway is the greatest. These points are illustrated by performing the industrially useful Vilsmeier formylation on a microfluidic reactor where uncontrolled exothermic hydrolysis generates large quantities of acidic gas.

5.1. Potential advantages of microfluidic reactors

5.1.1. Scale-up

Synthetic chemistry is traditionally performed in the research laboratory on a scale with which the user is familiar. Usually this scale falls in the milligram to gram region and in industrial processes, anywhere between a kilo and several tonnes. Regardless of the scale in which the synthesis is performed, the typical processes involved remain fundamentally the same, and the resultant product must be produced in the desired quantity and of the appropriate quality. The scale-up of processes from the laboratory to bulk and fine chemical industries is often a complex and expensive operation where precautions must be taken to guard against reactive hazards and loss of reaction specificity. Most importantly the process must not endanger either the workers, or the plant in which the process is being performed, while the cost of ensuring these constants must not outweigh the benefits of performing the process. One of the most significant obstacles facing the industrial chemist is heat generation. The majority of manufacturing processes for chemical production are exothermic, the heat of which increases with scale. Once control of the reaction is lost, temperature can rise rapidly leaving little time for correction due to the exponential relationship between reaction rate and temperature. Without appropriate safety mechanisms and procedures in place, reaction runaway may cause severe disruption to production. In the absence of adequate safety mechanisms, thermal reaction runaway has the potential to lead to a major accident.[186] This was demonstrated by the incident at Seveso, where a burst valve resulted in loss of control over the synthesis of 2,4,5-trichlorophenol. The resulting explosive exothermic reaction led to the production and release in kilogram quantities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a poisonous carcinogen. Although incidents in chemical plants are rare, when they do occur the results are often catastrophic. In many cases these incidents are the result of a relatively small failure or accident; the sheer scale of the quantities of reagents being responsible for the often-disastrous consequences. In open vessels, loss of control over an exothermic reaction can result in the boiling over of the reaction mass or rapid

gas generation, either of which can have very severe consequences for plant operation and worker safety. In addition, elevated temperatures may initiate secondary, more hazardous runaways or decompositions.

In enclosed vessels, violent boiling may occur leading to the reaction vessel becoming over-pressurized. Failure of the reaction vessel may then result in a violent explosion causing blast and missile damage. Such violent exotherms are not only generated by a reaction in the synthetic sequence, they are equally often the result of a dilution or the quenching or decomposition of an excess reagent prior to disposal. If flammable materials are released, fire or a secondary explosion may result. Contamination of the workplace with hot liquors and toxic gases that can spread off-site may culminate in the injury or death of plant operators or the general public as well as severe damage to the local environment.

5.1.2. Thermal runaway

Since the majority of reactions are feed-controlled, careful addition of reagents prevents heat build-up by allowing heat to be dissipated faster than it is generated, thus preventing thermal runaway. However, this significantly extends the process time, which can lead to increased degradation of the system and unwanted by-product formation. The majority of incidents of thermal runaway can be attributed to a few key factors, the most significant of which are inadequate heat removal and a lack of reaction control. Both of these contributing factors are related to simple laws of geometry. The generation of heat is related to the volume of the reaction vessel, while heat removal is dependant on the surface area available for heat dissipation. As the scale of a reaction vessel increases, the surface to volume ratio rapidly decreases due to their square:cubed relationship. In larger reaction vessels, this results in inefficient heat transfer and inadequate cooling of a reaction, which can lead to loss of control or thermal runaway resulting in disaster. Although active cooling of reaction vessels is possible through the use of refrigerants or heat pumps, these

measures can often be expensive and become increasingly ineffective as the size of reactor grows.

5.1.3. Hotspot generation and by-product formation

As already established in Chapter 1, for reaction orders greater than one, poor mixing performance results in the formation of microscopic concentration gradients and segregation. This stifles the reaction and the potential for forming secondary products is greatest.[187]

As the reactor size increases, mixing becomes less trivial and mass transfer less efficient. This allows the concentration of reagents in localized volumes, which can lead to the formation of superheated zones or hotspots. The increased temperature experienced in these hotspots can initiate side reactions and an increase in undesirable by-product formation. Reactions in biphasic systems are additionally complicated as they are frequently mass transfer limited. In biphasic systems, micromixing on a molecular level rarely occurs and the reaction is limited to the interface boundary between the solutions. The generation of hotspots is exaggerated by this less efficient mixing and an optimal interfacial area is difficult to maintain in macroscale reaction vessels. This too may be addressed by the application of microfluidic reactors as previously discussed in Chapters 1 and 3 where novel mixing methods may lead to an increase in the interdiffusion area between phases in biphasic systems and consequently increase mixing efficiency.

5.2. Application of microfluidic devices

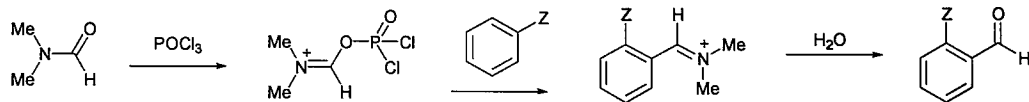
Each of the problems described above can be attributed to the independence of chemical rate constants and the dependence of physical parameters (such as heat and mass transfer) to the scale of the reaction vessel. Chapter 1 demonstrated that the high surface-to-volume ratios encountered within microfluidic systems result in exceptionally high heat transfer coefficients. With large surface to

volume ratios, microfluidic channels have the ability to dissipate heat extremely efficiently. In the case of exothermic reactions any heat so generated is dissipated rapidly such that the maximum temperature gradient experienced on a microfluidic reactor may be just 1°C.[51]

The application of microfluidic structures to exothermic reactions and reactions operating in explosive regimes has been investigated previously (see Chapter 1 for a review). The manipulation of hazardous (e.g. toxic, pyrophoric, explosive or unstable) compounds on the microscale allows chemical reactions to be undertaken with increased safety due to the volumes involved. Typically, a resultant spill of the entire contents of a characteristic microfluidic reactor generates volumes in the nanolitre to microlitre region. In contrast to traditional reaction scaling, increased production capacity in microfluidic systems can be achieved by operation of a number of reactors in parallel – ‘scale out’ as opposed to ‘scale up’.[4] With mass production techniques for microfabrication in metals, glass and plastics already well established, significant time savings can be attained during transfer from the laboratory to the commercial scale. Operation of a production plant in a modular fashion may result in increased production flexibility, while improved reaction efficiency may lead to substantially higher throughput per total system volume.

5.3. The Vilsmeier-Haack formylation

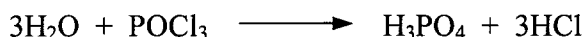
The Vilsmeier or Vilsmeier-Haack reaction is the most common method of formylating active aromatic rings.[188] It is a popular reaction in the chemical industry, especially in the production of organic photo-conductors[189] and backbone amide linkers in solid-phase chemistry.[190] An extension of the Mannich reaction, it involves the reaction of disubstituted formamides with phosphoryl chloride, phosgene or thionyl chloride to produce a thermally unstable iminium intermediate species (Scheme 5.1).



Scheme 5.1 The Vilsmeier-Haack formylation.

5.3.1. Exotherm control

Although many typical reaction exotherms may be feed controlled, “delayed exotherms” are much less open to control by such simple methods. As previously mentioned in Chapter 3, mixing in biphasic systems is limited by the interface boundary as in the case of phosphoryl chloride quenching in water (Scheme 5.2). Phosphoryl chloride is used in a number of syntheses including the generation of acid chlorides and the Vilsmeier-Haack formylation. Following completion of the reaction, quenching of excess POCl_3 must be performed with great care due to the delayed exothermic nature of the quench. Once the hydrolysis of POCl_3 at the interface boundary commences, the exothermic reaction generates heat. With an increase in temperature in the local area, diffusion becomes more rapid leading to an increase in the rate of hydrolysis. The reaction can also, when uncontrolled, evolve large quantities of gaseous hydrogen chloride. If allowed to continue unabated, this simple hydrolysis may result in an explosive thermal runaway.[191]



Scheme 5.2 The hydrolysis of phosphoryl chloride by water.

5.3.2. Microfluidic reactor design

To demonstrate the utility of microfluidic reactors to the control of highly energetic delayed exotherms, the hydrolysis of phosphoryl chloride was performed. For the execution of the hydrolysis, a glass microfluidic reactor was fabricated in-house using direct-write laser lithography, wet chemical etching and bonding techniques as previously described in Chapter 2.

To ensure symmetrical filling, the channel layout incorporated a ‘split and mix’ configuration (Figure 5.1). The first inlet channel (X) was divided into two separate streams. The second fluid channel (Y) was then introduced between these streams before their recombination, which created a layered stream (XYX). By introducing the aqueous phase into the first channel inlet (X), this arrangement ensured that only the aqueous phase interacted with the hydrophilic channel edges throughout the device. To allow for observation of the hydrolysis, an 80 mm serpentine reaction channel 150 μm wide and 50 μm deep followed the inlet channels with a total volume of 0.6 μL .

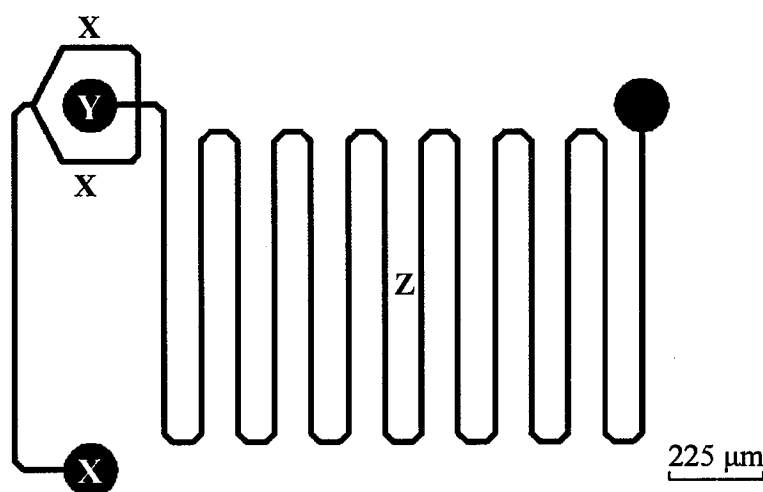


Figure 5.1 A microfluidic reactor incorporating a split and mix design for the quenching of POCl_3 (inlet Y) by NaOH (inlet X). Z indicates the placement of a thermocouple for temperature determination.

5.3.3. Experimental

To perform the hydrolysis, a 1% sodium hydroxide quenching solution was employed. This was introduced into the device through inlet X while phosphoryl chloride was introduced into inlet Y at a variety of flow rates. Through use of Fourier's number (Equation 1.9, Chapter 1) an estimation of the residence time required for mixing to be complete ($\tau = 1$) can be calculated for a volumetric flow rate of $1.6 \mu\text{Lmin}^{-1}$. However, by observation of the quenching process on

the microfluidic device, hydrolysis and the expulsion and reabsorption of gaseous hydrogen chloride appear complete at flow rates up to $10 \mu\text{Lmin}^{-1}$. This may be attributed to an increase in diffusion rate accompanying an increase in the local temperature during the hydrolysis. To monitor the device temperature during the hydrolysis, a Pt 100 k-type thermocouple connected to a HI 8757 microprocessor controlled thermometer accurate to $\pm 0.5^\circ\text{C}$ (Hanna Instruments Ltd., U.K.) was secured to the surface of the device with adhesive approximately 40 mm down the serpentine channel.

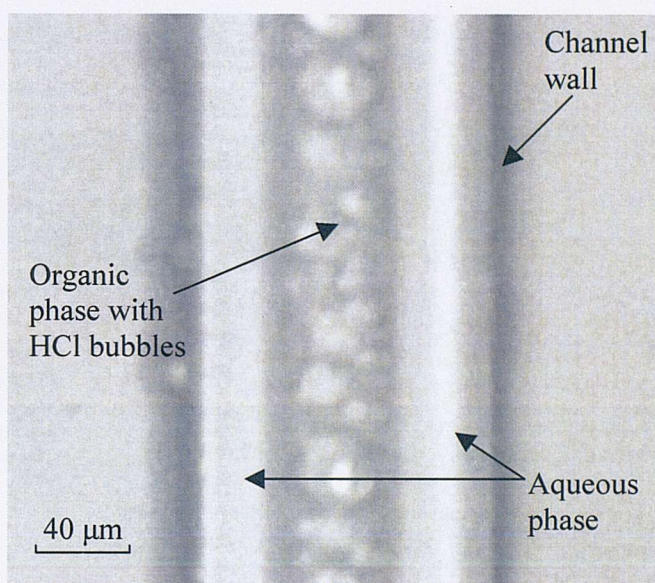


Figure 5.2 The generation of gaseous hydrogen chloride during POCl_3 quenching on a microfluidic reactor.

This method, although secure, did not provide adequate thermal contact to the surface of the device. As an alternative, temperature measurements were obtained by surrounding the thermocouple with insulating plastic such that the temperature sensor protruded by approximately 0.5 mm. By directly placing the thermocouple onto the device surface, good thermal contact was ensured while allowing a single temperature sensor to be applied to several microfluidic devices. However, it should be noted that the measurements thus obtained must not be taken as absolute values. This is firstly due to the proportionally large distance between the temperature sensor and the channel (1.5mm),

approximately 30 times that of the depth of the channel. Secondly, the medium between the thermocouple and the channel, in this instance glass, is an extremely poor thermal conductor. For these reasons, the temperature measured on the surface of the device is known to be significantly different to that within the microfluidic channel. At this time no alternative method for in-channel temperature determination was available, however the following discussion of observed trends remains valid.

Quenching of phosphoryl chloride was performed at a variety of flow rates and system temperatures recorded. Due to the continuous flow conditions, to ensure thermal equilibrium was reached prior to measurement of the temperature, each experiment was run for at least 15 minutes prior to each temperature measurement (Figure 5.3). Phosphoryl chloride is extremely corrosive, and for this reason experiments were strictly limited to the minimum number of runs required for data collection and analysis. Excessive exposure to POCl_3 led to the corrosion of syringe parts and degradation of PEEKTM tubing. Although PEEKTM may be substituted by PTFE, no facile method of replacement existed for the stainless steel components of the reagent syringes.

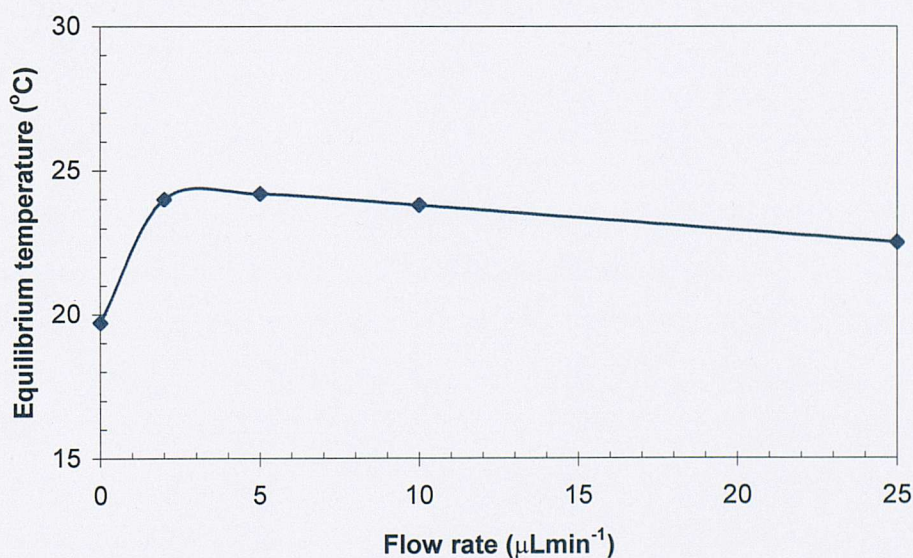


Figure 5.3 Heat generated during the quenching of POCl_3 with 1% NaOH on a microfluidic reactor.

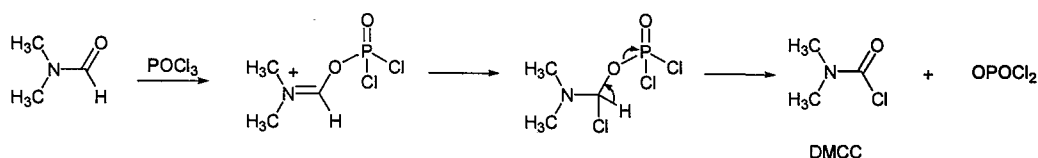
5.3.4. Discussion

From the data illustrated in Figure 5.3, a distinct relationship between system temperature and flow rate can be observed. As the flow rate was increased, a decrease in equilibrium temperature was obtained. This implied that maximum quenching occurs at lower flow rates. As the flow rate was increased, the maximum amount of quenching was no longer performed on the reactor, but beyond the area under examination, possibly in the exit pipe or collection vessel. However, from observation of mixing on the microfluidic device, quenching appeared to be complete, demonstrated by only a single phase present in the serpentine channel and an absence of hydrogen chloride bubbles. This implied that the quenching was complete at higher flow rates and that the decrease in temperature measured was associated with a movement in the zone of greatest quenching away from the fixed point of temperature detection. Furthermore, it was observed that the quenching of phosphoryl chloride was effectively a smooth reaction, with no delayed exotherm effects. The re-absorption of hydrogen chloride by the aqueous solution produced by the hydrolysis of phosphoryl chloride in microfluidic channels is of additional benefit. In the macroscale hydrolysis of POCl_3 , hydrogen chloride traps are required as a precautionary measure to remove gaseous hydrogen chloride produced during the hydrolysis.

5.3.5. By-product formation

During the scale-up of the Vilsmeier reaction, the formation of a particular by-product is of special concern. Dimethylcarbomyl chloride (DMCC), is a potential human carcinogen with stringently controlled exposure limits in the region of only a few parts per billion. Although DMCC formation has little effect on the overall reaction yield of the synthesis, its formation at levels of up to a few thousand parts per million is of serious concern. From the mechanism proposed in Scheme 5.3 for the formation of DMCC from DMF and POCl_3 , it is clear that analogous chlorinating agents such as thionyl chloride and phosgene with alternative dimethylamides may also form DMCC. Where reagent substitution also results in formation of the DMCC by-product, reaction control

may be achieved by the application of alternative mixing mechanisms, i.e. by the application of microfluidic reactors.



Scheme 5.3 The decomposition of the Vilsmeier electrophile to DMCC.

To investigate the effect of mixing mechanisms on the production of DMCC, the Vilsmeier formylation of phenol and 3,4,5-trimethoxyphenol was performed in a bulk laboratory format and in a microfluidic reactor for comparison. The microfluidic reactor utilised for these experiments consisted of a T shaped junction and serpentine channel for diffusive mixing. To accommodate the introduction of the aqueous POCl_3 quench as performed in section 5.3.1, initial experiments were performed on a modular design device consisting of two mixers linked via a short length of fused silica capillary (Figure 5.4). The glass microfluidic reactors were fabricated in-house using direct-write laser lithography, wet chemical etching and bonding techniques as previously described in Chapter 2.

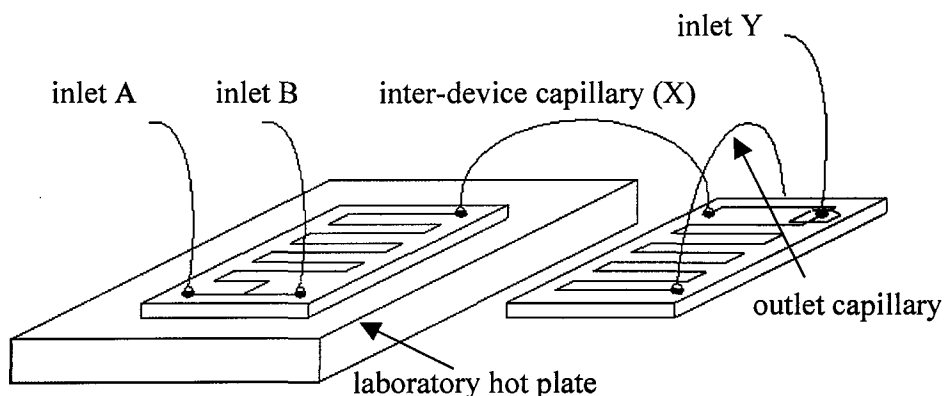


Figure 5.4 A modular design for the formylation of phenols by the Vilsmeier-Haack and subsequent POCl_3 quenching.

Using the fluidic network shown in Figure 5.4, solutions of phenol and DMF in DCM and POCl₃ in DCM were introduced into the first mixer which was held at an elevated temperature of 333 K by placement on a laboratory heating plate. A flow rate of 2 μLmin^{-1} was employed using a Harvard Apparatus PHD 2000 syringe pump (Fourier number = 0.4, Reynolds number = 0.6). A solution of 1% NaOH was then introduced into the second mixer at a balancing flow rate of 4 μLmin^{-1} (Fourier number = 0.2, Reynolds number = 1.3). Quenching of the POCl₃ was evident by the production of gaseous bubbles and the mixing of the two immiscible phases resulting in a single phase. After the reaction streams had stabilised, the reactors were run for extended periods to collect samples for analysis. It should be noted that collection of macroscale samples at this stage posed no hazard as the POCl₃ at this time had been fully quenched and all gaseous HCl produced during quenching reabsorbed by the solution.

Continuous flow synthesis within microfluidic environments naturally lends itself to on-line analysis and dynamic reaction control. This has previously been demonstrated by Jensen *et al.*[86, 105] where the conversion of benzophenone to pinacol was estimated from on-line UV spectral data as a function of flow rate. More detailed information on reaction progress by on-line analysis has been demonstrated by Mitchell *et al.*[82] where a microfluidic reactor was used in combination with ESI-TOF mass spectrometry for dynamic optimisation of the Ugi multicomponent reaction. Although UV spectroscopy is an important method of detection in macroscale analysis systems such as HPLC, it provides little specific information about the analyte in question. In comparison to UV and MS based techniques, Raman spectroscopy is an established method for real-time, on-line fingerprinting tool for chemical identification.[180] It provides structural and symmetry information via molecular vibrations due to the polarised nature of the scattered light and its use has been decisive in the debate on the exact structure of the Vilsmeier electrophile.[192]

Despite employing a range of flow rates and temperatures, low yields of the formylation product of both phenol and 3,4,5-trimethoxyphenol were obtained

compared to that of the bulk synthesis. It has been postulated that the reason for the disappointing yields observed on microfluidic reactors may be attributed to the very characteristics that, theoretically, lend them to organic synthesis.[35] Wootton *et al.* have recently suggested that the high surface area to volume ratios may act to quench reactions such as the Vilsmeier reaction and those exothermic reactions that may rely on the generation of hotspots for propagation of the reaction.[191] With efficient hotspot and radical quenching, conventionally high yielding, but dangerous reactions on the bulk scale, may prove unsuitable and ultimately inapplicable to microfluidic reactor technology. Similar difficulties have been experienced by Kricka *et al.* in the application of the polymerase chain reaction (PCR) to microfluidic devices. They observe that PCR inhibiting surface chemistry effects are significantly increased over macroscale PCR due to the high surface to volume ratios.[193] The low yields of formylated product indicates that low levels of the Vilsmeier electrophile are formed and correspondingly an absence of DMCC. Consequently, the analysis of levels of DMCC by conventional GC-MS and Raman spectroscopy in low concentrations proved extremely problematic. As a result, DMCC proved unsuitable as a marker for the comparison of microfluidic reactors and bulk scale vessels.

5.4. Conclusion

These initial results demonstrate that exothermic quenching reactions can be safely controlled in microfabricated environments. Even “delayed exotherm” type reactions can be smoothly and rapidly carried out due to the efficient mass transport of the glass microfluidic reactor. Due to the poor thermal conductivity of the glass material employed for these experiments, a temperature increase of less than 5°C was observed. Following fuller characterization of the quenching process on microfluidic reactors, transferal of the process to reactors fabricated in materials of higher conductivity may result in near isothermal operation. Although the throughput of the device detailed within this chapter is small, by application of the distributive mixing mechanism described in Chapter 1, the flow rate for the quenching may be increased to as much as 2 mLmin⁻¹ or 5 Kg

per day for a single microfluidic reactor. Scale-up of the process by the use of multiple reactors in parallel may result in efficient continuous flow quenching with significantly increased throughput and safety over the traditional bulk scale batch process.

The performance of the Vilsmeier formylation on microfluidic reactors has also been examined and the yields observed uncharacteristically low. The disappointing results obtained succinctly illustrate two points. Firstly, microfluidic reactors are not necessarily applicable to every synthetic reaction. The large surface to volume ratios responsible for efficient heat transfer preventing thermal runaway, may also act to quench the reaction with such efficiency that the desired product is not obtained. Secondly, to obtain a satisfactory yield on any scale, the application of heat in the required quantities is essential. In these experiments, where heat was applied through placement of the microfluidic reactor on a laboratory scale hotplate, temperature fluctuations, due to imprecise temperature determination, are likely to have proved detrimental to the overall yield. To allow an investigation into the quenching effects of microfluidic reactors to reaction propagation, a method for the direct determination of temperature within the microfluidic device must first be addressed.

5.5. Synthesis

5.5.1. General method for the laboratory synthesis of formylated phenols by the Vilsmeier-Haack reaction.[194]

A solution of phenol (0.23 g, 2.5 mmol) in dichloromethane (15 mL) and *N,N*-dimethylformamide (0.5 mL, 5 mmol) was warmed to 333 K in a 50 mL two necked round bottomed flask fitted with a dropping funnel and reflux condenser. Phosphoryl chloride (0.45 mL, 5 mmol) was added dropwise and the mixture stirred magnetically for 60 minutes. The mixture was then poured into cooled 1% sodium hydroxide solution and stirred thoroughly. Following the separation

of the aqueous and organic phases, product analysis was performed by GC without further purification by comparison to spiked standardised solutions giving a conversion by area analysis of 40%. GC-MS spectra were also obtained and showed concordance.

5.5.2. General method for the continuous-flow synthesis of formylated phenols by the Vilsmeier-Haack reaction.

Solutions of phenol (0.92 g, 1 mmol) and *N,N*-dimethylformamide (0.2 mL, 2 mmol) in dichloromethane (3 mL) and phosphoryl chloride (0.18 mL, 2 mmol) in dichloromethane (3 mL) were prepared. These solutions were introduced into inlets A and B of the first reactor chip at a flow rate of 2 μLmin^{-1} using a PHD 2000 syringe pump (Harvard Apparatus, Kent, U.K.). A solution of 1% sodium hydroxide was introduced into inlet X of the second reactor chip at a balancing flow rate of 4 μLmin^{-1} . Heating of the primary reactor was achieved by contacting the microfluidic reactor to a metal block held at 333 K.. Following the separation of phases, product analysis was performed by GC without further purification by comparison to spiked standardised solutions giving a conversion by area analysis of 2-9%. GC-MS spectra were also obtained and showed concordance.

6.

HEATED MICROFLUIDIC REACTORS

In organic synthesis, temperature has a significant role, as demonstrated by each of the syntheses performed during the course of this thesis. The application of heat for the initiation and propagation of a reaction is as essential in synthesis as the act of mixing. Heated microfluidic reaction systems presented elsewhere frequently incorporate Peltier[195] or cartridge heaters[77] with temperature sensing methods remote to the reaction channel. In order to produce a method of heating with a low thermal mass and fast heating/cooling cycle rates, microfabricated heaters were investigated. In addition, the application of thermochromic liquid crystals (LCs) was explored for the monitoring of in-channel temperatures. These concepts were then applied to the microfluidic Reimer-Tiemann synthesis where precise temperature control is of crucial importance

6.1. The effect of temperature

Several proposed mechanisms exist to describe the reaction, or breaking and formation of bonds, between two molecular species. Collision theory states that for a reaction to occur the molecules must be brought into intimate contact, a process normally referred to as a collision. In general, it is found that the rate of most reactions multiplies with a raise in system temperature. Increasing the temperature of a system leads to an increase in particle speed and therefore collision rate. The rate of a reaction however, is dependent not only on the number of collisions, but more significantly it is dependent on the kinetic energy of the particle. For a reaction to occur, the collision must occur with sufficient energy for the original bonds to be broken before new, more energetically favourable bonds are formed. This minimum kinetic energy requirement is known as the activation energy. The temperature of a system is actually a measure of the average kinetic energy of the particles within the system and therefore the application of heat has a significant effect on the rate of reaction.

Many reactions have rate constants that follow the Arrhenius equation (Equation 6.1).

Equation 6.1
$$k = Ae^{-E_a / RT}$$

Where k is the rate constant ($\text{Lmol}^{-1}\text{s}^{-1}$), A is the pre-exponential factor or frequency factor (s^{-1}), E_a the activation energy (kJmol^{-1}), R the universal gas constant (8.314) and T the absolute temperature (K).

It can be seen from Equation 6.1 that temperature has an exponential effect on the rate of reaction. In general, an increase in temperature of 10 K leads to an increase in rate of between 1.8 and 4.1 times. Although many reactions occur at ambient temperature, their rate of reaction is often slow. For a reaction to proceed at an acceptable rate, the temperature must often be increased. Alternatively, the activation energy of a reaction is such that heat must be applied for a reaction to be initiated.

Once begun however, such reactions are frequently exothermic and the heat source must be removed or controlled to prevent over-heating and thermal runaway, as discussed in Chapter 5. In microfluidic reactors, the removal of energy from reactions is rarely required due to their excellent inherent heat transfer[2] and radical termination properties[3] (see Chapter 1). Subsequently, heat energy generated by the reaction, which on the bulk scale is absorbed by the surrounding solution, is efficiently dissipated and additional heat may be required for the reaction to proceed satisfactorily. To this end, previous experiments incorporating a heating operation in this thesis (Chapters 4 and 5), utilised conventional laboratory heating plates. It was observed during these experiments that the temperature in the microfluidic reactor fluctuated with time as the bimetallic strip controlled heating plate strived to maintain a constant temperature. These heating devices have considerable thermal masses and high conductivity limiting heating and cooling cycle times.

6.2. Microheaters

In order to produce a simple, complete microfluidic reactor system, additional components to reactor vessels need to be examined; such as valves, pumps, separators and integrated heaters. Heated microfluidic reaction systems frequently incorporate Peltier[195] or cartridge heaters.[77] These systems however have a large thermal mass and have slow heating/cooling cycle rates. As an alternative, heating and cooling fans have been applied in the field of microfluidic PCR amplification.[196] Although suitable for miniaturised electronic components, their size and opacity makes them unsuitable for microfluidic reactors where visualisation of the reaction provides essential information on the process performance. For a non-contact, transparent heating method, infrared heating using a tungsten lamp or similar has great potential, however such systems are bulky and have a high power consumption.[197] Thin film resistive heating elements for heating in microfluidic systems have been investigated by several groups.[44, 198-202] Due to the similarity in fabrication methods, resistive heating elements naturally lend themselves to integration in

microfluidic devices. By incorporation of the thin film resistive element between substrate layers, heat can be supplied directly into a channel allowing the reaction to proceed at a satisfactory rate. In this way, excessive heating of the surrounding environment is avoided resulting in low power consumption and rapid heating and cooling cycle times.

6.2.1. Microfluidic reactor design

In each of systems mentioned above,[44, 198-202] silicon has been used for the fabrication of integrated heaters for microfluidic reactor systems. Due to the electrical conductivity (low breakdown voltage) of silicon, the metal resistive element pattern has to be isolated from the silicon by a polymer or the growth of an oxide layer. Since silicon has a high thermal conductivity of $45 \text{ Wm}^{-1}\text{K}^{-1}$, the conduction of heat to a fluidic channel and throughout the device is excellent thus providing near isothermal operation. However, in addition to conducting heat towards a channel, silicon is excellent at dissipating heat. In the case of silicon microfluidic reactors incorporating metallic interfacing methods, several hours were required to bring the device to a set point of 70°C with a power input of 15 W.[198] As previously discussed in Chapter 2, glass has been chosen in this work as the preferred material for chemical microfluidic reactor fabrication due to its chemical compatibility, ease of fabrication and optical transparency. In comparison to silicon, a material frequently used in microfluidic reactor fabrication, glass is thermally insulating with a thermal conductivity of $0.937 \text{ Wm}^{-1}\text{K}^{-1}$. The effect of a decreased heat transfer efficiency of glass over silicon is twofold. By incorporating resistive heating elements into a thermally insulating material, heat transfer to the reaction channel is less efficient, resulting in an increased heating and cooling cycle time. However, at the same time, dissipation to the surrounding environment is also reduced for the same reason. This reduction in heat loss results in a significantly lower power consumption over an uninsulated silicon device.

For the application of thin film heaters to glass microfluidic reactors, the permanent incorporation of heating elements into the glass structure surrounding the reaction channel was investigated. By the incorporation of electrodes into the middle of the device, the characteristic dimension for heat transfer or the distance between the heat source and the fluidic channel, is reduced, minimizing power consumption. In addition, by placing the heating elements at the centre of the device, the resistance of the heating elements themselves can be used as an indicator of the internal chip temperature and enable feedback temperature control by use of a computer controlled power supply.[199] To ensure an even temperature distribution and efficient transfer of heat to the microfluidic channels, the resistive heater elements were positioned in such a way that each part of the fluidic network was equidistant to the heating element. Each turn of a meandering fluidic channel was separated from its neighbouring electrode by 250 μm . The intermediate space provided a clean flat surface area sufficiently large for diffusion bonding between the two substrates and provided mechanical stability for the chip. Should an insufficient area be provided for diffusion bonding, repeated temperature cycling may result in the premature destruction of the device. In addition, the in-plane design removes the need for an insulating material to protect the electrode from exposure to reagents, this in contrast to devices proposed by Losey *et al.*[198] Where the application of a high electrical charge or the presence of a metal surface may adversely effect the reaction, this arrangement is highly undesirable. Insulation of electrodes has been demonstrated by Burns *et al.*[203] and Santini *et al.*,[204] but at the cost of simplicity as each design incorporates either thin film polymers or CVD oxides to act as an insulating layer and prevent electrode corrosion.

To achieve a fluidic seal between bonded glass substrates, the thickness of the metal film must remain small. Large features obstruct contact between the two glass substrates and prevent sealing. Thermal bonding may be performed at higher temperatures and allow the glass to ‘flow’ to accommodate such surface features, however this normally results in deformation of the channel profile.

Electrode thickness is also limited by the maximum current density of the electrode material before electromigration leads to failure of the film.

6.2.2. Fabrication

Microfluidic channels were fabricated from glass as previously described in Chapter 2. The microfluidic reactor consisted of a plain featureless serpentine channel 140 mm long incorporating two inlets (A and B), a T-shaped mixing area and an exhaust (C) at which samples may be collected for analysis (Figure 6.1). The etched channels have an average depth of 50 μm and an average width of 150 μm with a total volume of 1.05 μL .

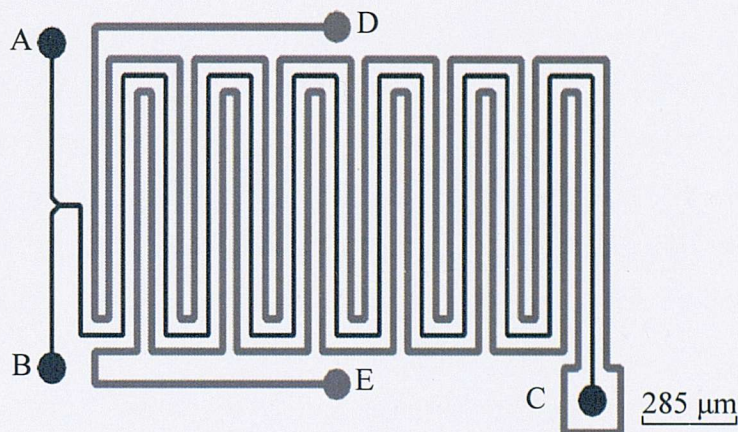


Figure 6.1 Schematic of a microfluidic reactor incorporating a thin film resistive element.

Due to the harsh conditions experienced during the fusion bonding of the reactor, gold was chosen for the permanent incorporation of thin film heating element (D to E). To assist the adhesion of the gold to the clean glass substrate a nickel layer was initially laid down. The nickel/gold electrode was evaporated onto a glass substrate using an Edwards 306A Coating System and a specially fabricated brass mask, each layer being approximately 40 nm thick. The mask was fabricated from a 150 μm thick sheet of brass coated with photoresist (Microposit S1818, Shipley Microelectronics, U.K.) by spin coating at 3000 rpm (Coros

OP15, Siemens, U.K.). The electrode design was then transferred to the photoresist using direct-write lithography as described in Chapter 2. The design was then etched into the brass substrate by immersion in a solution of iron (III) chloride (12.5 g, 75 mmol) and 10% hydrochloric acid (75 mL). Once the brass mask was formed it was fixed into a copper supporting structure, which also acted to accurately locate the glass substrate over the brass mask.

6.2.3. Results

To allow characterization of the heating properties of the microheater and its comparison with other heating devices, a series of experiments were performed. To determine the effectiveness of incorporated thin film resistive heaters as a heat source for glass microfluidic reactors, the relationship between power and temperature was assessed. This was achieved using a Pt 100 K-type thermocouple placed on the surface of the device prior to bonding and connected to a HI 8757 microprocessor controlled thermometer accurate to $\pm 0.5^{\circ}\text{C}$ (Hanna Instruments Ltd., U.K.). To simulate the presence of the sealing top plate, a borosilicate plate of identical dimension was placed on top of the device, the thermocouple being passed through a 500 μm hole in the centre of the plate. The heating of the resistive elements was then achieved using a 300V DC power supply (Powerpac 300, Bio-Rad Laboratories Ltd., U.K.). The voltage was increased and the corresponding temperature rise measured; at each measurement the temperature was allowed to come to equilibrium for five minutes before a reading was recorded (Figure 6.2). In this way a maximum temperature of 330°C was achieved before internal thermal stresses led to device failure. To compare the temperature consistency properties of the microfabricated heater and that of a heating plate, each device was raised to 55°C and the temperature maintained and monitored over a 100 hour period to confirm the reliability of the device. No insulating material or lagging was incorporated at any stage to improve heating performance of the device. The use of a draft excluder however was utilised to limit the effect of irregular laboratory air currents.

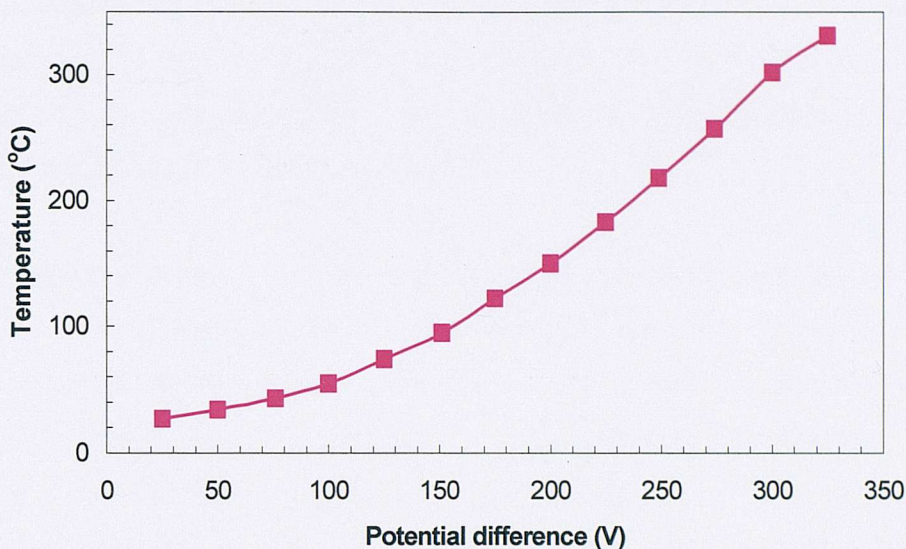


Figure 6.2 The relationship between applied potential difference and temperature for a thin film microheater

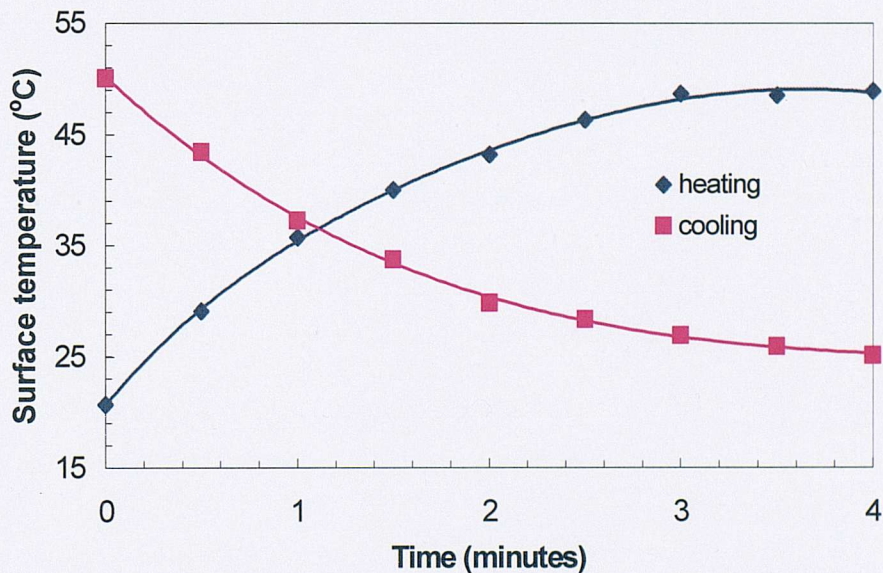


Figure 6.3 Heating and cooling rates of a thin film microheater, set point = 50°C.

From Figure 6.3, it can be seen that the microheater required 4 minutes to reach the desired temperature. This, and the somewhat slow cooling times, may be attributed to the size and low conductivity of the glass device. The low thermal transfer by conduction to the surrounding environment however, also reduces the

power consumption of the device. The silicon based thin film heater device presented by Losley *et al.* required 15 W to achieve a set temperature of 70°C.[198] In comparison, 70°C was achieved by the microheater described herein with a consumption of only 0.84 W.

Prior to introduction of fluids into the chip, the temperature increases at approximately $7^{\circ}\text{Cmin}^{-1}$. On introduction of the reaction mixture, the heat capacity of the solution may cause a slight decrease in the temperature of the reaction channel. Since the microfluidic reactor operates in a continuous flow regime at a constant flow rate, there will be no variation in in-device temperature during operation and thus the power supplied can be adjusted to compensate. To compare the temperature consistency of the microheater, a temperature of 50°C was set and the temperature of the device monitored over a 15 minute period and compared to that of a laboratory heater (Figure 6.4).

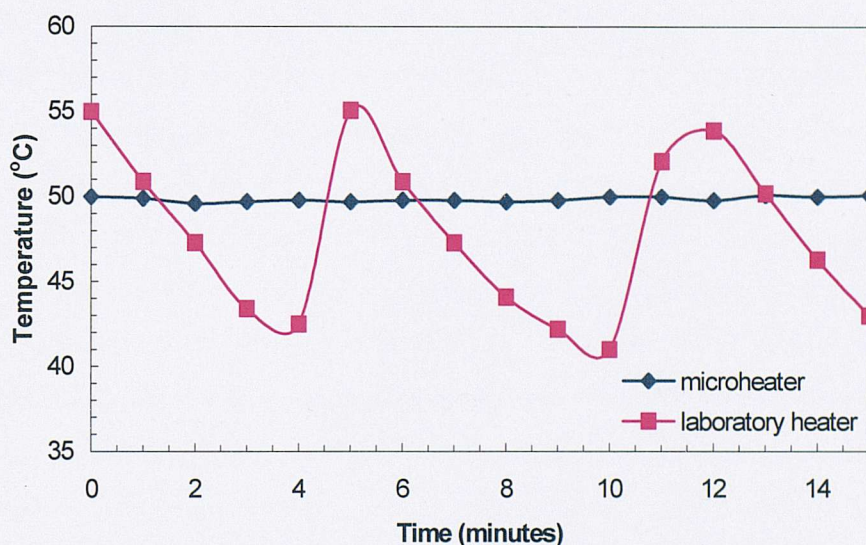


Figure 6.4 Comparison of temperature consistence of a thin film microheater and a laboratory hotplate, set point = 50°C.

During the course of the experiment, the temperature of the laboratory heater varied by a total of 14°C, the temperature of the temperature sensor (an integrated bimetallic strip) continually lagging behind that of the surface

temperature. In comparison, by setting a constant current based on the calibration represented in Figure 6.2, the thin film heater varied by a total of only 0.5°C, temperature fluctuations only occurring as a result of surrounding environmental changes.

6.3. In-channel temperature detection

Although macroscale temperature probes may be used to measure the surface temperature and microfabricated electrodes may indicate the internal device temperature, neither method accurately represents the temperature experienced inside a microfluidic channel. In heated synthesis, due to the exponential relationship between temperature and reaction rate, it is important to determine if the measured temperature accurately reflects the actual temperature in the reaction channel. In a situation where the temperature sensing mechanism is remote to the channel, a greater or lesser amount of heat may be applied to the reaction than desired. In the case of an exothermic reaction, the reaction may progress more aggressively than predicted. For example, at elevated temperatures during the nitration of toluene, secondary and tertiary substitutions occur during the course of the reaction. Due to the exothermic nature of the process, the temperature must be precisely controlled to prevent the formation of these *bi*- and *tri*- nitro substituted toluenes. The measurement of microfluidic device temperatures has been performed previously by integration of platinum[44, 202] and chromium-platinum[199] temperature sensitive resistors. However, in microfluidic channels, where heat transfer coefficients are high, the temperature difference between the channel and the bulk material may be significant. Additionally, this may be accentuated by the difference in the thermal conductivities of the device material and the fluid filled portion of the device.

The direct measurement of in-channel temperature has been performed previously by Sun *et al.* by the measurement of fluorescence from a temperature sensitive dye.[200] However, in the absence of a secondary calibration dye, the

method cannot be used as a reliable measure of the absolute temperature. In addition, fluorescent dyes can be affected by a number of factors including pH and the presence of metal ions as well as suffering from photodegradation. Although the authors are able to determine the in-channel temperature of their system by application of temperature sensitive dyes, no facility is provided for temperature determination during the operation of the device, the fluorescent dye being replaced by a polymerase chain reaction (PCR) reagent mixture.

For the determination of in-channel temperature of the glass microfluidic heaters presented here, thermochromic liquid crystals (LC) were chosen. LCs are used extensively in a number of industries in applications from determining the location of laminar and turbulent boundary layer transitions aeronautics[205] to the study of thermal performance of gas turbine components.[206] Thermochromic liquid crystals are organic molecules that demonstrate properties of both liquids and solids and exhibit a number of phases between these two extremes. The transition between phases is triggered by heat and results in a colour change due to the chiral nature of the molecule. Temperature-sensitive liquid crystals exhibit a range of colours reversibly from colourless (black against a black background) through the colours of the visible spectrum in sequence from red to violet before turning colourless again beyond their thermal range. Standard products are available covering a range of start temperatures from -30°C to $+120^{\circ}\text{C}$ and a colour bandwidth from 0.5°C to 40°C allowing either a broad or precise temperature indication for the rapid accumulation of experimental data.

As with the thermally sensitive dyes employed by Sun *et al.*, LCs can be introduced into the reaction channel for temperature calibration of heating elements, as previously demonstrated for the thermal mapping of a microfabricated array for PCR.[9] In this situation, the PCR mixture was replaced by LC beads for studies of thermal transfer between the heating elements and the microarray. In the absence of a spectroscopic interpretation system for hue measurement, the authors were reliant on the ability of the human

eye to distinguish between shades of colour for temperature determination. As with the fluorescent dye method, once the LC is removed from the microdevice to allow an assay to be performed, all temperature information is lost.

The integration of LCs into a microfluidic reactor for in-channel temperature measurement was achieved by the introduction of an additional collaterally running microfluidic channel occupying the same location and pattern as the permanently incorporated gold electrode (Figure 2. D to E). With similar dimensions and thermal properties to the reaction channel, the temperature experienced by the LC occupying the second channel should be identical to that of the reaction channel assuming the characteristic dimension for heat transfer is maintained. Inclusion of a LC slurry into a second microfluidic channel allows each LC to be injected and removed repeatedly without significant loss (Figure 6.5).

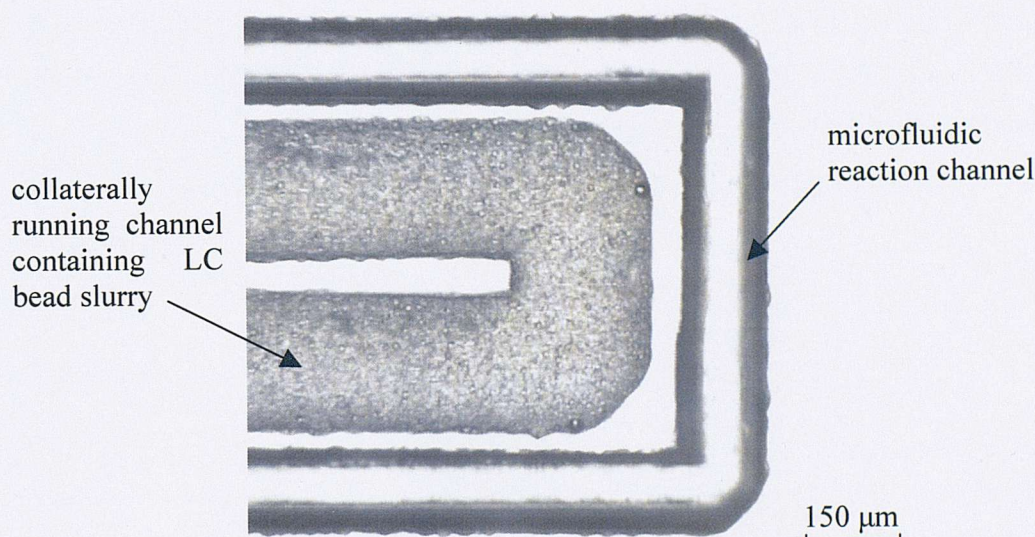


Figure 6.5 A polymer bead encapsulated LC in microfluidic channels surrounding the reaction channel.

Encapsulation in a polymer shell protects LC mixtures from the atmosphere and any residual solvents, which may adversely effect their performance, whilst use of the beads in an aqueous slurry facilitates handling and offers similar thermal conductivity properties to that of the filled reaction channel. In addition,

encapsulated LCs are relatively independent of the viewing angle. In this way a range of liquid crystals with differing thermal properties could be incorporated enabling different temperature regimes to be studied. Alternatively, the use of chemically inert polymers such as PTFE may also allow the LC mixtures to be included directly in the reaction mixture for temperature determination *in situ*.

For ease of production, the collaterally running channel for the encapsulation of the LC slurry was fabricated in the same plane of the device as the fluidic channel. To maintain an equal distance between the reaction and temperature determination channels, the heating element can no longer be fabricated in the plane of the channel. As an alternative, the heating element is placed on an additional non-bonded layer of the device. By placing the electrode on a separate layer, the geometry of the electrode is no longer restricted by the layout of the microfluidic channels. Moreover, using a system of separate heating and reacting modules reduced reactor specialisation providing additional operational flexibility. By vertically displacing the electrode from the reaction channel to an additional device layer, as opposed to the horizontal spacing described in section 6.2.1, the characteristic diffusion dimension is actually reduced to only 100 μm providing greater heat transfer efficiency.

6.3.1. Fabrication

Microfluidic channels were fabricated from glass as previously described (Chapter 2). In contrast to formerly fabricated microfluidic reactors, the etched channels in this case were sealed by a 100 μm thick top plate as described in section 6.3 to minimize the distance for heat transfer. The collaterally running microfluidic channel for the incorporation of the LC slurry consists of a single serpentine channel 263 mm in length, 250 μm wide and 50 μm deep and a total volume of 3.3 μL . The encapsulated LC slurry was introduced into the device by injection through inlet D until the channel was completely filled, the excess LC collected at the outlet (E). Electrodes for modular heating chips were fabricated from borosilicate glass substrates precoated with an 80 nm thick chromium layer

and an AZ photoresist (Nanofilm, U.S.A.). The design was transferred using a direct-write lithography system before development and etching of the chromium layer in the usual way. Once formed, an electrical connection was made to the surface by fixing a length of 0.5 mm copper wire to the thin film by the use of electrically conductive epoxy resin (CircuitWorks CW2400, Chemtronics, U.S.A.). To ensure efficient thermal transfer between the heating and reacting/temperature sensing devices firm contact is essential. To maintain firm contact, four spring loaded clamps were employed at the furthest corners from the heating zone. These clamps incorporated a serrated contact edge to prevent unwanted insulating/conduction effects, which may act to skew experimental data.

Although a large variety of LCs are available covering a wide temperature range, initial experiments were focused on maintaining a temperature set point of 60°C. A LC slurry was purchased from Hallcrest Inc., U.S.A., exhibiting a red onset temperature of 60°C and a bandwidth of 12°C incorporating the remaining colour range (Figure 6.6).

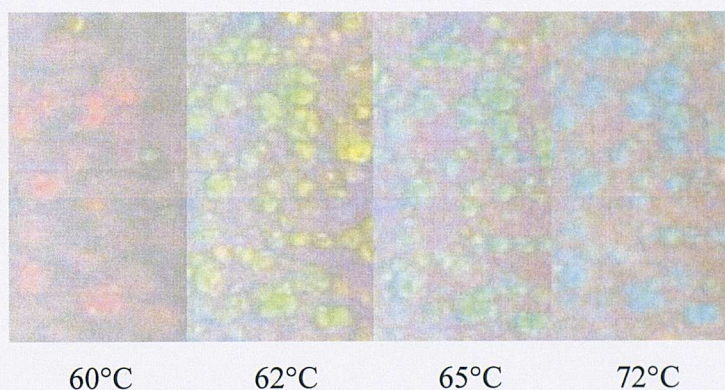


Figure 6.6 LC beads in a microfluidic channel at a range of temperatures.

The red onset colour is the most easily observed of the colour range and also has the narrowest bandwidth, typically between 0.5°C and 10% of the total bandwidth. By selecting a LC with a narrow bandwidth, the required

temperature can be maintained accurately (to within $\pm 10\%$ that of the bandwidth). In the case of the selected LC, the red colour play disappears with a temperature fluctuation of more than $\pm 1.2^\circ\text{C}$. Temperature calibration of each colour play is performed by the manufacturer prior to shipment, and is accurate to $\pm 20\%$ of the bandwidth, in this case $\pm 2.4^\circ\text{C}$. Although the colour plays of the liquid crystal are divided by the broad categories of red, green and blue, the colour spectrum exhibited by the LCs spans the visible spectrum between 400 and 750 nm.

To allow for precise temperature monitoring, reflectance spectra of the LCs were acquired using a high sensitivity fibre optic spectrometer (AVS-S2000, Avantes Inc., U.S.A.). As the reflected colour can alter with wavelength and incidence angle, a halogen lamp fitted with a light guide (Leica GLS100) was used to illuminate the device. A 100 μm OD fibre optic was positioned perpendicular to the LC using a micrometer controlled translation stage and reflectance spectra were recorded at a resolution of 0.5 nm and integrated over 75 ms using 00Ibase2 software (Ocean Optics Inc., U.S.A.) running on a Pentium PC.

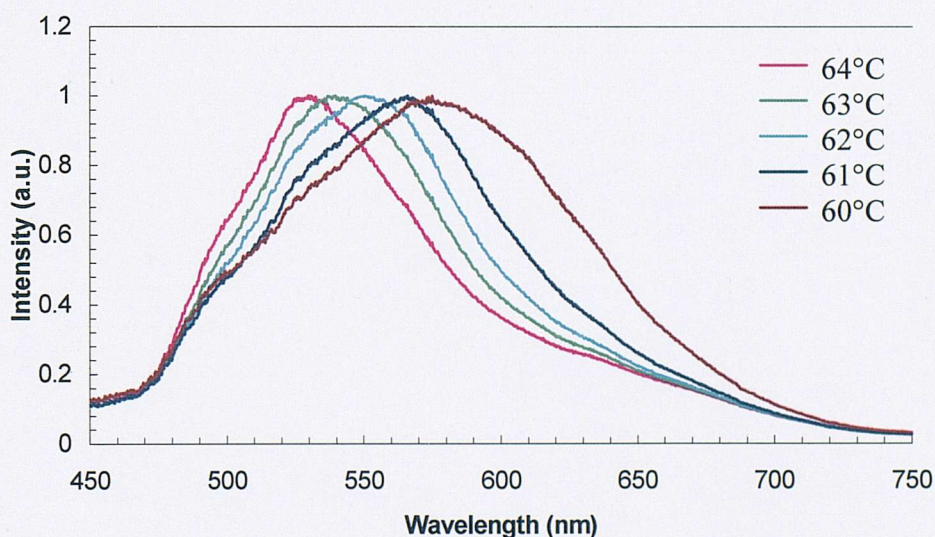


Figure 6.7 The reflectance spectra of the thermochromic LC between 60 and 64°C .

Calibration data for temperature versus the wavelength of peak reflectance were obtained by recording spectra of a chip containing LC which was immersed in an oil bath and heated over a range of temperatures.

6.3.2. Results

From the reflectance spectra of the LC over a range of temperatures, an exponential relationship between peak reflectance wavelength and temperature was observed between 60 and 70°C (Figure 6.8). Beyond 65°C, temperature resolution decreases as the rate of peak reflectance wavelength change with temperature lessens. However, each spectrum is composed of a number of overlapping peaks. These peaks could be analysed using a multi-wavelength algorithm as opposed to a single peak maximum, leading to improved temperature resolution across a broader temperature range. Although feedback control for these experiments was performed manually, the use of a miniaturised spectrometer allows for the facile integration of a computer regulated feedback loop for automated temperature control.[8]

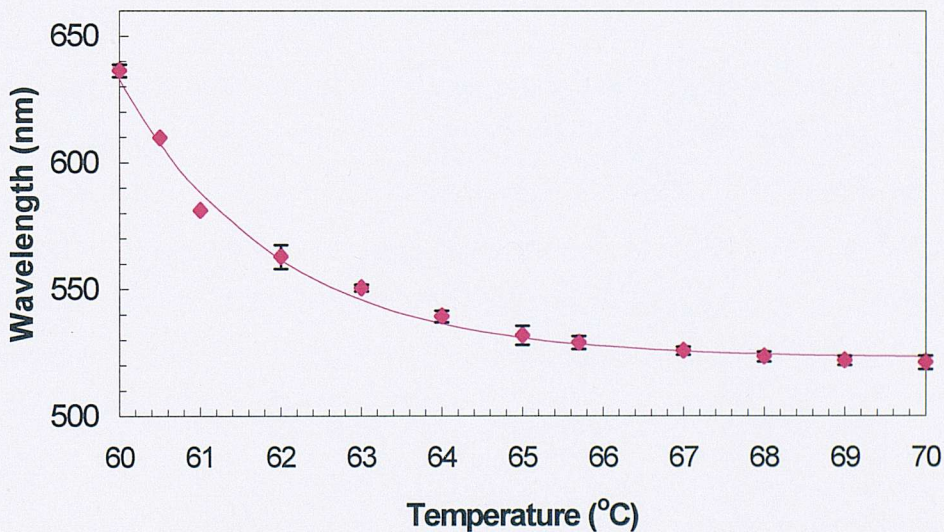
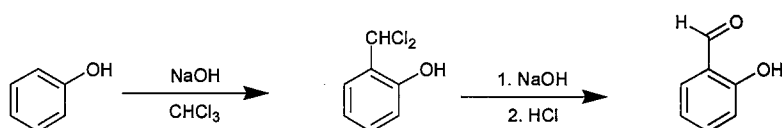


Figure 6.8 The variation in peak reflectance of a thermochromic liquid crystal with temperature. Error bars are standard deviations where $n = 2$.

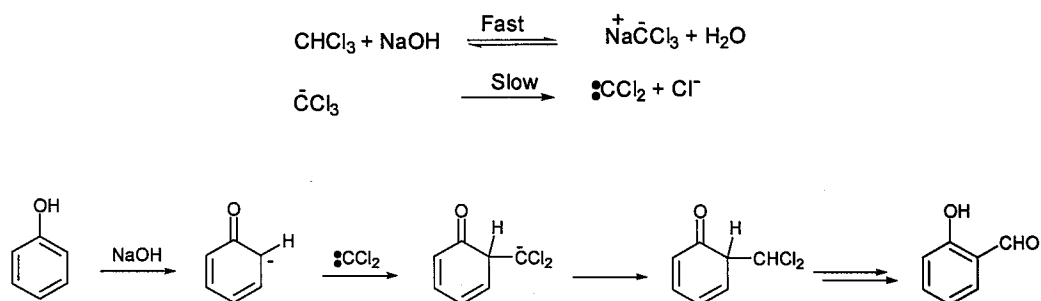
6.4. The Reimer-Tiemann formylation

The Reimer-Tiemann reaction is utilized in the formylation of activated aromatic rings, namely phenols and pyrroles.[207] Direct introduction of an aldehyde group into an aromatic ring is possible with a variety of other methods including the Gattermann,[208] Gattermann-Koch,[209] Vilsmeier-Haack[210] and Duff[211] reactions. However, each method has strict limitations and requirements. For example, only the Vilsmeier, Gattermann and Duff reactions are applicable to phenols and then only with some restrictions. The entering aldehyde group in the Gattermann reaction usually enters in the *para* position and the Duff reaction fails with phenols carrying electron-donating substituents. For these reasons the Reimer-Tiemann reaction is occasionally the only practical method available for the direct formylation of phenols (Scheme 6.1).



Scheme 6.1 The Reimer-Tiemann direct formylation of phenol.

Even 105 years after its discovery, the conditions of the reaction have not yet been optimized, despite yields frequently falling below 40%. Yields of abnormal products (e.g. ring expansion due to carbene insertion) of 3-10% are also common. The Reimer-Tiemann reaction proceeds in two steps. The first is the rate determining generation of dichlorocarbene by the ipso elimination of chloroform in alkali conditions. The second involves the reaction of a phenol with the carbene (Scheme 6.2).



Scheme 6.2 The mechanism of the Reimer-Tiemann reaction.

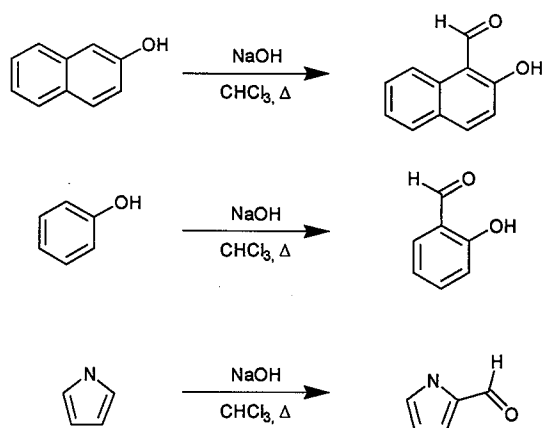
The Reimer-Tiemann reaction suffers from two major drawbacks, both of which may lead to the low yields often observed. Firstly, the dichlorocarbene reacts with water to generate carbon monoxide, which in turn hydrolyses in the sodium hydroxide solution to form sodium formate. The hydrolysis of carbene competes with the formylation reaction and additionally consumes alkali. Second, and more significantly, is the limited reaction interface induced by the biphasic nature of the system. Biphasic reaction systems such as the Reimer-Tiemann are often mass transfer limited. In biphasic systems, ‘micromixing’ on a molecular level never occurs and the reaction is limited to the interface boundary between the solutions. Where macromixing does occur, the coalescence of the phases causes a reversal of the mechanical splitting performed by the mixing process, decreasing the area available for reaction. Due to the presence of the phenolic substrate primarily in the aqueous alkaline layer and the carbene’s preference for the chloroform layer, inefficient transfer of reagents between these layers may lead to stifled reaction rates and subsequently decreased yields.

To overcome the inefficient transfer of reagents between phases in other systems, phase-transfer catalysts have been employed and have enjoyed great success.[212, 213] In application to the Reimer-Tiemann reaction however, phase transfer catalysts have proved disappointing.[214] In a microfluidic mixer, the inherent high surface area to volume ratio provides a large interface boundary for efficient mixing. In addition, operating in a continuous flow regime prevents coalescence of the two phases and the interface boundary may be maintained. In

this way microfluidic reactors may have application in increasing reaction yields over bulk scale processes.

To obtain a satisfactory reaction rate on the bulk scale, the Reimer-Tiemann reaction is usually performed at reflux. Firstly, the reaction solution is warmed to 60°C. Once the chloroform is added, the exothermic nature of the reaction is sufficient to maintain the reaction at reflux without the need for an additional heat source and the addition of the chloroform is adjusted to prevent over-boiling. In a microfluidic reactor temperature control is critical. If the temperature is allowed to approach the solvents boiling point, expansion and eventually boiling may occur. As solvents boil they expand rapidly, leading to the premature expulsion of the reaction mixture from the chip and a significant reduction in the residence time of the reaction. If the temperature is not sufficiently high the reaction rate is low and the time to completion exponentially increases. The generation of vapour pockets also prevents the use of down stream on-line analysis by Raman spectroscopy or similar analysis techniques. To maximise the reaction efficiency without boiling, the temperature within the microfluidic channels must be carefully maintained as close to the boiling point as possible without exceeding it.

To study the Reimer-Tiemann reaction in a continuous flow microfluidic device, the formylation of phenols was performed (Scheme 6.3). A series of reactions were performed at set flow rates over a range of temperatures for β -naphthol, phenol and pyrrole. The highly coloured nature of the intermediates and products rendered detection facile and allowed reaction monitoring by optical detection. Heat was applied either by a traditional laboratory heater or thin film resistive elements and the temperature monitored by LC reflectance spectra.



Scheme 6.3 The formylation of β -naphthol, phenol and pyrrole.

6.4.1. Results and discussion

Traditionally, a concentrated solution of alkali hydroxide is utilised for the hydrolysis of chloroform to generate the dichlorocarbene reactive intermediate and for the hydrolysis of the neutral intermediate (Scheme 6.2). This poses additional problems when performing the Reimer-Tiemann reaction in microfluidic reactors due to the viscosity of the reagents (50% aqueous sodium hydroxide = 78 mPas). Utilising highly viscous reagents generates large backpressures whilst operating under hydrodynamic pumping regimes. For micropumps and even conventional laboratory syringe pumps, the backpressures encountered during the hydrodynamic pumping of such viscous reagents can lead to pump failure. Consequently the sodium hydroxide concentration was reduced to 25%.

Due to the high heat transfer coefficient of the microfluidic reactor there is no appreciable exotherm during the performance of the reaction. In the absence of additional applied heat, the reaction failed to proceed to any substantial degree. On the microfluidic reactor it is possible to follow the progress of the Reimer-Tiemann reaction due to the colouration of the intermediate (Figure 6.9).

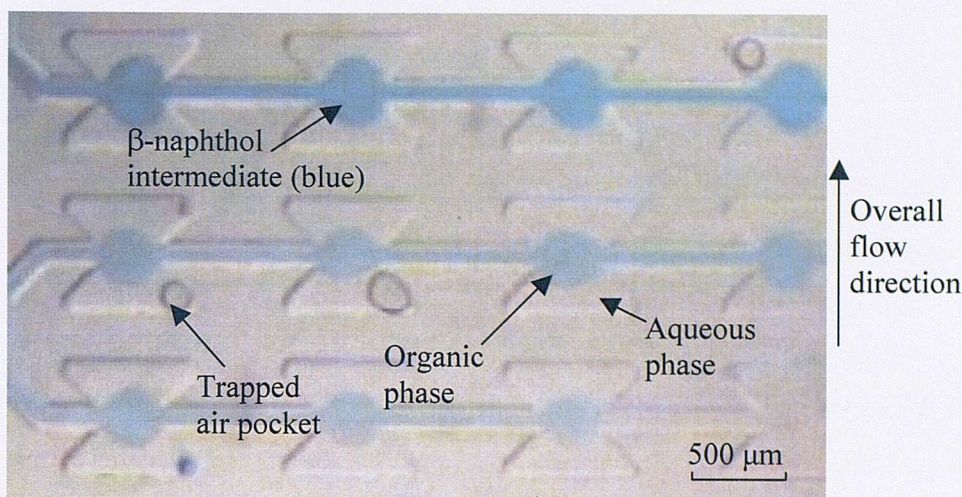


Figure 6.9 The Reimer-Tiemann formylation of β -naphthol at $5 \mu\text{Lmin}^{-1}$ on a glass microfluidic reactor at room temperature.

In the case of the bulk scale formylation of β -naphthol, a deep blue intermediate was formed almost immediately on contact of the reagents and continued to darken as the reaction progressed. On the microfluidic reactor, in the absence of heat, only a very pale blue colour was formed shortly prior to exiting the chip.

For the reaction to be completed, the phenoxide ion is protonated by hydrochloric acid in an acidic quench. However, during the quench of the reaction mixture, substantial quantities of the alkali salt are produced. If the exhaust tubing is placed directly into the quenching solution, the desired red oily product is formed, however, the salt produced precipitates around the exit of the capillary leading to blockage of microfluidic reactor device. If the product is allowed to drip from the end of the tubing into the quench mixture, rapid evaporation of the solvent leads to solidification of the reaction mixture and blockage of the exit capillary. To overcome these difficulties, the exit pipe was placed directly into an acidic quench with the addition of a magnetic follower. The exit tube was then placed in such a way that any salt collected on the capillary exit pipe would be removed with each rotation of the follower. Ideally, both the reaction and the quench would be performed on a monolithic microfluidic reactor, however, this would require major deviation from the

traditional methodology of the Reimer-Tiemann reaction and consequently results thus gained would be incomparable to previous syntheses. By the utilizing a microfluidic reactor, the Reimer-Tiemann reaction was performed at a range of flow rates at a constant temperature of 53°C (Figure 6.10). From Figure 6.10 a significant rise in product yield can be observed for an increased residence time. However, due to the precipitation effects previously discussed, flow rates below 5 μLmin^{-1} were unviable. From the experiments performed, it could be predicted that yields comparable to those achieved on bulk scale syntheses could be attained either by increasing the reaction channel length, thus increasing residence time without decreasing the flow rate, or by the application of an alternative microfluidic reactor design.

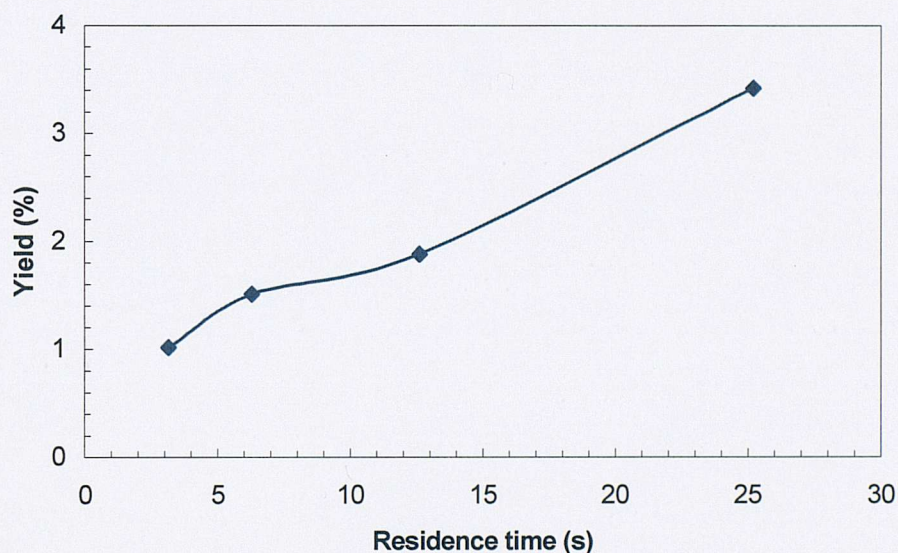


Figure 6.10 The optimisation of residence time of the Reimer-Tiemann reaction on a microfluidic reactor at a constant temperature.

The low to moderate yields expected for the Reimer-Tiemann formylation of phenols were observed in the laboratory scale formylation of phenol, β -naphthol and pyrrole. When performed on a microfluidic reactor in combination with a traditional hot plate, yields of all three substrates were substantially decreased (Table 6.1).

Substrate	Bulk	Yield (%) Microfluidic reactor (Hotplate)	Microfluidic reactor (Microheater)
Phenol	20	2	5
β -naphthol	36	3	10
Pyrrole	31	2	7

Table 6.1 Comparison of yields for the formylation of a range of aromatic phenols by laboratory and microscale synthesis heated by a hotplate and a microheater.

This is believed to be due to the boiling effects observed as previously discussed in section 6.4 where the residence time of each reaction is dependent on the amount of boiling and therefore on the heat supplied by the laboratory hot plate, and not the flow rate defined by the pump. In comparison, a microheater, monitored by LC reflectance spectra, was used to effect reaction at a range of temperatures both above and below that of the boiling point of the reaction system (Figure 6.11).

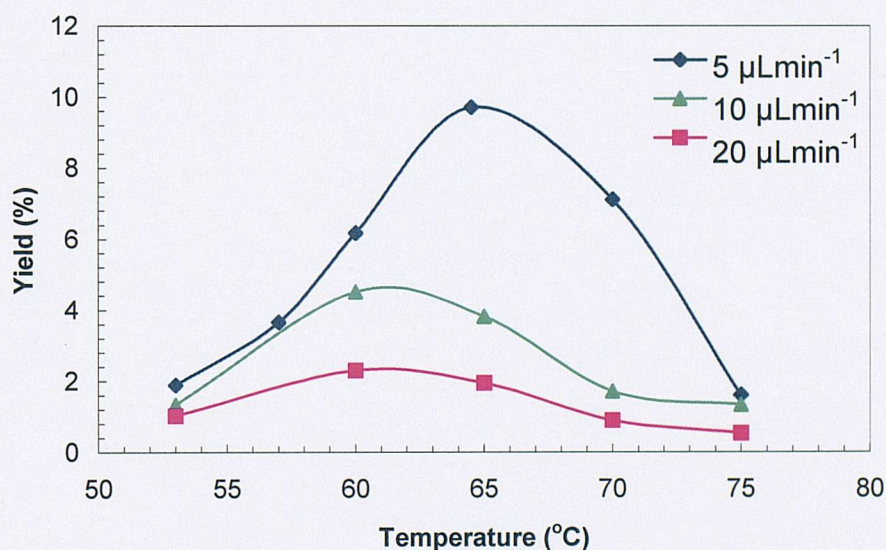


Figure 6.11 The effect of temperature on yield of the Reimer-Tiemann formylation of β -naphthol in an enclosed microchannel at a range of flow rates.

Upon an increase of the reaction temperature, a corresponding increase in reaction rate was observed as predicted. Once the boiling point of the solution was exceeded however, thermal expansion and boiling led to severely decreased residence times resulting in a decrease in yield for a given flow rate. Although yields of only 10% were obtained during these experiments, no optimisation of chip design or reagent concentration was performed. To allow for a more direct comparison with a laboratory or bulk scale reaction vessel, the space/time yield of the device is calculated to be $2,500 \text{ molm}^{-3}\text{h}^{-1}$ for the synthesis of 2-hydroxy-1-naphthaldehyde. A corresponding space time yield of $41 \text{ molm}^{-3}\text{h}^{-1}$ can be calculated for a 1 L scale synthesis, assuming a total yield of 40%.^[184] The space time yield for the microfluidic reactor quantifies the hourly throughput of the device as a function of the total volume of the device. For this calculation, the volume of the channel itself was used and not that of the entire glass substrate. In the prototype devices presented herein, the ultimate volume of the device is determined by the substrate material and fabrication methods. In a final production microfluidic reactor, mass microfabrication methods would allow for multiple channels to occupy the same volume, as illustrated by comparison of the total size of the devices detailed in sections 2.10 and 2.12 in Chapter 2.

6.5. Conclusion

The utility of thermochromic liquid crystals for accurate in-channel temperature determination and control in microfluidic devices has been demonstrated by application to the Reimer-Tiemann formylation. LCs are ideal for microfluidic applications as they enable temperature measurement of microenvironments, such as a fluidic channel, to be monitored. In addition, LCs potentially offer *in situ*, direct temperature determination in-channel, by incorporation into the reaction mix as an inert bead or into sub-micron fluidic channels as a liquid. In these initial experiments a resolution of 0.5°C was obtained over a temperature range of only 5°C . Although this temperature range is not large, it was adequate to indicate the critical temperature such that reflux of the Reimer-Tiemann reaction was avoided. This resulted in an overall increase in yield of two to

three times over the reaction performed on a microfluidic reactor in conjunction with a macroscale heater. Despite the application of increased temperature control, overall yields obtained on the microfluidic reactor were lower than usually obtained on the bulk scale. This can be attributed to the experimental difficulties experienced and an absence of optimisation of the fluidic structure. Although yields were low, a high space time yield of $2,500 \text{ molm}^{-3}\text{h}^{-1}$ can be calculated for this device.

6.5.1. Synthesis

6.5.1.1. General method for the laboratory synthesis of formylated phenols by the Reimer-Tiemann reaction

To a 100 mL round bottom flask, fitted with a reflux condenser and magnetic follower containing 3 mL of ethanol, 1.25 g (8.7 mmol) of β -naphthol was added and stirred until dissolved. With rapid stirring a 25% solution of NaOH (2.5 g, 62.5 mmol) in water (5 mL) was added and heated to 75°C in a water bath. Through the reflux condenser 1.6g (13.4 mmol) of chloroform was added dropwise to maintain reflux. Once the addition was complete, the mixture was stirred for an additional 10 minutes. Once complete, the solution was neutralized to pH 6 by the addition of conc. HCl with vigorous stirring. Sufficient water was added to dissolve the sodium chloride prior to separation and concentration by evaporation, yielding a deep red oil. Product analysis was performed by GC without further purification by comparison to spiked standardised solutions. GC-MS and ^1H NMR spectra were also obtained and compared.

6.5.1.2. General method for the continuous-flow synthesis of formylated phenols by the Reimer-Tiemann reaction.

Solutions of β -naphthol (1.25 g, 8.7 mmol) in ethanol (3 mL) and chloroform (1.6g, 13.4 mmol) and 25% sodium hydroxide (2.5g, 62.5 mmol) in water (5 mL) were prepared. These solutions were introduced into inlets A and B respectively of the heated reactor chip at flow rates between 5.0 and $20 \mu\text{Lmin}^{-1}$ using a PHD 2000 syringe pump (Harvard Apparatus, Kent, U.K.). The reaction was

quenched immediately upon exiting the chip outlet (C) in a continually stirred solution of 12% hydrochloric acid. Once collected, the aqueous and organic layers were separated and product analysis was performed by GC, without further purification, by comparison to spiked standardised solutions. GC-MS spectra were also obtained and compared.

7.

CONCLUSION

The application of the concepts of miniaturisation to analytical methods has shown great promise over recent years. Microfluidic structures with feature sizes in the low micron range have been shown to offer several advantages over traditional analytical devices. The application of this same precept to synthetic chemistry however, remains at the periphery of the μ -TAS concept. This thesis has described the design and fabrication of novel microfluidic reactors and their application to small molecule organic synthesis, where mixing of reagents over reduced distances and efficient heat transfer has led to increased yields and substantially higher throughput.

7.1. Summary of achievements

The research undertaken during the course of this thesis concentrated on two main areas, firstly the design, fabrication and interfacing of microfluidic devices and secondly, their application to small molecule organic synthesis. Prior to the utilisation of microfluidic reactors for organic synthesis, the fundamental operation of sample introduction and removal was addressed. In processes which employ valuable or hazardous components, the fidelity of this process is of utmost importance. To this end, the reversible coupling of microfabricated structures to the real world (e.g. capillary tubing, sample reservoirs and pumps) was examined. Subsequently a series of clamped interfaces were designed and fabricated, culminating in the fabrication of an economical, gasket free interface of good chemocompatibility and low dead volume.

Mixing in microfluidic reactors is an area of great importance. The mechanisms of mixing in single-phase microfluidic systems have been thoroughly investigated with numerous papers on the subject appearing in the literature.[129, 138, 139] On the subject of microfluidic mixing in two component flows however, there are significantly fewer examples available.[147, 148, 150] Consequently, a study on the effect of expansion/contraction ducts on mixing efficiency in biphasic flows on planar microfluidic devices was performed. Fluorescence derivatisation reactions were employed as a sensitive method to detect and quantify the extent of mixing in a series of devices of varying duct geometries and volumes. The mixers described proved suitable for both single and biphasic fluid regimes over a range of flow rates and the rate of mixing was shown to improve in each case over a straight, featureless channel of comparable dimensions and materials.

The second part of this thesis has examined the effect of microenvironments on the synthesis of small molecules. One of the most important benefits that miniaturisation has to offer the organic chemist is the facility to perform synthetic procedures that would otherwise be unavailable due to safety concerns.[39, 54] The ability of microfluidic reactors to render potentially

dangerous reactions safe is demonstrated by the synthesis of unstable diazonium salts and their subsequent *in situ* chlorination in anhydrous conditions. The precept that reactions performed in microenvironments are intrinsically safe is further strengthened by the rapid, continuous flow quenching of phosphoryl chloride. Conventionally an energetic delayed exotherm, the hydrolysis of POCl_3 on a microfluidic reactor is performed rapidly and smoothly without the evolution of acid gas and exhibiting a temperature rise of only 5°C . Furthermore, the use of Raman spectroscopy for on-line detection and optimisation during synthesis on a microfluidic reactor is performed for the first time, succinctly demonstrating the applicability of microfluidic reactors to automated, dynamic feedback control and reaction optimisation.

The application of heat in organic synthesis has a significant role in the initiation and propagation of a reaction. However, if precise control is not employed, heating in enclosed environments has considerable repercussions. These include localised boiling and the premature expulsion of the reaction mixture from the chip leading to a reduction in the residence time of the reaction. To provide a method of accurate temperature monitoring on a microfluidic format, thermochromic liquid crystals were investigated for application to in-channel temperature determination. The combination of thermochromic liquid crystals with reflectance spectroscopy and thin film resistive heating elements provided rapid and uniform heating of microfluidic devices with in-channel temperature determination to within 0.5°C . These concepts were then applied to microfluidic synthesis where precise temperature control ensured that overheating of the Reimer-Tiemann formylation reaction mixture was avoided. This resulted in an overall increase in yield of two to three times when compared to the reaction performed on a microfluidic reactor in conjunction with a macroscale heater. Although accurate temperature monitoring was only possible over a narrow temperature range, the combination of two or more LCs and the use of a multi-wavelength algorithm for the analysis of overlapping peaks could lead to improved temperature resolution across a broader temperature range.

7.2. Future applications

7.2.1. Nanoparticles and quantum dots

The synthesis of nanocrystalline semiconductors is an intriguing area and one that could benefit from the outstanding levels of process control that are available in microfluidic systems. Although some work has already been performed in this area,[9, 10] such is the scientific and commercial interest in nanoparticles, that the application of microfluidics to their synthesis may yet become the main driving force behind microfluidic reactor development.

To investigate the application of microfluidic technologies to the synthesis and production of structurally perfect, monodisperse nanocrystalline materials the following points may be addressed.

- The design and manufacture of novel mixing architectures for the synthesis of nanoparticles on a microfluidic format.
- The effect of physical parameters such as heat, magnetic fields and flow regime on particle size and monodispersity.
- The effect of concentration and phase boundary interfaces on nanoparticle formation.
- The application of surface passivation techniques to nanoparticles generated *in situ* for improved quantum efficiency.

The effect of flow rate on nanoparticle formation has already undergone preliminary investigations in this laboratory, where a decrease in polydispersity was observed with increasing flow rate on a distributive micromixer employed in the synthesis of cadmium sulfide nanoparticles.[9] By transferring the synthesis of nanoparticles to a microfluidic format, it may be possible to efficiently synthesize nanoparticles of a desired size directly and without the need for

subsequent recrystallization. This will grant maximum flexibility to the manufacturer, while simultaneously providing the end-user with complete control over particle design and production.

7.2.2. Photochemistry

Photochemistry is an extremely useful area of synthetic chemistry finding applications in isomerisation, rearrangement, addition, cleavage, substitution and elimination reactions, to name but a few. The seemingly trivial process of exciting a molecule from its ground state to an excited singlet or triplet state by the absorption of light on an industrial scale however, is inherently difficult. Problems typically encountered during an industrial scale photochemical reaction derive from two sources. Firstly, achieving adequate light permeation such that the whole of the reaction volume is submitted to illumination is problematic. This is especially difficult where photosensitising molecules, which are typically strongly absorbing, are required, in which case intense tungsten light sources may be necessary to achieve the desired level of illumination. The second area of difficulty comes from the light sources themselves, where over-irradiation of the target may lead to unwanted by-products. Furthermore, due to the size and intensity of the illumination source, large quantities of heat may be generated necessitating the addition of complex refrigeration systems.

Initial studies performed in this laboratory indicate that each of these difficulties encountered during the transfer of photo processes from the laboratory to the industrial scale can be countered by the application of microfluidic reactors.[84] Since microfabricated channel depths are typically in the low micron range, radiation can easily penetrate through the entirety of the reaction volume, even in the case of highly absorbing photosensitising dyes. The removal of the intense light source immediately negates the additional problems it creates. Moreover, due to the efficient heat transfer and radical termination properties of microfluidic reactors, potentially explosive industrial scale photochemical

reactions such as the generation of singlet oxygen may be performed in complete safety. [11]

7.2.3. High throughput screening

Combinatorial style synthesis and high throughput screening has huge potential for the rapid generation of compound libraries, where potentially thousands of compounds may be generated in very short times. However, such is the rate at which large libraries of compounds can be produced using combinatorial chemistry, it is no longer the synthesis and purification, but the analysis of the produced compounds that becomes the rate-limiting step in the discovery process. One example where the combination of combinatorial chemistry with microfluidic reactors could lead to significant advances is the discovery of enantioselective catalytic processes. For instance, the asymmetric reduction of prochiral ketones and imines to chiral alcohols and amines provides a convenient route to a variety of important compounds where introduction of chirality is essential for biological activity. Although the determination of enantiomeric excess can be achieved in many ways, for example; optical rotatory dispersion (ORD), circular dichroism (CD), chiral GC and NMR in combination with either chiral solvents or chiral lanthanide shift reagents, few lend themselves to high throughput screening.[215, 216]

Recently, the determination of the enantiomeric excess (ee) of alcohols and amines has been successfully achieved on nanomolar scale by Guo *et al.* by kinetic resolution.[217] By employing an equimolar mixture of pseudo-enantiomeric “mass-tagged” chiral acylating agents that differ in a substituent remote to the chiral centre, ee can be determined by mass spectrometry. Since electrospray- (ESI) and nanospray-MS are already equipped to deal with a continual flow of small volumes of sample, the integration of microfluidic reactors with these systems could lead to a continuous flow synthesis of chiral compounds with on-line real-time enantiomeric excess determination.

7.3. Summary

Throughout this thesis, microfluidic reactors have been shown to be beneficial to small molecule organic synthesis in areas such as throughput, mixing, product yields and safety. In particular, microfluidic reactors have been shown to demonstrate increased yields over laboratory scale syntheses involving reactive diazonium salts in otherwise prohibitively dangerous anhydrous conditions. The applicability of microfluidic reactors to automated dynamic feedback control for process optimisation is also demonstrated by the use of Raman spectroscopy for on-line detection and optimisation during synthesis in a microfluidic device. In addition to the application of microfluidic reactors to organic synthesis, reversible interconnection technology was investigated, culminating in a novel interface of low dead volume and cost with a potentially high ultimate pressure threshold. Moreover, novel microfluidic reactor designs incorporating expansion/contraction ducts demonstrated that channel geometry can be manipulated on the microscale for increased mixing efficiency in two component reaction systems.

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8.

APPENDIX

This chapter contains reprints of published material on the subject of microfluidic reactors resulting from the collaboration with colleagues from Imperial College London.

Microfluidic Routes to the Controlled Production of Nanoparticles

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A microfluidic procedure for the controlled production of cadmium sulfide nanoparticles is described.

Nanocrystalline semiconductors are of considerable scientific and commercial interest owing to their tuneable optical and electronic properties, and potential applications in a wide range of electronic devices¹. Physical characteristics of nanocrystallites are determined primarily by spatial confinement effects with properties such as the optical band gap often differing considerably from the bulk semiconductor. As these properties are ultimately determined by the physical size and shape of the crystallites, there is considerable interest in processing routes that yield nanoparticles of well-defined size².

To date, techniques for producing highly monodisperse nanoparticles have been relatively complex and of a post-hoc nature³⁻⁵ (A good review article by Trindade *et al* describes various approaches to synthesis of nanocrystalline semiconductors⁷). They are commonly produced in two stages: a poly-disperse sample is first obtained using standard synthetic routes, and nanoparticles of a given particle size are then isolated by means of repeated recrystallisations. This approach, whilst appropriate for small-scale production of nanoparticles, may not be ideally suited to large-scale production owing to the low product yield and the time consuming nature of the recrystallisation process⁶. At the time of writing there have been few – if any – *direct* (single-stage) approaches to the fabrication of monodisperse nanocrystallites. Fischer and Giersig⁸ have previously used a fast flow (~1 ml/min) mixing chamber in conjunction with a chromatographic column to produce ultrasmall CdS nanoparticles. In this paper, we take an established synthetic procedure for cadmium-sulfide nanoparticles and demonstrate how adaptation to a continuous flow microfluidic format leads to an immediate reduction in polydispersity. The procedure may offer an effective approach to synthesising nanoparticles *directly* and without recourse to subsequent recrystallisations.

Following Mahteb *et al*, nanoparticles of cadmium sulfide were obtained by mixing aqueous solutions of cadmium nitrate and sodium sulfide⁹. In conventional approaches to nanoparticle synthesis, the reactions are carried out in static macroscopic reaction vessels. Any variations in physical conditions across the reaction chamber (e.g. concentration or temperature gradients) are liable to influence the nature of the chemical product and in particular are likely to affect the size of the crystallites. In order to improve polydispersity, it is therefore necessary to eliminate local variations in reaction conditions, which is not trivial in a bulk reactor. Miniaturisation on the other hand affords a direct means of eliminating these local variations as the entire reaction volume can be held at a constant (uniform) temperature with near-absolute chemical homogeneity pertaining throughout¹⁰.

For bulk production of chemicals, the miniature reaction vessel must be used in a continuous flow format with the product being continuously extracted and the reactants continuously

replenished. In order to preserve chemical homogeneity, it is essential that the entrant reagents should mix rapidly (faster than the time-scale of the reaction). Owing to the absence of turbulence in microengineered structures efficient mixing is a non-trivial process¹¹. Mixing in microstructures is mediated almost exclusively by molecular diffusion and can be relatively slow. This leads to departures from chemical homogeneity and in the present context increases the polydispersity of the nanoparticles produced. To overcome this problem, a fast micromixer must be used (see Electronic Supplementary Information). Specifically, we use the continuous flow micromixer developed by Bessoth *et al.*, which has been described in detail elsewhere¹². In short, two inlet flows (containing either sodium sulfide or cadmium nitrate) are split into a series of separate multichannel streams (16 partial flows) before bringing them into final contact; as the diffusion time is proportional to the diffusion distance, splitting each reagent stream into n substreams of similar width decreases mixing times by a factor n^2 . After mixing, the channels are sequentially combined in a reverse network until all partial flows are united in one outlet channel. The entire mixing volume for the micromixer is less than 600 nl.

An additional cause of polydispersity is coalescence of the newly formed nanoparticles to form larger crystallites. This may be discouraged to some extent by stabilising the nanoparticles with another semiconductor or chemical species¹³. However it is nevertheless important to extract the nanoparticles from the reaction volume as quickly as possible. It is therefore to be expected that varying the flow rate of the reagents through the micromixer will have a considerable influence on the size distribution of the nanoparticles.

To summarise the overall experimental procedure: CdS nanoparticles were obtained by directly mixing 4×10^{-4} M aqueous solutions of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and Na_2S . To aid stabilisation of the resulting nanoparticles, an equal quantity of sodium polyphosphate was added to the cadmium nitrate solution prior to mixing. A syringe pump was used to deliver the reagents into the microfluidic channel network at various flow rates (10–300 $\mu\text{L}/\text{min}$). The outlet flow from the mixer chip was coupled to a quartz flow cell (10 mm path length) and absorption spectra were obtained using a Perkin-Elmer, Lambda 15 UV-Vis spectrometer.

The inset of Figure 1 shows an absorption spectrum for CdS nanoparticles obtained by mixing bulk (750 μL) solutions of Na_2S and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. The shallow profile of the absorbance – which shows a gradual increase from 2.4 eV to 3.6 eV rather than a sharp onset at a specific energy – indicates that a broad range of crystallite sizes are present in the sample. A simplistic application of Brus' Equation would suggest that the nanoparticles range in size from roughly 12 nm ($\approx$ 2.4 eV) to roughly 3.2 nm ($\approx$ 3.6 eV); however we stress the highly approximate nature of this calculation. (In passing we note that,

as with all the data reported here, no attempt was made to optimise the synthesis procedure. Reaction concentrations of 4×10^{-4} M were taken directly from the literature⁹, and it is likely that systematic variation of reaction conditions might yield nanoparticles of lower polydispersity.)

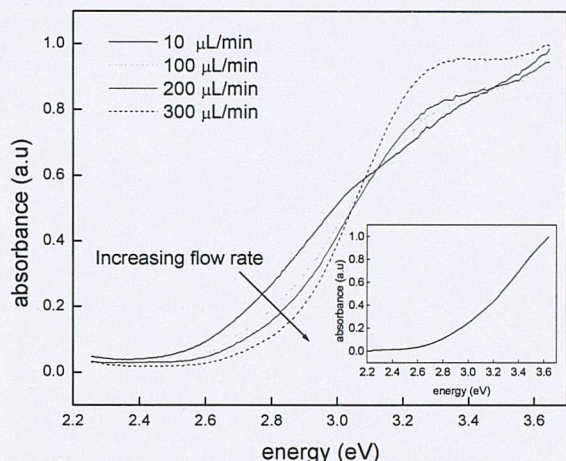


Figure 1: Absorption spectra of fluid streams exiting the micromixer chip subsequent to mixing of cadmium nitrate and sodium sulfide solutions as a function of volumetric flow rate. Inset: Absorption spectrum of nanoparticles produced by mixing bulk solutions.

Figure 1 shows, for a variety of flow rates, absorption spectra of fluid streams exiting the micromixer chip after mixing of the cadmium nitrate and sodium sulfide solutions; the reagent concentrations were 4×10^{-4} M in all cases. Two important features are evident: firstly, the slopes of all absorption profiles in Figure 1 are steeper than in the inset; and, secondly, there is a progressive steepening of the absorption profile as the volumetric flow rate is increased from 10 to 300 $\mu\text{L}/\text{min}$. The first observation indicates that the process of miniaturising the reaction volume is sufficient to lower the polydispersity of the crystallites. The second observation illustrates that increasing volumetric flow rates leads to further improvements in crystallite monodispersity. Both observations are consistent with the discussion above: miniaturisation improves homogeneity across the reaction volume; and increasing the flow rate lessens the likelihood of nanoparticle coalescence.

An interesting feature of the data presented in Figure 1 is the existence of an approximate isosbestic point at 3.12 eV. This (and the variation in absorption profiles) may again be rationalized in terms of the polydispersity of the nanoparticles. If $N(E)$ represents the relative occurrence of particles with bandgap E and $A(E, E_G)$ represents the absorption spectrum of monodisperse particles of bandgap E_G , the average absorption spectrum of the population $\langle A(E) \rangle$ may be approximated as $\langle A(E) \rangle = A(E, E_G) \otimes N(E)$ where $\otimes$ denotes a convolution. (Here we have assumed that the absorption spectra of the variously sized nanoparticles are essentially identical in form, differing only in the location of the band edge.) In principle, if $A(E, E_G)$ is known, the underlying size distribution $N(E)$ may be inferred using deconvolution. In practice though this is seldom possible owing to noise in the input data and hence we make no attempt to extract size distributions here. We note however that the various absorption spectra will yield an isosbestic point only in special circumstances: *underlying population distributions must be of similar form – with the same modal energy gap & skewness – but different variances*. This is illustrated in Figure 2 where we

have taken an arbitrary non-symmetric absorption profile and performed convolutions with a series of skewed distributions with different second moments but identical first, third and fourth moments. The spectra are all seen to pass through a common point with the steepest absorption profile corresponding to the narrowest size distribution. The exact location of the isosbestic point is close to the modal energy gap of the particle distribution. Importantly, an isosbestic point is not obtained if the distributions have different third moments (skewness).

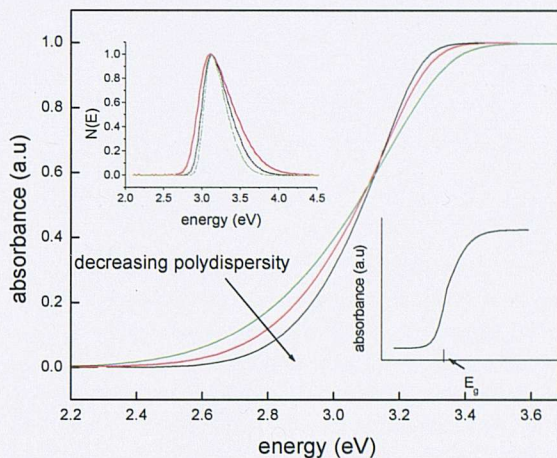


Figure 2: Theoretical absorption spectra for samples of nanoparticles obtained by convolving an arbitrary single-particle absorption spectrum (inset right) with three crystallite distributions of similar shape but different second moments (inset left). E_G is defined as the energy at which the absorbance = $1/2$.

Relating this observation to the experimental data, it is apparent that varying the flow rate provides a means of improving monodispersity (reducing the second moment of the size distribution) without affecting the modal energy gap or altering the symmetry of the size distribution.

In conclusion, it can be seen that the use of microfluidic synthesis procedures offers distinct advantages over bulk scale fabrication procedures. The small size of the microreactor ensures that thermal and chemical homogeneity pertain throughout the entire reaction volume, leading to well controlled reaction conditions. Moreover, performing the reaction in a continuous flow format discourages coalescence of the newly formed nanoparticles. It should be noted that no effort has been made to optimise the microreactor in the current application. It is expected that refinements will lead to further improvements in resultant size distributions.

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A Microfabricated Nanoreactor for Safe, Continuous Generation and Use of Singlet Oxygen

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Abstract:

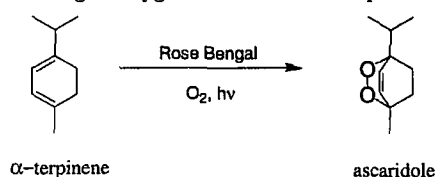
Singlet oxygen was effectively and safely generated in a nanoscale reactor and used for the synthesis of ascaridole. The technique allows for the generation of singlet oxygen without the inherent dangers of large quantities of oxygenated solvents. The methodology allows for facile scale-out of the process.

Introduction

The use of photosensitisers to effect the singlet oxygen oxidation of terpenes and conjugated dienes has a long history, particularly in the perfume industry.¹ Despite the utility of the functionality introduced by this transformation there are inherent difficulties in adapting the process for large-scale production. These include the difficulty of introducing adequate intensities of the required wavelength of light into the reactor and achieving sufficient oxygen saturation. The challenge for the process chemist in achieving adequate illumination is made more difficult by the strong absorption of sensitizer dyes. This leads to a shortened effective path length even under quite powerful irradiation and also the formation of dimers and higher aggregates, many of which are insoluble.² Furthermore, the use of tungsten lamps or sunlight in this regard brings with it difficulties for scale-up and unwanted sample heating, necessitating the use of collimators or refrigeration. The explosive nature of oxygenated organic liquids³ is well-known and can lead to significant health and safety issues.⁴ Even aerated organic solvents can be extraordinarily hazardous on a laboratory scale and more so on an industrial scale. Recent developments in this area have addressed the problem of adequate illumination but still leave the safety issues associated with large quantities of oxygenated organic solvents unsolved. For example, most “small tube” flow devices involve recirculating feedstock from a large reservoir through an illuminated tube, leaving large quantities of aerated solvents in reservoir.⁵ Yields for this type of synthesis are as high as 90% for terpene oxidation, but the problems posed by accidental pipe rupture are still of concern.

The application of nanoreactor technology to address difficulties in synthetic chemistry is an area of increasing

Scheme 1. Singlet oxygen addition to α -terpinene



interest to the chemical community.⁶ The term “nanoreactor” denotes reactors with an instantaneous reaction volume most conveniently measured in nanolitres. Because of increased efficiencies of mixing and separation combined with high rates of thermal and mass transfer, nanoreactors are ideal for processing valuable or hazardous reaction components and in many instances for improving reaction selectivities.⁷ Moreover, the low-Reynolds-number environments encountered within most nanofluidic devices afford a high degree of control over processes performed in continuous-flow formats.⁷ Importantly, continuous-flow alternatives to traditional batch processes can be scaled with facility by the use of a multiparallel (or scale-out) approach.⁶

Motivated by these advantages, we describe herein the application of nanoreactor technology to the safe, efficient, continuous-flow synthesis of ascaridole from α -terpinene, as shown in Scheme 1. Miniaturising the reactor footprint takes advantage of the small length scales and high surface-to-volume ratios of microfabricated devices, and since the microfluidic channels fabricated are approximately 50 μm deep, radiation can easily penetrate through the entirety of the reaction environment. Importantly, the technique allows for the generation of singlet oxygen without the inherent dangers of large quantities of oxygenated solvents.

Nanoreactor Fabrication

The glass microchip (footprint 5 cm $\times$ 2 cm) was made in-house using direct-write laser lithography, wet chemical etching and bonding techniques as previously described.⁸ Briefly, a positive photoresist (S 1818, Shipley Corporation, Whitehall, PA) was spun onto the surface of a glass substrate, and the channel design transferred to the substrate into the photoresist using a DWL system (DWL2.0, Heidelberg Instruments, Heidelberg, Germany). After the photoresist was developed (Microposit 351, Shipley Europe Ltd, Coventry, UK), the channels were etched into the glass substrate using a buffered oxide etching solution (HF/NH₄F). A glass cover

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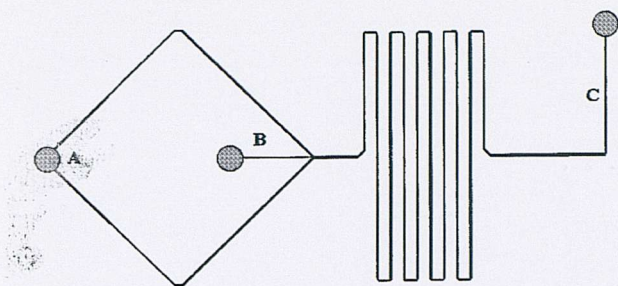


Figure 1. Schematic of reactor chip for singlet oxygen reactions.

plate, with holes drilled for external access, was cleaned in $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ and aligned before contact with the etched glass substrate. To form enclosed channels, the plates were thermally bonded in an oven.

The reactor channel pattern is schematically described in Figure 1. The chip consists of a divergent inlet channel (A), a secondary inlet channel (B), a serpentine irradiation sector, and an outlet channel (C). The etched channels have an average depth of $50\ \mu\text{m}$ and an average width of $150\ \mu\text{m}$. The serpentine section has a total length of 50 mm.

Results and Discussion

Under the reaction conditions optimised for the reactor chip no presaturation of the α -terpinene solution with oxygen was necessary, meaning that at any given instant the total volume of oxygenated solvent could be measured in picolitres. This small volume of oxygenated solvent present at any one time minimises the risks involved in the synthesis. Furthermore, nitrogen degassing of chip effluent can be undertaken as soon as the effluent is collected, to avoid accumulation of oxygenated solvents or the elevation of oxygen saturation in the reactor surroundings.

To effect the synthesis of ascaridole from α -terpinene the delay section was irradiated using an unfiltered, 20-W, 6-V overhead tungsten lamp on an inverted microscope stage (Leica DMIL, Milton Keynes, UK). A solution of sensitizer dye and terpinene in methanol was introduced through A, and a stream of oxygen was introduced through B. The effluent was collected at C and worked up by diluting 10:1 with diethyl ether, passing through a silica plug, and evaporating off the excess solvent with a stream of nitrogen. Transformation was monitored using capillary gas chromatography (GC), utilising a HP Innowax stationary phase. GC samples were introduced using an autosampler and a HP 68190 autoinjector array to avoid operator errors. Samples were compared to a sample of ascaridole prepared by standard methods that had been characterised by spectral comparison to literature data.⁹ Samples thus prepared co-ran in GC analysis with chip-produced products. Crude material from the microchip effluent was submitted to NMR analysis and demonstrated ascaridole present as the majority product.

In common with laboratory-scale procedures pure oxygen and a relatively concentrated sensitizer solution were chosen

for use. It should be noted that oxygen and air are considered interchangeable for many (although not all) singlet oxygen preparations, although an adjustment in flow rate would have to be made to compensate for the lower partial pressure of oxygen in air. Rose Bengal is readily recyclable, so that relatively high concentrations of sensitizer are not necessarily a cost issue. Furthermore, since the effective optical path length is $50\ \mu\text{m}$, a high sensitizer concentration does not pose any experimental problems. The molar extinction coefficient of Rose Bengal in methanol is $99\,800\ \text{cm}^{-1}\ \text{M}^{-1}$ at 550 nm. Despite this high value a concentration of $5 \times 10^{-3}\ \text{M}$ Rose Bengal yields a total absorbance of 0.025 (equating to a transmittance of approximately 95%). This means that molecules at any position within the irradiation zone will experience a broadly similar photon flux. Consequently, the use of a microfabricated reactor actually facilitates the use of high sensitizer concentrations, which is desirable in most circumstances.

In addition, it should be noted that the small cross section of the reactor channels results in effective irradiation even from relatively low-intensity light sources. This leads to reduced sample heating and therefore reduced radical recombination. Efficient transformation ($>80\%$ conversion of feedstock by GC area analysis as described) can be achieved with an irradiation (reaction) time of less than 5 s. Figure 2 shows typical GC traces of the chip feedstock solution and the chip product after work-up. Although the method of analysis is crude, the identification of the product peak by separately synthesised authentic ascaridole renders it less ambiguous.

It is likely that the majority of microfluidic devices will eventually be structured from polymeric materials (rather than silicon or glass) due to the wide availability of base materials, commercial chip manufacturers, and highly cost-effective fabrication methods (such as injection molding and hot-embossing). This means that the reactor chips described herein may be "printed" and bonded *en masse* at low unit cost.¹⁰ Furthermore, since light can penetrate the substrate material from any direction and the chips function perfectly in any orientation, large arrays of chips can be linked together to form three-dimensional structures surrounding light sources. Altering the size of the serpentine section will also allow for faster flow rates with similar irradiation times.

In conclusion the use of a microfabricated nanoreactor to effect the safe, continuous generation and reaction of singlet oxygen has been demonstrated. The technique avoids the major dangers inherent in the method and allows for reliable reaction even with relatively low-intensity light sources.

Experimental Section

Reagents and solvents were obtained from commercial sources and used as received.

Continuous Flow Synthesis of Ascaridole. A solution of α -terpinene (85%, 0.6 mL) and Rose Bengal (certified, 0.1 g) in methanol (20 mL) was prepared. This solution was introduced into inlet A of the reactor chip at a flow rate of

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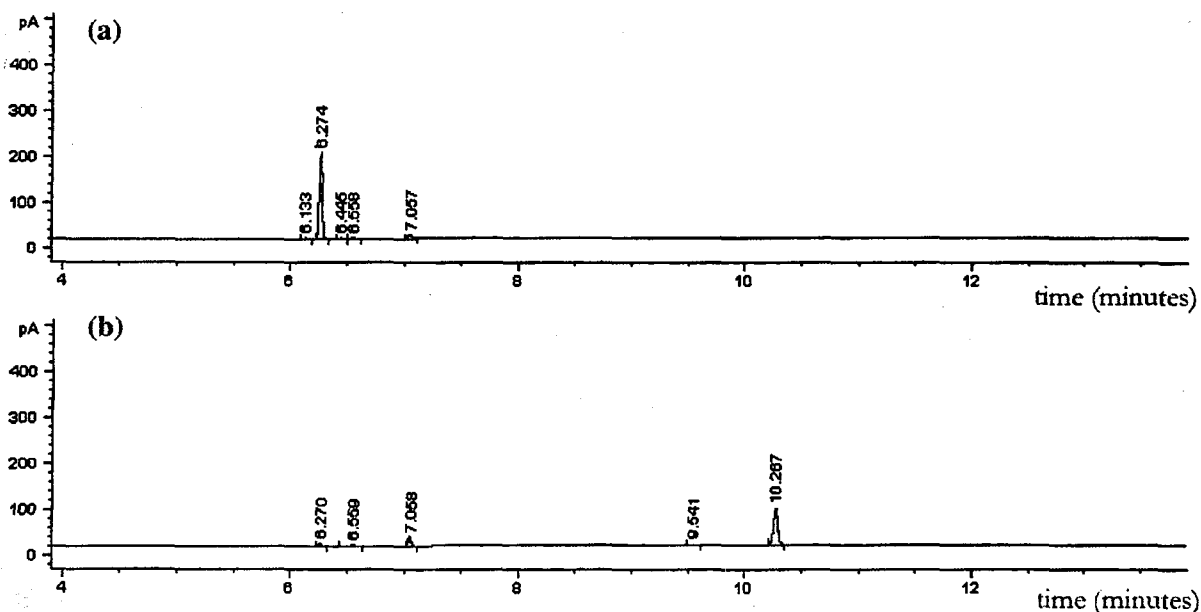


Figure 2. GC traces of (a) chip feedstock solution, (b) chip effluent.

1 $\mu\text{L}/\text{min}$. Pure oxygen was introduced into inlet B of the reactor chip at a flow rate of 15 $\mu\text{L}/\text{min}$. Both were introduced using a Hamilton PHD 2000 syringe pump. The serpentine section of the chip was irradiated at full power using the microscope overhead lamp as described at a distance of 10 cm from the reactor surface and the product collected in a brown glass vial. The product was diluted 10:1 with diethyl ether and passed through a short silica plug, and the solvent was evaporated using a nitrogen stream. The product was analysed by GC giving a conversion by area analysis of 85%, and a crude NMR spectrum was obtained.

Laboratory Synthesis of Ascaridole. α -Terpinene (1 mL), Rose Bengal (0.2 g), and methanol (40 mL) were combined in a 100-mL round-bottomed flask fitted for reflux. Oxygen was bubbled through the mixture for 5 min and then the flask was illuminated with a 500-W tungsten lamp at a distance of 20 cm from the flask. Illumination and oxygenation were continued for 4 h. The mixture was evaporated, and then the residue was dissolved in diethyl ether (20 mL) and filtered through a short silica plug. The resulting solution was concentrated and purified by flash chromatography on silica (hexane eluent, $R_f = 0.65$) giving ascaridole (0.7 g, 67%).

NMR (Bruker 250 MHz) δ 0.99 (6H, d, $J = 6$ Hz), 1.37 (3H, s), 1.49–1.56 (2H, m), 2.01 (1H, hept, $J = 6$ Hz), 2.02–2.05 (2H, m), 6.41 (1H, d, $J = 8$ Hz), 6.49 (1H, d, $J = 8$ Hz) ppm.
MS (C. I. Ammonia) m/z 186 (MNH_4^+), 169 (MH^+) 152, 135, 121, 109, 93
GC HP Innowax capillary GC column, 30 m in length. Helium carrier gas operating at a flow rate of 1.3 mL/min
 inlet temperature: 300 $^\circ\text{C}$
 column temperature: 60 $^\circ\text{C}$, maintained for 3 min, then ramped at 40 $^\circ\text{C}/\text{min}$ to a maximum of 230 $^\circ\text{C}$, maintained for 10 min
 retention times: α -terpinene 6.30 min, ascaridole 10.26 min

Acknowledgment

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Supporting Information Available

NMR and MS spectra for ascaridole (bulk method), crude NMR for ascaridole (continuous synthesis). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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