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Electrochemical effects related to synthesis in micro reactors operating under electrokinetic flow

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Abstract

We have demonstrated that peptides may be prepared in quantitative conversion in a micro reactor by reaction of the pentafluorophenyl (PFP) ester derivatives of protected amino acids. It was found that performing these reactions in a micro reactor operating under electrokinetic control resulted in an increase in reaction efficiency compared with the traditional batch method. By addition of an electrode to batch reactions, we propose that the enhancement in reaction rate is due to an electrochemical effect.
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Keywords: Pentafluorophenyl ester; Micro reactor; Electrokinetic flow

1. Introduction

Micro reactors generally consist of a series of interconnecting channels formed in a planar surface, in which small quantities of reagents are manipulated [1,2]. To perform a chemical reaction, reagents are brought together in a laminar or slug flow diffusive mixing regime and are allowed to react for a specified time in a controlled region of the reactor. The ability to manipulate reagent concentrations and reaction interfaces in both space and time within the channel network provides a level of reaction control, which is not attainable in traditional bulk reactors. The spatial and temporal control of reactions in the chemically intensive environment of micro reactors, coupled with the features of very small reaction volumes and high surface interactions has been demonstrated to give faster reactions and improved product yields, with greater product selectivity compared with conventional bench top methodology [3–12].

To illustrate the principles of electroosmotic flow (EOF) [13], one can consider a micro channel fabricated from a material having negatively charged functional groups on the surface. If a liquid, displaying some degree of dissociation, is brought into contact with the surface, positive counterions will form a double layer. Application of an electric field causes this layer to move towards the negative electrode, thus causing the bulk liquid to move within the channel (Fig. 1). Importantly, when using EOF plugs of fluid are transported without significant hydrodynamic dispersion, which is not the case with hydrodynamic pumping.

When operating micro reactors under electrokinetic control, two unique processes occur which influence the reaction properties that are fundamentally different to those associated with bulk reactions. The first mechanism relates to properties associated with the electroosmotic and electrophoretic mobilities of solvents and individual species, which in addition to offering excellent spatial and temporal control, enables localised thermal, electric field and concentration gradients to be generated in pre-defined sections of a channel network. The second process is more specifically associated with the presence of an electrical field and relates to the possibility of performing electrochemically based processes within a micro reactor channel.

In order to exploit more fully the electrochemical effects within the channels of a micro reactor, operating under electrokinetic control, high voltages (typically up to 1500 V) need to be applied across electrodes placed in the reagent reservoirs. The conductivities for common solvents are of the order of 500 $\mu S\,cm^{-1}$, with the result that the electrical resistances of the channel sections are typically of the order of tens of megaohms. Hence, nearly all the voltage drop occurs within the channel of the reactor. This has the consequence that the voltage drop across the electrode–solution interface is usually very small and may or may not be sufficient to drive an electrochemical reaction. The micro reactor situation is therefore very different to the usual electrochemical cell in which a small voltage (e.g. 2 V) is

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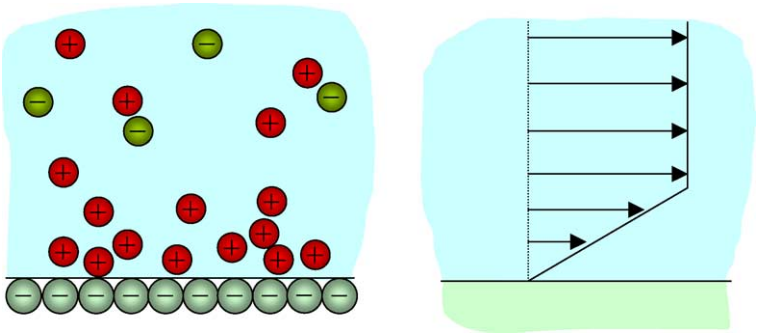


Fig. 1. Principle of electroosmotic flow.

mainly localised across the electrode–solution interfaces in a cell where the resistance of the intervening solution is low [14,15]. Experimental observations when using micro reactors show that electrochemical reactions can be induced at high voltages, particularly when the resistance of the channel section between the electrodes is relatively low. Thus, at low operating voltages when electrochemical effects are absent, the progress of chemical reactions in micro reactors are controlled solely by the localised concentrations. At high operating voltages, additional effects due to electrochemical processes may be present. In this paper, we report the effect of reaction rates when performed in micro reactors operating under electrokinetic control. It should be noted that Yoshida and coworkers [16,17] have recently reported electrochemical reactions in micro reactors, however, they hydrodynamically pumped the reactants through an electrochemical cell.

2. Experimental

The borosilicate glass micro reactors used in this work were prepared using standard fabrication procedures developed at Hull [18]. The reactions were carried out in a T-shaped micro reactor with approximate channel dimensions of 200 mm × 50 μm and outer dimensions of 20 mm × 20 mm × 25 mm. Micro porous silica frits were placed within the channels in order to minimise hydrodynamic effects [19]. Prior to synthesis, the micro reactor channels were primed with anhydrous solvent to remove air and moisture from the channels and the micro porous silica frits. Platinum electrodes were placed in each of the reservoirs of the micro reactor and an external voltage was applied to the channels inducing electroosmotic flow of the reagents. The power supply was manufactured by Kingfield Electronics (Sheffield, UK) and was controlled using LabVIEW^{TM} software.

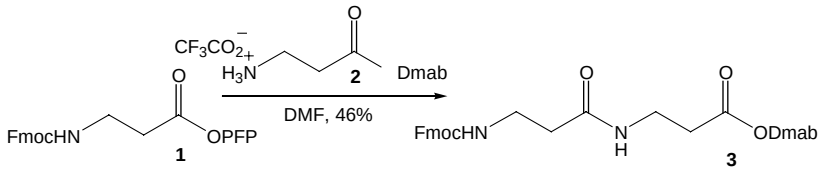
The reactions were conducted at room temperature for a period of 20 min, in order to acquire sufficient volume of product to determine the conversion of the reaction. Reaction products were determined by HPLC via comparison with retention times and spectra with those obtained from synthetic standards. Analysis was achieved by high performance liquid chromatography (Jupiter C_{18} 10 μm, 4.6 mm × 250 mm, mobile phase composition: 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, using a gradient system of 30% aqueous to 70% aqueous over 20 min, with a flow rate of 2.5 ml min^{-1} at room temperature). Product conversions were based on the amount of pentafluorophenyl (PFP) ester remaining in the sample.

3. Results

We have recently demonstrated that the multi-step synthesis of peptides may be performed within micro reactors [20,21]. In a series of examples we demonstrated that peptide bonds were produced in quantitative conversion in much shorter periods of time when prepared in the micro reactor environment in comparison to batch reactions. Furthermore, when reactions involving α-amino acids were conducted less racemisation was observed compared with batch reactions and this is attributed to the enhanced speed of the reactions [22]. In this paper, we report a more detailed study on the aforementioned observations.

In at batch reaction between pentafluorophenyl ester **1** and amine **2** we found that dipeptide **3** was produced in 46% yield, using *N*,*N*-dimethylformamide (DMF) as solvent (Scheme 1).

In a micro reactor a standard solution of the pentafluorophenyl ester of Fmoc-β-alanine **1** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A, a solution of amine **2** (50 μl, 0.1 M) was placed in reservoir B and anhydrous DMF (40 μl) was placed in reservoir C, which was used to collect the products of the reaction (Fig. 2). Platinum electrodes were placed in each of the reservoirs (A and B positive, C ground) and an external voltage was applied to the channels inducing electroosmotic flow of the reagents. The reactions were conducted at room temperature for a period of 20 min, in order to acquire sufficient volume of product to determine the conversion of the reaction by HPLC. It was found that using continuous flow of both reagents in the micro reactor, where reservoir A was maintained at 700 V and reservoir B was maintained at 600 V, dipeptide **3** was produced in 100% conversion in 20 min. This represented a significant increase in conversion compared with the traditional batch synthesis, for which 46% yield was obtained in 24 h.



Scheme 1. Preparation and reaction of PFP esters.

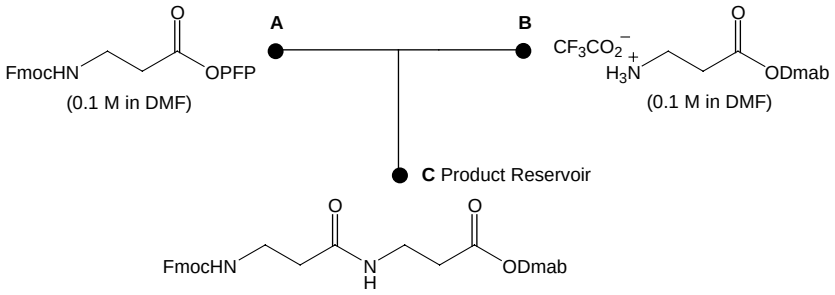


Fig. 2. Schematic of micro reactor manifold.

We were interested to observe that the reaction between pentafluorophenyl ester **1** and amine **2** appeared to be much faster in a micro reactor than when performed in batch. However, batch reactions are generally performed at much higher concentrations than in micro reactors. In order to make meaningful comparison between rates of reaction we monitored the conversion of pentafluorophenyl ester **1** (200 μl, 0.1 M) and amine **2** (200 μl, 0.1 M) into peptide **3**, at the same concentration as used in the micro reactor studies. It can be seen from Fig. 3, that the bulk reaction is very slow with approximately 60% conversion to product in approximately a week. The data suggests that in 20 min, the duration of a micro reactor reaction, only 10% conversion to dipeptide should be obtained.

In an attempt to further understand the enhancement in rate of reaction the pentafluorophenyl ester **1** (200 μl, 0.1 M) and amine **2** (200 μl, 0.1 M) were again mixed, but in this case a 10 V electrode and a ground electrode were placed in the reaction mixture. The two electrodes were approximately 5 mm apart. It can be seen from Fig. 3, that the electrodes have the effect of enhancing the rate of reaction such that 90% conversion was obtained in under 4 h. The data also illustrates that less than 30% conversion was observed in 4 h when the electrodes were absent. This implies that the enhancement in rate of reaction is due to an electrochemical phenomenon.

It should be emphasised that the voltage was set at 10 V in order to produce a current (ca. 5 μA) similar to that observed within the micro reactor situation. Nevertheless, the reaction conducted within the micro reactor, operating under electrokinetic control, still appears to be even faster than the batch reaction performed under electrochemical control. This can be attributed to the effect of increased diffusional mixing within the micro reactor environment.

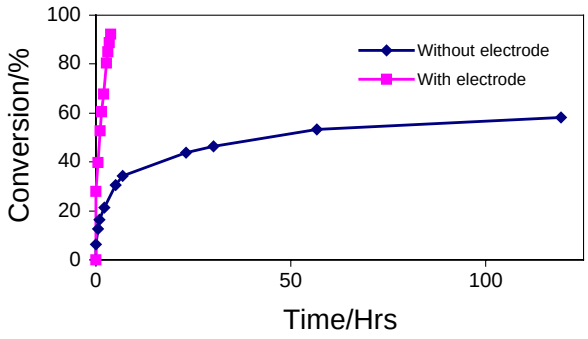
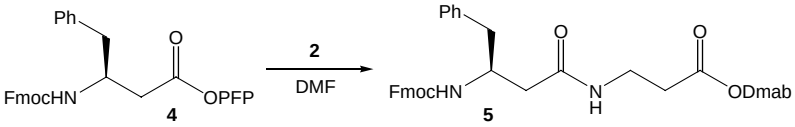


Fig. 3. Reaction of PFP ester **1** (0.1 M) with amine **2** (0.1 M) in a batch reaction at room temperature, with and without electrodes.

To illustrate that rate enhancement was always observed in such reactions the pentafluorophenyl ester **4** of Fmoc-L-β-homo phenylalanine was reacted with amine **2** to prepare a synthetic sample of dipeptide **5** in 35% yield in a batch reaction (Scheme 2). The reaction was subsequently investigated in the micro reactor. A solution of the pentafluorophenyl ester **4** in anhydrous DMF (50 μl, 0.1 M) was



Scheme 2. Batch reaction to study reaction kinetics.

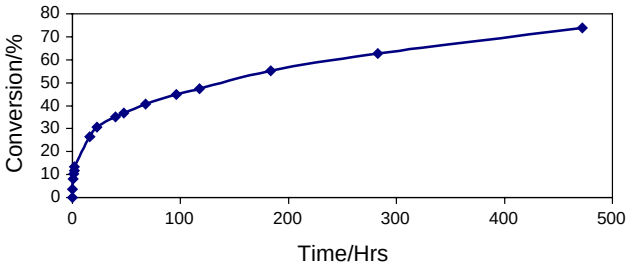


Fig. 4. Conversion of PFP ester **4** (0.1 M) to dipeptide **5** in a batch reaction at room temperature.

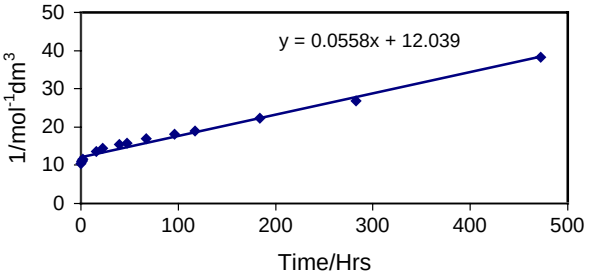


Fig. 5. Graph of pentafluorophenyl ester **1** concentration during the reaction to produce dipeptide **5**.

added to reservoir A, a solution of amine **2** (50 μl, 0.1 M) was placed in reservoir B and anhydrous DMF was placed in reservoir C (40 μl) to collect the product of the reaction. It was found that using continuous flow of both reagents, where the ester **4** was maintained at 900 V and the amine **2** was maintained at 600 V, dipeptide **5** was produced in 100% conversion in a 20 min period. Again this represented a significant increase in conversion compared with the batch reaction.

We monitored the conversion of pentafluorophenyl ester **4** into peptide **5**, where 0.1 M solutions of reagents were mixed in a batch reaction. It can be seen from Fig. 4, that the batch reaction is very slow with approximately 70% conversion to product in 500 h. The data in Fig. 4 was subsequently used to calculate how the concentration of the pentafluorophenyl ester **4** changes during the reaction. Fig. 5 illustrates that the reaction is indeed second order as expected, which implies that no other chemical species are involved in the reaction.

4. Conclusions

We have demonstrated that peptide bonds may be prepared in high conversion from pre-activated derivatives of amino acids such as pentafluorophenyl esters. It was found that performing these reactions in the micro reactor resulted in an increase in the reaction efficiency over the traditional batch method. By addition of an electrode to batch reactions we have demonstrated that the enhancement in rate of reaction is due to an electrochemical phenomenon. Subsequent analysis of amine **2** showed that the compound was actually the trifluoroacetate salt rather than the free amine as originally thought. It is proposed that the salt is converted into the free amine at the electrode, consequently the more reactive amine leads to a faster reaction. Nevertheless, the reaction is still faster when performed within the micro reactor rather than in batch which is attributed to the faster mixing which occurs within micro fluidic systems.

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