The Application of Micro Reactors for Chemical Synthesis: How small is required?

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ABSTRACT

The miniaturisation of chemical reactors offers many fundamental and practical advantages of relevance to the chemical industry, who are constantly searching for controllable, information rich, high throughput, environmentally friendly methods of producing products with a high degree of chemical selectivity.

In this paper a number of chemical reactions will be used to illustrate the advantages that micro reactors offer for the rapid optimisation of reactions, in which the product is typically produced in both higher yield and purity. It will be illustrated that compounds may be prepared and purified within an integrated system and that it is possible to generate intermediates *in situ* within the reactor, which may then be subsequently reacted to produce more complex products. More recently the incorporation of solid supported reagents and catalysts has been investigated.

It will be illustrated that the '*scale out*' of micro reactors may be used to generate larger quantities of compound without having to re-optimise the reaction, thus saving time and money whilst simultaneously making the process inherently safe. Furthermore, integration of the microfluidic system with analytical detection will be illustrated, enabling *in situ* process control to be achieved.

Keywords: micro reactor, organic synthesis, drug discovery

INTRODUCTION

The success of pharmaceutical companies depends largely on the synthesis and screening of novel chemical entities and their ability to optimise selected leads to marketable drugs. In an industry where development costs are extremely high, the ability to shorten optimisation cycle times would be desirable in order to reduce this cost. Furthermore, new technology that would enable a cost-effective upward step-change in the number of lead candidates that could be prepared would provide a distinct competitive advantage, as this would enable the discovery of a better lead with enhanced therapeutic properties with reduced side effects.

So how can a chemist make more compounds for biological evaluation? Clearly the pharmaceutical industry has invested heavily in automation; however one of the slowest steps is still the synthesis and purification of potential drug candidates. Several pharmaceutical companies have acknowledged that micro reactors offer many fundamental and practical advantages of relevance to automated, high throughput synthesis and product purification.

Micro reactors consist of a network of micron-sized channels etched into a solid substrate [1,2]. They may be fabricated from a range of materials including glass, silicon, quartz, metals and polymers using a variety of fabrication techniques including photolithography, hot embossing, powder blasting, injection moulding and laser micro forming [3]. For glass micro reactors, which are the most common for synthetic applications involving the use of organic solvents, photolithographic fabrication of channel networks is generally performed [4,5].

For chemical synthesis the channel networks are connected to a series of reservoirs containing chemical reagents to form the complete device with overall dimensions of a few cm, as illustrated in Figure 1. Reagents can be brought together in a specific sequence, mixed and allowed to react for a specified time in a controlled region of the channel network using a variety of methods, including electrokinetic or hydrodynamic pumping. For electrokinetically pumped systems, electrodes are fabricated into the reservoirs, to which specific voltage sequences can be delivered under automated computer control [6]. This control offers a simple but effective method of moving and separating reactants and products within a micro reactor, without the need for moving parts. In comparison, hydrodynamic pumping uses conventional or micro-scale pumps, most commonly syringe pumps, to manoeuvre solutions around the channel network. It should also be emphasised that although hydrodynamic pumping is relatively easy when reacting just two solutions, it becomes far more complex to accurately control the fluidics when introducing more than three reagents into the device.

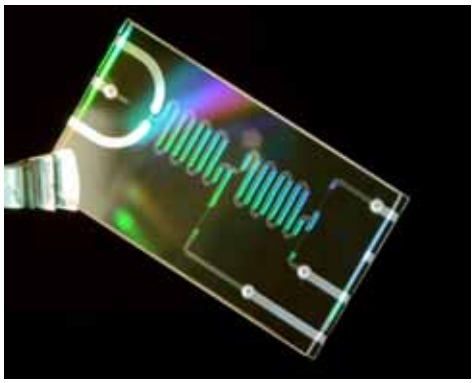


Figure 1: Borosilicate glass micro reactor.

A concerted effort is now underway to establish the benefits that micro reactors can bring to the field of reaction chemistry. Many reactions have been demonstrated to show enhanced reactivity, product yield and selectivity when performed in micro reactors as compared with conventional bench top glassware [6-8]. For instance, in the Wittig reaction the yield was of the order of 70 % for both batch and micro reactions, however in the micro reactor the product was generated in approximately 6 seconds compared to several hours for the batch reaction [9]. Comparable results were observed in the Suzuki reaction (Table 1) [10]. Clearly this would enable more compounds to be prepared in a given period of time; one of the aims if the pharmaceutical industry wishes to screen more compounds.

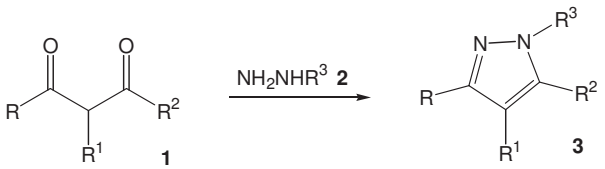
	In flask		In micro reactor	
Reaction	Time	Conv (%)	Time	Conv (%)
Suzuki	8 h	70	6 sec	78
Wittig	3 h	48	3 sec	60

Table 1: Comparison of reaction rates and yields for batch and micro reactions.

In addition to the enhanced speed of synthesis, a microchannel system also provides a potential separation column and a non-turbulent environment for partition between solvents. Integration of a micro reactor device, *via* purification, to one of the many highly sensitive microchannel-based biological assay systems would enable the compounds to be screened. Apart from the greatly reduced reaction times demonstrated for the micro reactors, handling times to assay and chemical reagent costs would be virtually eliminated [6].

SYNTHESIS IN MICRO REACTORS

In a move to develop the above methodology, Garcia-Egido *et al.* have recently reported the synthesis of a combinatorial library of pyrazoles within a glass micro reactor system operated using hydrodynamic control [11]. A T-shaped micro reactor was used to react seven 1,3-dicarbonyl compounds **1** sequentially with three hydrazine derivatives **2** to produce a 21-member library of pyrazoles **3** (Scheme 1).

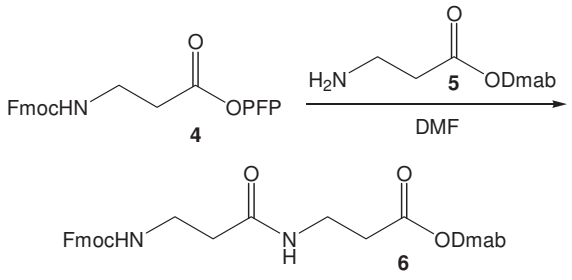


Scheme 1

The automated system consisted of an autosampler to introduce small volumes of reagents into the chip, a pump to move the reagents through the micro reactor and a dilution system to enable a small sample to be diverted to an LC-MS instrument for analysis. In most cases the pyrazoles were obtained in 99% conversion, but clearly a further chromatography step would allow products to be further purified. However it should be mentioned that in order to obtain these high yields the authors found it necessary to use 80 equivalents of the hydrazine derivative. In order to achieve their final aim, the purified products would need to be analysed using an integrated flow-through bioassay system in order to enable *in situ* screening to be performed.

Clearly this advanced type of hydrodynamically pumped system is suitable for drug discovery applications and could be applied to a range of other reactions performed within micro reactors. Although the above system successfully enables combinatorial synthesis for the desired application, cynics argue that the overall system is hardly miniaturised; the micro reactor itself is tiny but the overall system is still composed of several pieces of large bench top instrumentation. This is where EOF-based systems are potentially advantageous as external pumps are not necessary and purification could be achieved using on-chip electrophoretic separation, rather than using large external instrumentation, such as the HPLC-MS described above.

In an attempt to develop an EOF based system, Watts *et al.* have recently conducted an extensive study on peptide synthesis, where they prepared a library of dipeptide derivatives within a computer controlled micro reactor system operating under EOF [12,13]. The authors demonstrated that dipeptides could be prepared from pre-activated carboxylic acids. They optimised the reaction using the pentafluorophenyl (PFP) ester of Fmoc-β-alanine **4** with amine **5** to give dipeptide **6** quantitatively in 20 minutes (Scheme 2). This represented a significant increase in yield compared with the traditional batch synthesis where only a 50% yield was obtained in 24 h. The authors then used the methodology developed to consecutively react alternative pentafluorophenyl esters with amine **5** to produce a library of dipeptides. In addition, it was also reported that other amines could be used in a similar way, demonstrating the versatility of the technique to very rapidly prepare a large number of related compounds.



Scheme 2

More significantly, the first example of a multi-step synthesis in a micro reactor was demonstrated, where the

authors extended the approach to the synthesis of tripeptides [13].

Although all dipeptide bond-forming reactions produced the appropriate dipeptide **7** in 100 % conversion, based on consumption of the pentafluorophenyl ester **8**, the product was still contaminated with residual amine **5** and pentafluorophenol (the by-product of the reaction). George *et al.* have reported that the dipeptide **7** may be separated from the reaction mixture using the reactor manifold shown schematically in Figure 2, where the reaction mixture was collected in the ground reservoir during the synthesis and then the peptide is purified by electrophoresis and collected in reservoir D [14]. Hence this methodology enabled the synthesis and separation to be efficiently conducted within an integrated micro reactor without the need to have large peripheral equipment attached. Clearly this method of integrating reaction and purification could be applied to a range of other reactions that have been performed within EOF pumped devices [6].

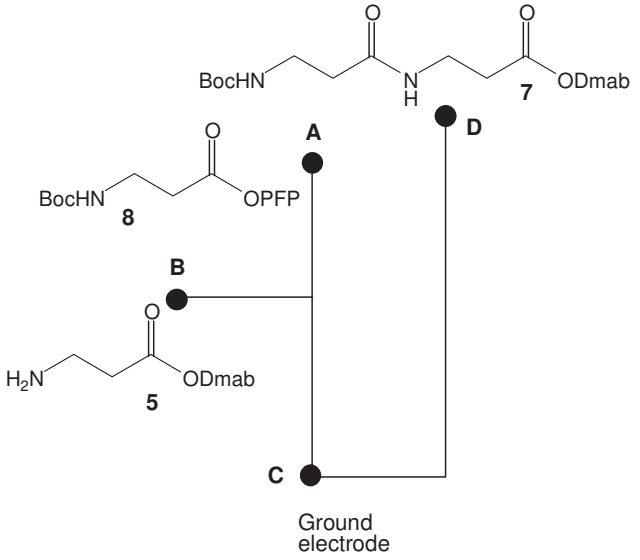


Figure 2: Micro reactor manifold for synthesis and electrophoretic purification of peptides.

The question still remains however, is it necessary to formerly 'purify' all reactions? Wiles *et al.* have reported the solution phase Knoevenagel reaction within an EOF based micro reactor, using the reaction manifold illustrated in Figure 3. Reaction of diketone **9** with dimethylamine **10** formed the enolate, which was reacted *in situ* with an aldehyde **11** to give the Knoevenagel adduct **12** in high conversion. However, the product was still contaminated with stoichiometric quantities of dimethylamine.

In order to overcome this problem the authors conducted an investigation using solid supported bases. The authors found that flow reactors could be packed with a variety of solid support bases and used in the Knoevenagel reaction (Figure 4). Using EOF to move regents through the catalyst bed it was possible to prepare the product **12** in greater than 99 % conversion and in 99 % purity [15]. Potentially this approach to synthesis would mean that the product could be screened for biological activity without having to formally purify the reaction mixture, which would make the overall process even more efficient.

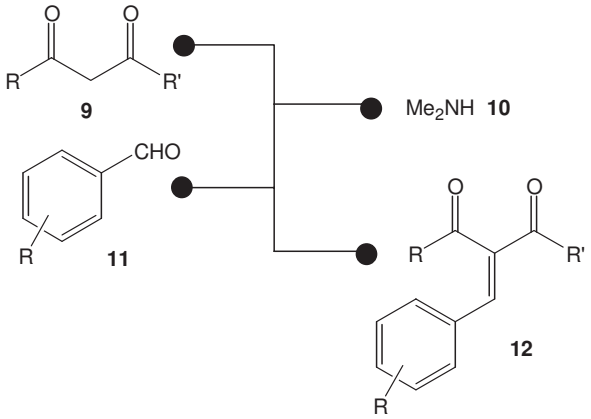


Figure 3: Micro reactor manifold for solution-phase Knoevenagel reactions.

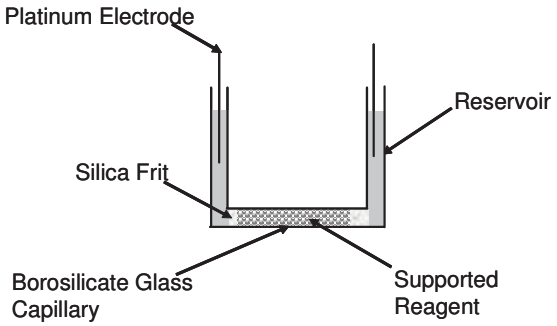


Figure 4: Schematic of apparatus used in solid-supported Knoevenagel reactions.

In addition, the authors demonstrated that the immobilised reagents suffered less physical damage within the flow-through system compared to batch reactions. The authors conducted fourteen sequential reactions obtaining an average conversion of 99.1 % with an RSD of 0.65. Furthermore it should be emphasised that EOF pumping means that no backpressures are generated, which means it is far easier to use this approach compared to a hydrodynamically pumped system. Another advantage of this type of '*micro reactor*' is that larger quantities of compound can be readily prepared. Clearly this approach to synthesis allows more moderate quantities of compounds to be prepared if required, and full instrumental characterisation may be easily performed.

LARGE SCALE MANUFACTURE

Current production technology is based on the scaling-up of successful laboratory scale reactions by firstly

constructing a pilot plant, followed by a final increase in scale to enable production. This approach is however fundamentally flawed, as at each stage changes are made to the overall surface to volume ratio of the reactors, which in turn affects mass and heat transfer processes. These variations in reactor conditions therefore result in changes to the process, meaning that it is necessary to evaluate the process and reoptimise it at each stage of scale up process. Consequently the route from bench to large scale production is both costly and time consuming. It is therefore postulated that through the application of micro reactor technology, the transfer from laboratory to production would be both rapid and cost effective as processes would initially be optimised on a single device and in order to increase the production capacity more devices would be employed; a technique referred to as 'numbering up' or 'scale out'. With the number of techniques amenable to mass production increasing, the commercial availability of parallel reactors is starting to be realised. Along with the ability to reduce the transfer time between initial discovery and production, the scale out approach is also advantageous as it enables access to an array of features not commonly used in traditional scale out approaches, such as reduced reaction times and the ability to work in the explosive limit.

From a production perspective, the scale out approach is advantageous as it enables changes in production volume by simply increasing or decreasing the number of devices employed, therefore meeting the customer demand. Also through the use of generic reactor designs custom syntheses could be performed with relative ease. Compared to a production plant whereby reactors are configured for a single function, this flexibility is both advantageous and cost effective.

CONCLUSIONS

Micro reactor chemistry is currently showing great promise as a novel method on which to build new chemical technology and processes. Reactions performed in a micro reactor invariably generate relatively pure products in high yield, in comparison to the equivalent bulk reactions, in much shorter times. One of the immediate and obvious applications is therefore in combinatorial chemistry and drug discovery, where the generation of compounds either with different reagents or under variable conditions is an essential factor. An interesting twist to the chemistry carried out to date is the opportunity to separate reactants and products in real time which would enable rapid screening to be facilitated. As an extension of the technology it has been demonstrated that kilogram quantities of products can be produced without have to '*scale up*' the synthesis.

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