The application of micro reactors to synthetic chemistry

Stephen J. Haswell,*[a] Robert J. Middleton,[a] Brian O'Sullivan,[a] Victoria Skelton,[a] Paul Watts[a] and Peter Styring[b]

[a] *Department of Chemistry, University of Hull, Cottingham Road, Hull, UK HU6 7RX. E-mail: s.j.haswell@chem.hull.ac.uk*
[b] *Department of Chemical and Process Engineering, University of Sheffield, Mappin Street, Sheffield, UK S1 3JD*

Received (in Cambridge, UK) 20th October 2000, Accepted 19th December 2000
First published as an Advance Article on the web 5th February 2001

A feature article describing the fundamental characteristics and emerging applications of micro technology in the field of synthetic chemistry.

Introduction

It is interesting to observe that despite the many advances made in synthetic chemistry over recent decades the basic practical methodology used remains unchanged. This situation arises primarily because reactions tend to be carried out on a bulk scale using a batch approach which chemists feel comfortable manipulating. At the molecular level however, it makes little difference fundamentally whether a reaction takes place in a 10 ml or 10 pl container. By applying technology developed for the electronics industry, it is now possible to produce reactors in which one can manipulate and analyse materials on a micron to nanometer scale. It is our belief that so called micro reactor technology can do for synthetic chemistry what the solid-state transistor has done for computing, vastly increasing the versatility and the amount of chemical information that a single person can generate. In short it represents a paradigm shift, changing the way we think about the way we work.

Stephen Haswell is Professor of Analytical Chemistry at the University of Hull. His current research activities are in the areas of micro reactors including analytical developments, microwave enhanced reaction chemistry, trace elemental speciation and process analysis. He is author of over 100 research papers, a number of books and patents and is widely known nationally and internationally for his enthusiastic lectures. For a number of years one of the underlying principles of Professor Haswell's research has been to break down the sectorial walls which exist in science, in particular, the integration of analytical science with main line chemistry, physics, engineering and biology. Many of these ideals are encompassed in his research into micro-chemical reactors the subject of this feature article.

Robert Middleton obtained a B.Sc. (Hons) from the University of Nottingham and carried out postgraduate studies in synthetic organic chemistry with Professor David Knight at Cardiff University focusing on the synthesis of highly substituted tetrahydrofurans via *electrophilic cyclisation. Now working at the University of Hull as part of the 'Lab on a Chip' Consortium, developing enzymatic and other catalytic reactions in micro reactors.*

Brian O'Sullivan obtained a Ph.D. in physical organic chemistry from the University of Exeter, and has spent some time at the University of Reading researching heterogeneous catalysis and organic synthesis in supercritical fluids. He is currently working at the University of Hull as part of the 'Lab on a Chip' Consortium, studying metal-catalysed carbon–carbon bond forming reactions in micro reactors and using electro-osmotic flow to control reagent mobilisation. Brian is also interested in modelling currents within micro reactors, in order to achieve a greater understanding of the fundamental processes underlying this technology.

Victoria Skelton graduated from the University of Hull in 1997 with a B.Sc. Hons. degree in Chemistry with Analytical Chemistry and toxicology. This included a year of industrial pharmaceutical experience in the analytical research and development department within Pfizer Central Research, Kent. Vikki obtained her Ph.D. at Hull University in 2000, investigating the role of micro reactors for organic synthesis and combinatorial applications and has continued a collaboration with GlaxoSmithKline. She is currently developing a number of chemical reactions and detection systems in micro reactors in order to establish the physical and chemical requirements of such devices.

Paul Watts graduated from the University of Bristol in 1995 with a B.Sc. in chemistry. He continued his studies at Bristol, obtaining a Ph.D. in bio-organic chemistry in 1999 under the supervision of Professor Tom Simpson and Professor Chris Willis. His Ph.D. focussed on the synthesis of isotopically labelled compounds for use in the determination of biosynthetic pathways to polyketide-derived natural products of biological interest. Paul is currently researching methods of peptide synthesis using micro reactor technology at the University of Hull. The project is funded by Novartis Pharmaceuticals, Basel, Switzerland.

Peter Styring is Senior Lecturer in the Process Fluidics Group at the University of Sheffield, Department of Chemical and Process Engineering. Previously he was the Thorn-EMI—BNR and DERA Lecturer in Chemistry at the University of Hull between 1990–2000. Peter gained his B.Sc. and Ph.D. from the University of Sheffield, Department of Chemistry. He has a background in Liquid Crystal Chemistry but moved into the field of Chemical Micro Reactors in 1997 where he addresses aspects of high throughput organic chemistry and catalysis within miniaturised devices.

DOI: 10.1039/b008496o

Basic concepts of micro reactors

A micro reactor is generally defined as a series of interconnecting channels (10 to 300 microns in diameter) formed in a planar surface in which small quantities of reagents are manipulated. The reagents can be brought together in a specified sequence, mixed and allowed to react for a specified period of time in a controlled region. The product may then be analytically monitored and if necessary separated for further steps in a reaction, or collected for analysis or testing.

In what is basically a diffusion limited environment, where laminar flow characteristics dominate, the micro reactor confers many advantages over conventional scale chemistry. The decrease in linear dimensions allows heat transfer coefficients to exceed those of conventional heat exchangers by an order of magnitude.[1] Micromixers can reduce mixing times to milli- or nano-seconds.[2] The increased surface to volume ratio in micro reactors (10 000 to 50 000 $m^2\ m^{-3}$, compared to 1000 $m^2\ m^{-3}$ in conventional laboratory vessels) has implications for surface-catalysed reactions.[3] Other properties include localised control of concentration gradients, separation of reaction products and the possibility of eliminating unwanted side reactions. For example, when Ehrfeld *et al.*[4] prepared hydrogen cyanide in a micro reactor *via* the Andrussow route, the rapid cooling of the products by a micro heat exchanger prevented hydrolysis of the HCN to ammonia. Jensen and coworkers[5] demonstrated that the synthesis of organic peroxides from acid chlorides and hydrogen peroxide may even be carried out beyond the 'explosion limit', as the transfer of heat energy from the area of reaction is rapid enough to prevent explosion.

In addition the small scales used reduce exposure to toxic or hazardous materials, and the enclosed nature of the micro reactors means greater ease of containment in the event of a runaway reaction. The greatest contribution to safety is the fact that hazardous materials can be synthesised as required at the point of use, in precisely defined quantities, thus eliminating the problems associated with transportation and storage.

Although the small size of the micro reactors would seem to preclude industrial scale synthesis, it has been shown[6] that only 1000 micro reactors operating continuously could produce 1 kg of material in a day. This so called 'scaling out' concept has clear implications in process development where the costly and time-consuming process of going from lab to pilot plant to full-scale production is by-passed simply by optimising the reaction on a single chip and replicating it 1000 or 1 000 000 times. The main attraction of this approach is not only the elimination of the problems associated with the scaling up procedure but also the ability to maintain the high level of control and selectivity made possible through using micro reactor technology.

The micro total analysis systems (μ-TAS)

In recent years, research in the area of miniaturised analytical systems has become well established with a large rapidly growing number of publications reflecting this trend.[7–14] The first fully miniaturised system fabricated was a gas chromatographic device reported by Terry *et al.*[15] at Stanford University in 1979. This micro device was constructed using a silicon wafer, which included a sample inlet port, a 1.5 m long column, an injector and thermal conductivity detector allowing the separation of a mixture of hydrocarbons within 10 s. However, it took a further 10 years before Manz and colleagues[16] at Ciba-Geigy laboratories in Switzerland fabricated a micro capillary electrophoresis device. The μ-TAS was fabricated from glass and allowed the rapid separation of two fluorescent dyes. During the past decade, the main research thrust in academia and industry has centred on the separation and characterisation of DNA.[17–22] This has now led to commercially available micro analytical devices such as the DNA analyser from Agilent, formerly Hewlett–Packard. More recently, a number of research groups worldwide have shifted the focus of research from μ-TAS to developing micro reactor technology building on the already existing μ-TAS concept. Some of the unique features of such devices will be described in the remainder of this paper but it is worth stressing that integration between μ-TAS and micro reactors is essential if chemical and biochemical reactions, at the micro scale or less, are to be effectively monitored and controlled.

Fabrication techniques

Many of the existing fabrication methods described for the μ-TAS systems have been successfully transferred to the field of chemical micro reactors.[23] A number of materials such as silicon, glass, quartz, metals and some polymers can be used to construct micro reactors. Glass and certain polymers have been particularly useful because of their physical properties and chemical inertness. These substrates also allow the mobilisation of organic reagent and aqueous solutions using a number of pumping mechanisms such as hydrodynamic pumping and electro-osmotic flow (EOF).[24–26] A range of fabrication methods such as photolithography, hot embossing, powder blasting, injection moulding, laser micro forming and LIGA, from the German *Lithographie*, *Galvanioformung* (electroforming) and *Abformung* (moulding), are available and can be both versatile and relatively low cost processes.

Fig. 1 shows the steps that are involved in the popular technique of photolithography and wet etching to produce

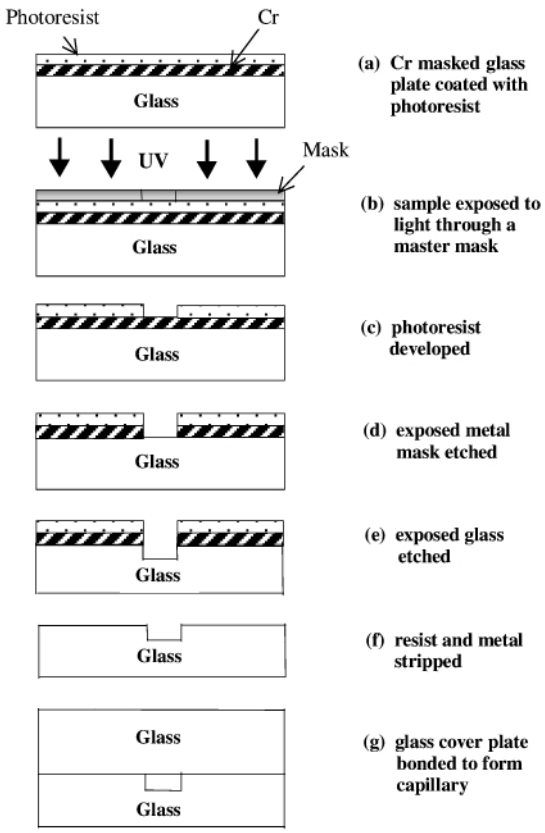


Fig. 1 Sequence of processes in photolithographic fabrication.

channels in a glass micro reactor. A thin layer of metal, such as chromium, is deposited on the surface of a glass plate to control the degree of undercutting during the etching process. A layer of positive photoresist is then spin coated on top of the chromium to a depth of 0.5 to 2.0 μm. The pattern of the required network of interconnecting channels is transferred to the photoresist layer using photolithography. After exposure, the photoresist is developed and removed together with the chromium layer to

reveal the areas of glass to be etched. The plate is then heated to allow volatiles to evaporate, before performing the chemical etch. The channels are then etched using, for example, a mixture of 1% HF and 5% NH_4F in water at 65 °C, resulting in an etch rate of 0.3 to 0.5 μm min^{-1}. A glass top block, with pre-drilled holes to act as reservoirs and if necessary electrode supports, is aligned with the channel geometry and thermally bonded to the glass base plate, producing an all glass device. An example of such a micro reactor, produced by the photolithographic, wet etch and thermal bonding method outlined above is shown in Fig. 2. It should be noted that a range of alternative fabrication

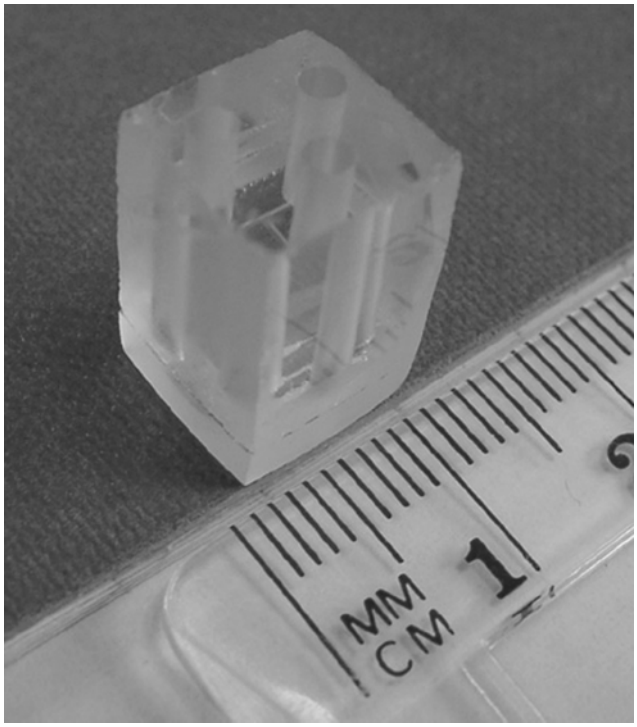


Fig. 2 A simple all-glass micro reactor.

techniques have been reported describing a number of different masking layers, etchant solutions, low temperature bonding, anodic bonding, and polymer based substrates. A recent review of these may be found in ref. 3.

Chemical control in micro reactors

Flow mechanics of liquids in micro channels

One of the main areas that liquid based micro reactor research has focused on to date has been the accuracy with which fluids in capillaries can be manipulated. Owing to the microlitre flow rates that are generally required, some groups have used methods such as syringe pumps, HPLC pumps and peristaltic pumps with high reproducibility being achieved through computer control. Syringe pumps can also be used to infuse and withdraw fluids through channels in both directions. These techniques provide a relatively quick and simple method for pumping reagents through a micro reactor in a controlled manner. However, these systems can build up high back-pressure due to capillary effects, which may lead to pulsing in the flow: this could be a particular problem when using peristaltic pumps.[24] Another problem is the cost associated with HPLC and syringe pumps. These pumps can also be intolerant of mixed-phase liquid systems, or systems that contain particulate matter.

Several companies have developed pumps specifically for micro reactor applications. These pumps are typically based on a piezoelectric driven one way valve to mobilise liquids.[27] For example, the Institut für Mikrotechnik, Mainz has developed a membrane pump that operates with microlitre volumes, but can also pump at up to 0.4 ml min^{-1}. These pumps, which are very small, can deliver the microlitre volumes that are required for managing the movement of liquids in typical devices. However, as they have been constructed from a polymer, practical difficulties may arise when using organic solvents, and depending upon the micro channel geometry, excessive back pressures may be generated.

As one of the attractive features of using micro reactors is their capacity to perform high throughput parallel processing, the use of hydrodynamic pumping may become impractical due to the large number of different solutions that will be required within the reactor. To overcome the need therefore for a large number of pumps and to simplify the construction of micro fluidic systems, electro-osmotic flow (EOF) which has no moving parts, has proved to be a widely preferred method for reagent and solvent pumping.

EOF can be used to move reagents and solvents around a system of channels as a function of applied voltages, with a very high degree of control and allowing the processes to be readily automated. In addition, due to the high electric field (*e.g.* 200 V per centimetre of channel) associated with the EOF, variations in the electrophoretic mobility of individual species enables separation to be achieved. The combination of EOF and electrophoretic mobility can be used to both model and practically control the spatial and temporal position of components in a micro reactor system.[28]

To illustrate the principles of EOF, one can consider a microchannel fabricated from a material (*e.g.* glass), having naturally negatively charged functional groups on its surface. If a liquid, displaying some degree of dissociation, is brought into contact with the material, positive counter ions will form a double layer such that the positively charged ions are attracted to the negatively charged surface. If an electric field is now applied through the liquid phase, the positive mobile ions will migrate to the negative electrode inducing a drag on the bulk liquid. In an aqueous buffered system (pH 3–9) the solution flows towards the cathode with volumetric flow rates in the order of nl min^{-1} to μl min^{-1} depending on the channel dimensions and applied field. The flow velocity achieved with EOF is given by eqn. (1)

$$\upsilon_{EOF} = \frac{V}{L}\mu$$

where V is the applied field, L is the length of the channel and μ is electro-osmotic mobility (dependent on factors such as zeta potential, ionic strength and pH).

Since V and L are controlled by the user, a very high level of control is achievable. Furthermore, this control can be automated and a relatively simple LabVIEW™ program, such as that developed at Hull which allows one to control the output from a power supply to a number of channels in a micro reactor (Fig. 3) has been developed. By varying the potentials across each channel section, it is possible to rapidly optimise the relative flows of different reagents, or to inject plugs of one reagent into a stream of another, or to introduce a number of reagents in a specified sequence for multi-step reactions.

Unlike conventional (hydrodynamic) flow systems, solutions that are moved by EOF have a flat velocity profile across the channel. This, together with an absence of back pressure effects and an inherent low Reynolds number, affords minimal band broadening and efficient electrophoretic separation of reactants and products.

Although EOF has mainly been used in applications with aqueous solutions, it is not restricted to these systems and EOF may be applied to reagents in polar solvents such as methanol, tetrahydrofuran, acetonitrile and dimethylformamide. For example, Harrison and coworkers[29] used EOF to achieve valveless

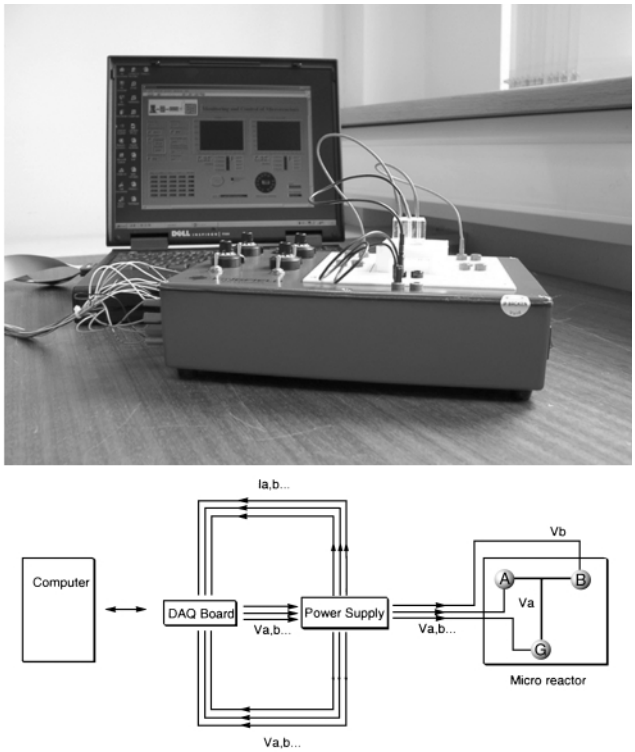


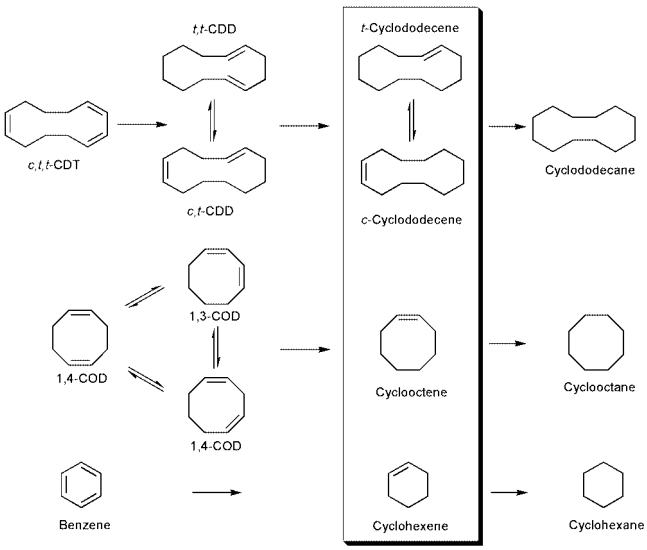
Fig. 3 An automated computer controlled chemical reaction, showing the hardware and a schematic of the system. Using the configuration shown, the duration and magnitude of voltage applied to each reservoir can be selected and the resulting current monitored.

pumping of acetonitrile reagent solutions during the synthesis of an azo dye in a glass micro reactor. This degree of solvent choice greatly extends the types of EOF-controlled chemistry that can be carried out in micro reactors. Obviously the solvent systems used must exhibit some level of polarity, and strictly non-polar solvents cannot be pumped by EOF unless a polar modifier is added.

Applications of micro reactors in synthesis

The inherent benefits of micro reactors, namely rapid generation of small but detectable quantities of reaction products, efficient heat transfer and fluidic control, are now being applied successfully to synthetic chemistry. In theory, these factors might give a research worker using a micro reactor the ability to greatly increase the rate at which new compounds are produced. The work highlighted in this section demonstrates how some of the initial findings obtained by research groups developing micro reactor systems could be applied to high throughput synthesis. There are also some operating characteristics of the micro reactor environment that result in fundamental differences in chemistry. Of more immediate and perhaps significant impact to the research community is the opportunity micro reactors offer in terms of performing a large number (many hundreds) of reactions to explore and optimise a single reaction or a series of chemical reactions. For example, the capability to generate information about reaction conditions, kinetics and product selectivity is now readily accessible using micro reactors, an option not easily available using conventional methodology.

Micro reactor systems have so far been successfully deployed in gas and liquid phase chemistry, including catalyst testing. A recent example of the application of micro reactors to gas phase chemistry was reported at the IMRET 4 conference. Hönicke and coworkers reported the gas phase partial hydrogenation of cyclododeca-1,5,9-triene (CDT), cycloocta-1,5-diene (COD) and benzene over palladium and ruthenium/zinc catalysts (see Scheme 1).[30] The micro reactor system consisted of alumina wafers with mechanically etched channels, which were then



Scheme 1 The mild reaction conditions and the unique mass transfer properties of micro reactors allow hydrogenation of cyclic trienes and dienes to industrially important monoalkenes (ref. 26).

activated by anodic oxidation and impregnation with an organic solution of palladium(II) acetylacetonate. This gave an 18 μm thick activated layer with 0.18 wt% palladium. Twenty four of these wafers were then stacked to give 672 micro channels with internal geometries of 200 μm × 200 μm × 30 mm. A similar process was used for the construction of the Ru/Zn reactor, which contained 0.2 wt% each of ruthenium and zinc. The organic solvent was then removed *via* oxidation in air at 417 °C followed by hydrogen reduction at 150 °C to give the activated catalyst. Although palladium showed no conversion of benzene to cyclohexene, CDT was converted with high yield and selectivity to cyclododecene at 150 °C, with the catalyst bed giving > 80% conversion to cyclododecene for over 20 h. The COD conversion went from 75 to 100% at 150 °C by increasing the residence time in the reactor from 40 to 115 ms. This system proved to be robust, in that throughput could be increased ten-fold from 50 to 500 mg h^{-1} whilst conversion to cyclooctene remained above 80%. Partial hydrogenation of benzene by Ru/Zn was less successful with conversion falling rapidly, and only low yields of cyclohexene were obtained, with the major product being cyclohexane. This work shows that high conversions may be achieved given only a short residence time. By controlling the rate of flow, conversion rate and product yields may be selected or rapidly optimised. This micro reactor system also allows easy re-activation of the catalyst, and it would be readily possible to allow the mixture and velocity of gases to be adjusted automatically in real-time *via* feedback control from analysis of exhaust gases.

Micro reactors using heterogenous catalysts have been applied in liquid-phase organic synthesis. An early, though still comparatively recent development was reported from the Micro Reactor Group in Hull by Greenway *et al.*.[31] The micro reactor utilised EOF to mobilise the reagents and allowed the catalytic synthesis of 4-cyanobiphenyl using a modified Suzuki coupling reaction (Scheme 2). The incorporation of micro porous silica frits[32] within the reactor manifold enhanced EOF and allowed the immobilisation of the heterogeneous catalyst (1.8% palladium on silica). The catalyst immobilisation method produced a leaching rate in the region of ppb (1.2 to 1.6 ppb) removing the need for subsequent purification from metal residues. The micro reactor device was optimised using flow injection analysis principles producing a 67 ± 7% (n = 6) yield of the 4-cyanobiphenyl product at room temperature within 25 min.

The flow injection method adopted allowed the periodic injection of the aryl halide (5 s injection length with a 25 s injection interval) into a continuous flow of phenylboronic acid. Flow was maintained using an external applied voltage of 200 V. The yield obtained using the device was comparable with Suzuki reactions performed on a large (batch) scale using homogeneous catalysts. One of the interesting observations of this reaction was that, unlike conventional Suzuki couplings performed in a flask, base was not required. Although the reason for this is as yet unclear, it is thought that the applied electric field may be sufficient to cause localised ionisation of solvent water to H^+ and OH^- at the metal surface. It may be this so-formed hydroxide that performs the function of a conventional inorganic or amine base. However, the micro reactor demonstrated the potential application of such devices to perform chemical reactions, allowing high throughput screening, rapid method development or reaction optimisation.

The Hull group have also demonstrated that a superacid catalyst (sulfated zirconia) could be immobilised onto the surface of a polydimethylsiloxane (PDMS) micro reactor top plate. This was achieved by dusting the pre-cured PDMS surface with activated catalyst and baking the plate at 100 °C for 1 h. The PDMS top plate (containing the catalyst) was clamped to a glass base plate (with etched micro channels) and syringe pumps were used to mobilise the hexan-1-ol, which underwent dehydration to hex-1-ene. The micro reactor featured an *in situ* resistive heater wire cast into the PDMS top plate, which was operated at 155 to 160 °C.[33]

An attractive feature of micro reactors is their ability to carry out chemical processes that may be hazardous. For example Burns and Ramshaw[34] at the University of Newcastle have described the nitration of toluene and benzene in stainless steel or PTFE micro reactors, demonstrating the approach is suited to a hazardous processes involving organic solvents and concentrated acids. In addition, they are also investigating the challenge of manipulating bi-phasic liquid–liquid systems and the control of product distribution to avoid hazardous trinitrated aromatic products. Their studies have yielded some elegant ways to control immiscible liquid layers in capillary systems that include (i) segmented flow, in which plugs of alternate phases travel down a capillary and (ii) parallel laminar flow, where similar amounts of two phases run together through the capillary producing an interfacial contact zone.

Burns and Ramshaw's studies on benzene nitration also demonstrated that conversion, while showing a near linear relationship with temperature, can be increased substantially by the use of smaller capillaries that enhance diffusion effects by reducing the size of the slugs of material in the channel. Halving the capillary diameter from 250 to 130 μm more than doubled the rate of nitration. Flow rates were also found to be important, with faster flow rates giving rise to higher conversion as they promoted internal circulation of the liquid plugs travelling down the capillary.

In comparison with conventional nitration techniques, the results showed that rate constants for the micro reactor process (1–8 min^{-1}) in 178 μm capillaries were similar to those in the published literature (1–5 min^{-1}). It is expected that further optimisation of the micro reactor device and its operation, particularly by increasing the sophistication of the technology to decrease droplet size, will result in substantial improvement to the efficiency of the devices.

To demonstrate the advantages that micro reactors offer when dealing with potentially hazardous reagents, Chambers and Spink[35] recently reported the development of a micro reactor fabricated from a block of nickel, which was used for the elemental fluorination of organic substrates. Conversions compare well with results from conventional reactors. The small amount of fluorine involved, together with the heat and mass transfer properties of the micro reactor, overcame many of the safety issues associated with this type of reaction.

Optimisation of catalytic processes

To demonstrate the testing and optimisation of catalytic processes, the Hull group has developed a simple procedure for the immobilisation and introduction of supported reagents in micro reactors (Fig. 4). Such configurations enable solutions to

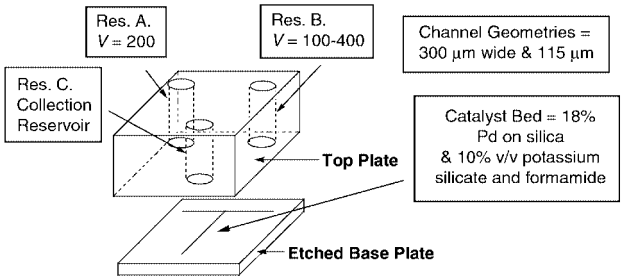
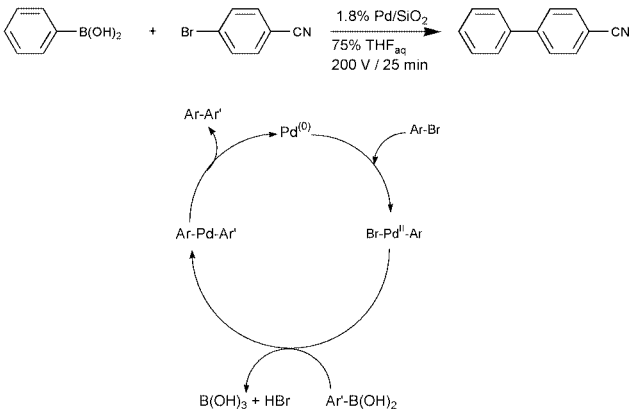


Fig. 4 A Hull micro reactor, configured for the Suzuki reaction. Reservoir A contains 100 μl of 4-bromobenzonitrile (0.1 M) and reservoir B 100 μl of phenylboronic acid (0.1 M), both in 75% $THF_{(aq)}$. Products from the reaction are taken from reservoir C and analysed by GCMS.

be passed over catalysts in either a continuous or plug mode with a high degree of fluidic control. Catalyst types under investigation include immobilised enzymes (such as lipases and esterase), metals, sulfated zirconia and zeolite-based materials. For example, in the case of an enzyme system based on porcine liver esterase, symmetrical diesters are passed over this catalyst bed to effect desymmetrisation to a chiral mono-ester, creating a high-throughput reactor for biocatalysis.

A second approach is to pulse several different reagents one by one over the catalyst bed. Given the computer-based flow-control possible with micro reactors, it is now relatively easy to achieve accurate and reproducible reaction sequencing. In the Suzuki reaction performed at Hull, the aryl halide and boronic acid were alternately pulsed over a catalyst bed of palladium on silica. This had the effect of increasing yields from < 5% to 68% by simulating the catalytic cycle (Scheme 2).[31] The catalyst was



Scheme 2 The catalytic cycle of the Suzuki reaction.

flushed with the aryl bromide to drive the oxidative addition to the metal, and then flushed with boronic acid to effect conversion to the biphenyls.

Using a system of five continuous flow micro reactors, the Suzuki reaction has been carried out on an industrial scale by Merck in Germany, where researchers found improvements over conventional batch reactors.[30] For example, in the reaction of 3-bromobenzaldehyde with 4-fluorophenylboronic acid, 90% yields were reported for the micro reactors, compared with 50% in stirred flasks.

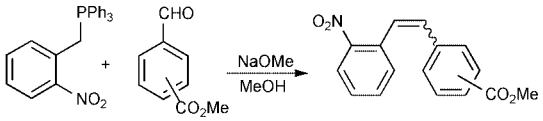
One of the chief limitations of the studies in Hull has been the high temperatures (680 °C) required to anneal the top glass plate

to the etched glass base plate as any organic material would be destroyed in these processes, hence metals on silica have been favoured as catalysts.

These early studies into catalytic processes demonstrate the potential of using micro reactor technology for a continuous production line approach to single compound production. However, the design of such devices could be easily modified to evaluate the performance of a catalyst across a broad range of substrates. Another opportunity would be in using multi-channel systems that would allow the evaluation of a number of different catalysts for a single reaction. These studies allow rapid evaluation of reaction conditions to allow the best catalyst for a given reaction to be studied, or the efficacy of a catalyst over a range of substrates to be evaluated.

Multi-step and analog based reactions

At the recent IMRET 4 conference, the Hull group reported a micro reactor device that allowed the synthesis of a number of nitrostilbene esters using a borosilicate micro reactor.[36] The micro reactor allowed the development of the Wittig reaction investigating a number of reaction features such as stoichiometry, stereochemistry and reaction diversity (Scheme 3).



Scheme 3 The Wittig reaction.

Initial investigations centred on using the device for synthetic method development and optimisation, allowing rapid reaction design in conjunction with EOF as the mobilisation method. With a 2 : 1 reaction stoichiometry (aldehyde in excess) a yield of 70% was achieved using the micro reactor in a continuous flow mode with an optimum voltage of 400 V. The micro reactor demonstrated an increase in reaction efficiency of 10% over the conventional batch method. The reaction stoichiometry was then reduced to 1 : 1 but the yield was poor (39%) so a flow injection technique was adopted. This resulted in the injection of the phosphonium salt into the continuous flow of the aldehyde compound at 400 V. A 59% yield was obtained, but more importantly it allowed a series of aldehydes to be reacted in sequential injections using the optimum conditions established at 1 : 1 stoichiometry. This demonstrated the micro reactors diversity and high through-put capability.

The above research has been extended to investigate the stereoselective control of the chemical reaction by applying electrical fields which generate controlled concentration gradients of the reagent streams.[37] The stereoselective synthesis of the *cis* (*Z*) and *trans* (*E*) isomers was controlled by varying the applied voltages to the reagent reservoirs within the device. The variation in the external applied voltage subsequently altered the relative reagent concentrations within the device producing *Z/E* ratios in the region of 0.57 to 5.21. In comparison, a traditional batch reaction was performed based on the same reaction length, concentration, solvent and stoichiometry resulting in a *Z/E* ratio of 3.0. The unique flow control created in the micro reactor system has allowed the localised concentration gradients, produced by a diffusion limited non-turbulent mixing regime, to generate the observed stereoselectivity. The control of these localised diffusion-limited concentration gradients is an important feature of micro reactors and one that can be effectively exploited for yield and product selectivity.

Multi-step reactions

So far, micro reactors appear to be limited to carrying out a single synthetic step. One of the thrusts of the research in Hull is to develop methodology that will give the chemist the ability to look as multi-step reactions, culminating in target or diversity based synthesis.

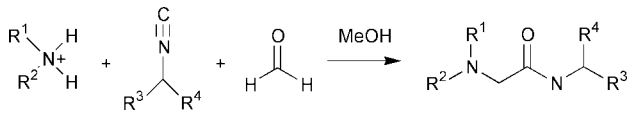
To extend the capability of performing multi step reactions in micro reactors, processes such as peptide synthesis represent a good model system. Peptides have been traditionally prepared combinatorially *via* solid supported techniques[38,39] but this approach has the disadvantage that a fairly expensive polymer support is required and that the product requires post-synthetic cleavage. In addition, extra steps are added to the synthesis as a result of having to initially link the amino acid to the polymer support. The preparation of peptides in micro reactors, using solution phase chemistry, offers the possibility of overcoming such problems.

Using solution phase chemistry there are several methods that may be used to form peptide bonds such as diethyl azodicarboxylate[40] (DEAD) or carbodiimide reagents such as dicyclohexylcarbodiimide (DCC)[41] or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI).[42] In addition acyl halides, anhydrides and azides may be utilised in the formation of peptide bonds.[43] Once the methodology for the formation of peptide bonds has been fully established, selective deprotection of either of the protecting groups will allow longer peptide chains to be assembled. This multi-step synthesis will clearly allow the rapid generation of libraries of peptides, which could then be used in determining their biological properties.

In situ detection methods in micro reactors

As indicated earlier, research focused on μ-TAS has developed a number of suitable detection methods for micron scale systems, with the most common method adopted being fluorescence. Other detection systems developed have included UV–VIS[44–48] and electrochemical[49–52] detection offering sensitivity and simple detection with environmental micro systems. The information provided is however generally insufficient for structural characterisation of unknown chemical species. For the micro reactor system to become truly versatile, the development of hyphenated techniques and specialised equipment such as NMR and Raman spectrometry would allow direct, real time characterisation and spatial determination of concentration and pH information. Currently a range of analytical techniques are being investigated by a number of research groups. These include mass spectrometry (MS)[53] and near infrared (NIR).[54–57]

One area of analysis which has been readily reported in the literature is the hyphenation of micro reactor to mass spectrometers.[58–61] Lazar *et al.*[58] from Oakridge National Laboratories have coupled a micro fluidic device with a nanospray tip for electrospray ionisation, allowing dilute peptide and protein solutions to be characterised using a time of flight mass spectrometer. The hyphenated system allowed the capture of spectra within milliseconds (10 to 20 ms) resulting in 50 to 100 spectra per second. The second study by Mitchell *et al.*, presented at the recent Micro Total Analytical Systems conference,[59] described the detection of a multicomponent reaction using an electrospray ionisation (EIS)-MS. The multicomponent synthesis investigated was the Ugi reaction (Scheme 4) in which a solution of formaldehyde and pure



Scheme 4 The Ugi reaction.

solvent was infused through one of the inlets whilst a multicomponent mixture (isocyanide, amine salt) was also added. The hyphenated micro reactor-MS system allowed the real time detection of the synthetic Ugi coupling reaction.

Other detection systems reported have been NIR and Raman spectrometry. A miniaturised NIR spectroscopic system fabri-

cated in borosilicate glass has been developed by Ache.[56] The micro device contains a circular wave guide covered by a sensing membrane. In addition, the micro NIR system contained an incandescent light source, a NIR micro spectrometer with a self-focusing reflection grating and NIR diode. At the University of Michigan, Reshni and co-workers[62] demonstrated one early example of Raman spectrometry on a micro device. The system was fabricated using a Raman microprobe stage coupled to a capillary electrophoresis chip. Traditionally, Raman spectroscopy has a limit of detection in the millimolar region, however Reshni's micro device achieved a limit of detection in the micromolar region and below. This was due to the addition of a preconcentration stage using isotachophoresis. This system has allowed the successful fingerprinting and quantification of reactions on-chip.

Commercialisation of micro reactor technology

A commercially available chemical synthesiser using micro reaction technology already exists, and is produced by IMM-Mainz. It consists of a pumping module, a micro-reactor that results in very efficient mixing of reagents, followed by a capillary to allow time for the reaction to go to completion. The outflow is then collected for further manipulation by the user. This could be just the first step along a road which will see the integration of automated reagent manipulation, reaction monitoring and product purification into a single instrument containing several interconnected micro reactors, or possibly a single micro reactor device. In common with microelectronic chips, once the facilities to fabricate micro reactors are in place, they become progressively cheaper to produce in quantity. This should make the production of chemicals in massive parallel arrays of reactors an economic possibility. It is likely that some of the peripheral equipment required will still represent a considerable cost, but this should be set against the potential increase in productivity per research worker. In addition, the effective production of molecules in terms of energy, safety and environmental impact will emerge as important factors in the future exploitation of micro reactor technology. One of the underlying features of any future commercially available automated synthesis system must be versatility. Research is now moving towards a 'plug and play' approach in which the reaction and detection configurations can be customised. The next couple of years will undoubtedly see significant development in this area of the technology. We should now prepare ourselves, including university courses for undergraduates, for the impact the micro reactor is going to have on the whole area of chemical research and production.

Acknowledgements

We wish to thank the EPSRC Lab-on-a-chip Consortium (B. O'S., R. J. M.), Novartis Pharmaceuticals (P. W.) and GlaxoSmithKline (V. S.) for financial support.

Further information

See the following websites:
http://analyticalsciencehull.org/
Further information about conferences relating to this topic may be found at the following websites:
IMRET 5 (Strasbourg, France, 27–30 May 2001):
http://www.aiche.org/conferences/cosponsored/#2001
μ-TAS 2001 (Monterey, CA, USA, 21–25 Oct 2001):
http://www.casss.org/tas2001

Notes and references

1 K. Schubert, W. Bier, J. Brandner, M. Fichtner, C. Franz and G. Linder, *Process Miniaturisation: 2nd International Conference on Microreaction Technology*, AIChE, New Orleans, 1998, p. 89.
2 J. B. Knight, A. Vishwanath, J. P. Brody and R. H. Austin, *Phys. Rev. Lett.*, 1998, **80**, 386.
3 W. Ehrfeld, V. Hessel and H. Löwe, *Microreactors*, Wiley-VCH, Weinheim, 2000, ch. 1.
4 W. Ehrfeld, V. Hessel, S. Kiesewalter, H. Löwe, T. Richter and J. Schiewe, *Proceedings of the Third International Conference on Microreaction Technology*, Frankfurt, Germany, 1999, p. 14.
5 T. M. Floyd, M. W. Losey, S. L. Firebaugh, K. F. Jensen and M. A. Schmidt, *Proceedings of the Third International Conference on Microreaction Technology*, Frankfurt, Germany, 1999, p. 171.
6 P. D. I. Fletcher and S. J. Haswell, *Chem. Br.*, 1999, **35**, 38.
7 S. J. Haswell, *Analyst*, 1997, **112**, 1R.
8 A. Manz, D. J. Harrison, E. Verpoorte, J. C. Fettinger, H. Ludi and H. M. Widmer, *Chimia*, 1991, **45**, 103.
9 A. Manz, D. J. Harrison, E. Verpoorte and H. M. Widmer, *Adv. Chromatogr.*, 1993, **33**, 1.
10 A. Manz, C. S. Effebhauser, N. Burggraf, E. Verpoorte, D. E. Raymond and H. M. Widmer, *Anal. Mag.*, 1994, **22**, M25.
11 *Proceedings of the Micro Total Analytical Systems 98' Workshop*, ed. D. J. Harrison and A. van den Berg, Kluwer Academic Press, Dordrecht, 1998.
12 A. van den Berg and T. S. J. Lammerink, *Top. Curr. Chem.*, 1998, **194**, 21.
13 D. J. Harrison, K. Fluri, K. Seiler, Z. H. Fan, C. S. Effenhauser and A. Manz, *Science*, 1993, **261**, 895.
14 S. C. Jacobson, R. Hergenroder, L. B. Koutny and J. M. Ramsey, *Anal. Chem.*, 1994, **66**, 1114.
15 S. C. Terry, J. H. Jerman and J. B. Angel, *IEEE Trans. Electron Devices*, 1979, **ED26**, 1880.
16 A. Manz, N. Graber and H. M. Widmer, *Sens. Actuators B*, 1990, **1**, 24.
17 A. T. Woolley, D. Hadley, P. Ladre, A. J. deMello, R. A. Mathies and M. A. Northrup, *Anal. Chem.*, 1996, **68**, 4081.
18 A. T. Woolley and R. A. Mathies, *Anal. Chem.*, 1996, **67**, 3676.
19 A. T. Woolley, G. F. Sensabaugh and R. A. Mathies, *Anal. Chem.*, 1997, **69**, 2181.
20 S. C. Jacobson and J. M. Ramsey, *Anal. Chem.*, 1996, **68**, 720.
21 C. S. Effenhauser, A. Paulus, A. Manz and H. M. Widmer, *Anal. Chem.*, 1994, **66**, 2949.
22 D. Schmalzing, A. Adourian, L. Koutny, L. Ziagura, P. Matsudaria and D. Ehrlich, *Anal. Chem.*, 1998, **70**, 2303.
23 T. McCreedy, *TrAC*, 2000, **19**, 396.
24 D. M. Spence and S. R. Crouch, *Anal. Chem.*, 1998, **358**, 95.
25 B. H. Schoot, S. Jeanneret and A. Berg, *Anal. Methods Instrum.*, 1993, **1**, 38.
26 P. K. Das Gupta and S. Lui, *Anal. Chem.*, 1994, **66**, 1792.
27 H. T. G. van Lintel, F. C. M. van de Pol and S. Bouwstra, *Sens. Actuators*, 1988, **15**, 153.
28 S. J. Haswell, P. D. I. Fletcher and V. N. Paunov, *Analyst*, 1999, **124**, 1273.
29 H. Salimi-Moosavi, T. Tang and D. J. Harrison, *J. Am. Chem. Soc.*, 1997, **119**, 8716.
30 E. Dietzsch, D. Hönicke, M. Fichtner, K. Schubert and G. Weißmeier, *IMRET 4: 4th International Conference of Micro Reaction Technology Topical Conference Proceedings, AIChE Spring National Meeting, March 5–9 2000*, Atlanta GA, USA, p. 89.
31 G. M. Greenway, S. J. Haswell, D. O. Morgan, V. Skelton and P. Styring, *Sens. Actuators B*, 2000, **63**, 153.
32 P. D. Christensen, S. W. P. Johnson, T. McCreedy, V. Skelton and N. G. Wilson, *Anal. Commun.*, 1998, **35**, 341.
33 N. G. Wilson and T. McCreedy, *Chem. Commun.*, 2000, 733.
34 J. R. Burns and C. Ramshaw, *IMRET 4: 4th International Conference of Micro Reaction Technology Topical Conference Proceedings, AIChE Spring National Meeting, March 5–9 2000*, Atlanta GA, USA, p. 133.
35 R. D. Chambers and R. C. H. Spink, *Chem. Commun.*, 1999, 883.
36 V. Skelton, G. M. Greenway, S. J. Haswell, P. Styring, D. O. Morgan, B. H. Warrington and S. Y. F. Wong, *Analyst*, 2000, **126**, 7.
37 V. Skelton, G. M. Greenway, S. J. Haswell, P. Styring, D. O. Morgan, B. H. Warrington and S. Y. F. Wong, *Analyst*, 2000, **126**, 11.
38 R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.
39 D. B. Whitney, J. P. Tam and R. B. Merrifield, *Tetrahedron*, 1984, **40**, 4237.
40 O. Mitsunobu, *Synthesis*, 1981, 1.
41 A. Hassner and V. Alexanian, *Tetrahedron Lett.*, 1978, 4475.
42 P. D. Bailey and K. D. Morgan, *Organonitrogen Chemistry*, Oxford University Press, 1996.
43 D. S. Perlow, J. M. Erb, N. P. Gould, R. D. Tung, R. M. Freidinger, P. D. Williams and D. F. Veber, *J. Org. Chem.*, 1992, **57**, 4394.
44 G. M. Greenway, S. J. Haswell and P. Petsul, *Anal. Chim. Acta*, 1999, **387**, 1.
45 G. N. Doku and S. J. Haswell, *Anal. Chim. Acta*, 1999, **382**, 1.

46 E. Verpoorte, A. Manz, H. Lüdi, A. E. Bruno, F. Maystre, B. Krattiger, H. M. Widmer, B. H. van der Schoot and N. F. de Rooij, *Sens. Actuators*, 1992, **6**, 66.
47 Z. Liang, N. Chiem, G. Ocvirk, T. Tang, K. Fluri and D.J. Harrison, *Anal. Chem.*, 1996, **68**, 1040.
48 H. Salimi-Moosavi, Y. Jiang, L. Lester, G. McKinnon and D. J. Harrison, *Electrophoresis*, 2000, **21**, 1291.
49 J.-J. Gau, E. H. Lan, B. Dunn and C.-H. Ho, *Micro Total Analysis Systems 2000*, ed. A. van den Berg, W. Olthius and P. Bergveld, Kluwer Academic Publishers, Dordrecht, 2000, p. 509.
50 A. T. Woolley, K. Q. Lao, A. N. Glazer and R. A. Mathies, *Anal. Chem.*, 1998, **70**, 684.
51 J. S. Rossier, M. A. Roberts, R. Ferrigno and H. H. Girault, *Anal. Chem.*, 1999, **71**, 4294.
52 R. S. Martin, A. J. Gawron, S. M. Lunte and C. S. Henry, *Anal. Chem.*, 2000, **72**, 3196.
53 A. Feustel, J. Muller and V. Reilling, *Micro Total Analysis Systems '95*, ed. A. van den Berg and P. Bergveld, Kluwer Academic Press, Dorchecht, 1995, p. 299.
54 J. P. Cozen, *Appl. Spectrosc.*, 1993, **37**, 753.
55 H. J. Ache, *Chem. Ind.*, 1993, **1**, 40.
56 H. J. Ache, *Micro Total Analysis Systems '95*, ed. A. van den Berg and P. Bergveld, Kluwer Academic Press, Dorchecht, 1995, p. 47.
57 R. J. Jackman, T. M. Floyd, M. A. Schmidt and K. Jensen, *Micro Total Analysis Systems 2000*, ed. A. van den Berg, W. Olthius and P. Bergveld, Kluwer Academic Publishers, Dordrecht, 2000, p. 155.
58 I. M. Lazar, R. S. Ramsey, S. Sundberg and J. M. Ramsey, *Anal. Chem.*, 1999, **71**, 3627.
59 M. C. Mitchell, V. Spikmans, F. Bessoth, A. Manz and A. de Mello, *Micro Total Analysis Systems 2000*, ed. A. van den Berg, W. Olthius and P. Bergveld, Kluwer Academic Press, Dordrecht, 2000, p. 463.
60 I. Ugi, R. Meyr, C. Fetzer and C. Steinbrückner, *Angew. Chem.*, 1959, **71**, 386.
61 J. Li, C. Wang, J. F. Kelly, D. J. Harrison and P. Thibault, *Electrophoresis*, 2000, **21**, 198.
62 K. A. Reshni, M. D. Morris, B. N. Johnson and M. A. Burns, *Micro Total Analysis Systems '98*, ed. A. van den Berg, W. Olthius and P. Bergveld, Kluwer Academic Press, Dordrecht, 1998, p. 109.