A Novel Approach Toward Microfluidic Drug Metabolite Synthesis – Electrosynthetic Methodology Simulating Cytochrome (CYP450) Oxidation

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Abstract:

A novel microfluidic technology and electrochemical synthesis method is demonstrated for the efficient generation of known drug metabolites. These metabolites are typically generated on first pass hepatic oxidation in vivo. The FLUX Module, a new microfluidic electrochemical cell manufactured by Syrris Ltd., has been employed to generate the metabolites of five commercial drugs: Tolbutamide, Chlorpromazine, Diclofenac, Primidone and Albendazole. It has been found that the electrochemical cell allows for clean oxidation and subsequent trapping of reactive intermediates with glutathione, thus mimicking mammalian hepatic transformation. In this particular study, the electrochemical cell enables four different types of reactions determined by the chemical structure of each drug: (1) aromatic hydroxylation, (2) aliphatic hydroxylation, (3) sulfoxidation and (4) glutathione conjugation. The metabolites are synthesized on the scale of 10 mg to 100 mg per hour of continuous flow, allowing for purification and full structural elucidation. This is particularly important because regioisomeric structural species are directly observed by NMR. This aspect is difficult to assess using typical in vitro microsomal or in vivo plasma bioassay with LC/MS analysis or emerging microscale EC/MS techniques. Therefore, the preparative nature of this new electrosynthesis module constitutes one of it great advantages, alongside its ease of use and versatility.

Introduction:

Electrochemical oxidation is a well known synthetic methodology and offers the advantage of atom efficiency since the reaction takes place on the electrode surface, from where the electron is sourced. But the substrate media must be conductive and the surface area to volume ratio must be as high as possible to enable the heterogeneous reaction to take place efficiently.

Historically, electrochemical oxidation reactions have been conducted in a 'batch' style (Fig. 1 below) which unfortunately, usually gives a range of oxidised and over-oxidised products.

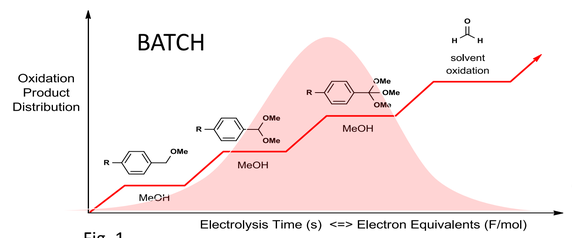

Fig. 1

Using Flow (Fig. 2) the material is constantly passed over the electrodes enabling a fine tuning of the oxidative product generated by varying the flow rate (and therefore residence time) of the reaction.

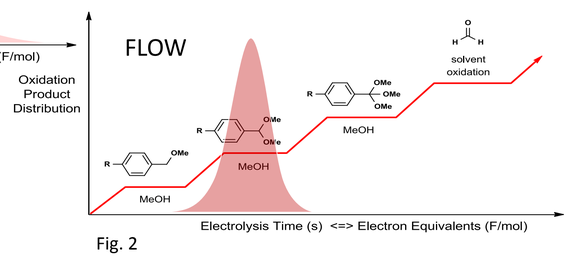

Fig. 2

The Syrris Ltd. Flow Electrochemistry module[1] provides an increased electrode surface area.[2] At a microfluidic scale the distance between the working electrode and the counter electrode is minimised which overcomes conversion limitations due to solution resistivity and can even enable electrolyte-free reactions.[3]

The Flow Electrochemistry module is shown below in Fig. 3 and allows a large number of reaction parameters to be monitored and/or modified during a reaction such as: Electrode materials, Reaction temperature, Electron equivalents (in F/mol) and Residence time (as a function of flow rate).

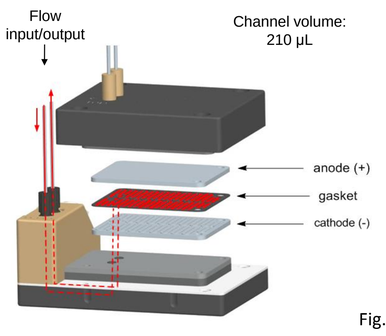

Fig. 3

Electron equivalents can be defined as: The number of electrons each molecule of substrate is exposed to during electrolysis (a function of applied current (mA), Substrate concentration (mM) and flow rate (mL/min))

Aim:

The aim of this study was to replicate hepatic (CYP450) oxidation of a number of commercial drugs that were chosen for their various oxidative chemical reactivity and well defined oxidative metabolite profiles through the use of microfluidic electrochemistry while retaining high throughput at a preparative scale of up to 100 mg/hr.

An overview of the hepatic oxidation of drug molecules in vivo is shown below, in comparison with the proposed simulated electrochemical oxidation pathway:

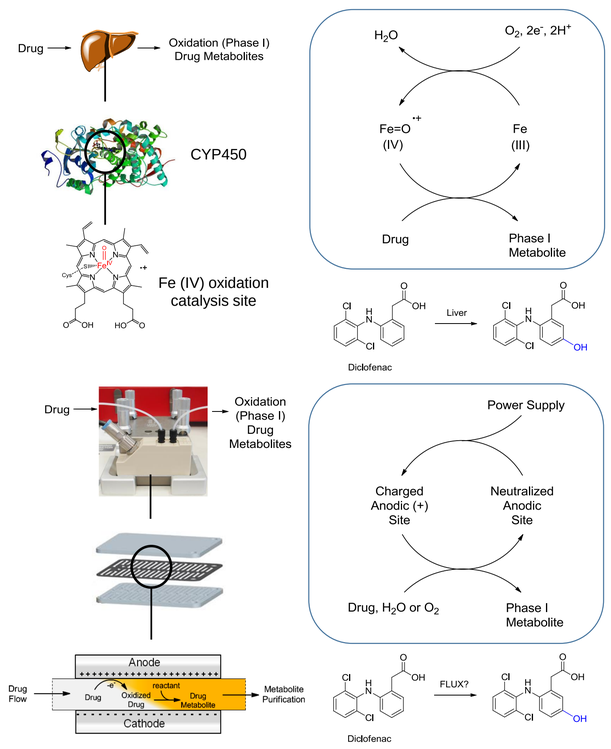

Fig. 4

Representative Method:

A 0.1 M Solution of Diclofenac in 1:1 MeCN and water was passed through the electrochemical cell at 113 mA with flow rates from 0.088mL/min to 0.235mL/min to give a range of electron equivalents (3 F/mol to 8 F/mol). ^{1}H NMR analysis was used to track the reaction progress and, after purification, 2D NMR was utilised to fully elucidate structures of the reaction products.

The phase I Diclofenac metabolite was then further reacted with GSH (Glutathione) to give the phase II GSH conjugate, which was then automatically separated with flow liquid-liquid extraction via the Syrris FLLEX module (as shown in Fig 5)

A similar method was carried out with solutions of Tolbutamide, Primidone, Albendazole and Chlorpromazine.

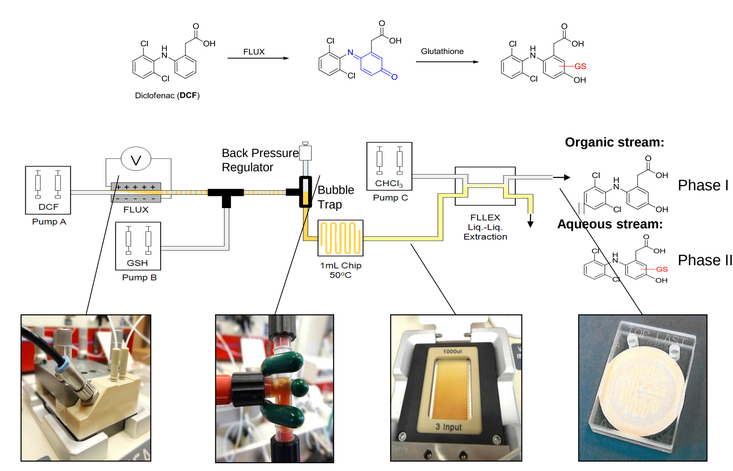

Fig. 5

Results:

The results from the electrochemical synthesis of the phase I metabolite of Diclofenac showed a dramatic increase in yield compared to other synthetic methods, increasing from <25% over 3 steps and 42% over 5 steps to 46% over 1 step, with an output of 85 mg/hr enabling rapid full structural elucidation (Fig. 6)

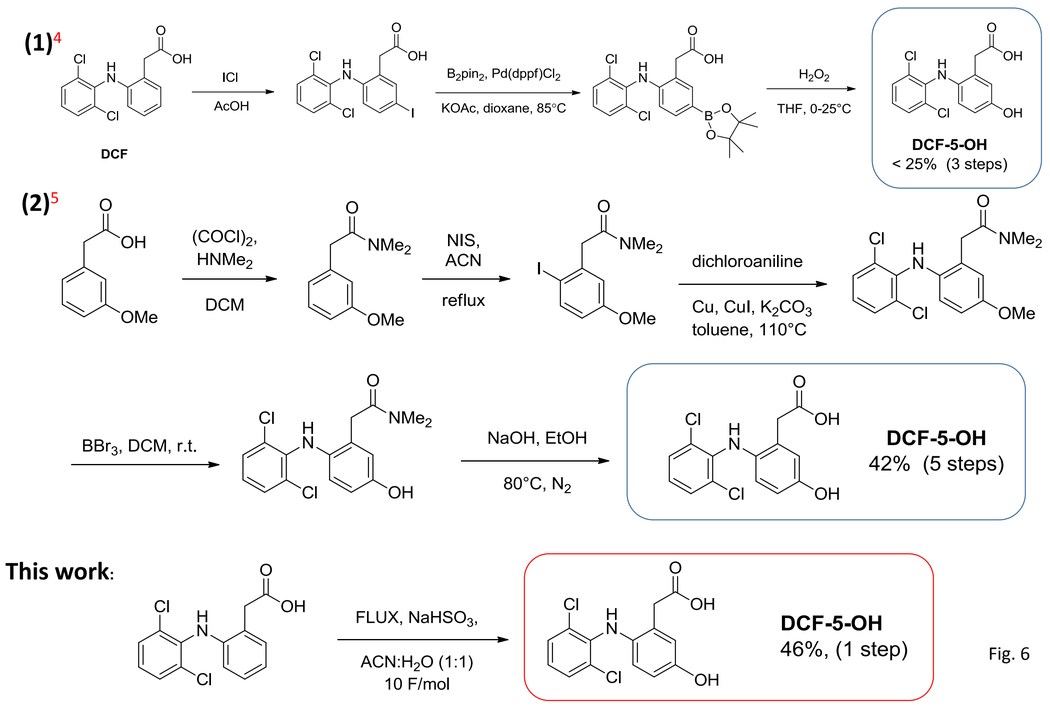

Fig. 6

The synthesis of other drug molecule phase I oxidative metabolites was also successful, and are shown in the Table below:

Drug	Electrode	Solvent	Electron Equiv. (F/mol)	Flow Rate (ml/min)	Isolated Yield (%)	Output (mg/hr)
Diclofenac (P1)	Pt/Pt	ACN:H_2O	10	0.2	46	85
Diclofenac (P2)	Pt/Pt	ACN:H_2O	10	0.2	28	100
Primidone	C/Pt	ACN:H_2O	6	0.1	24	7
Albendazole	Pt/Pt	ACN:H_2O	1.5	0.2	38	65
Chlorpromazine	C/Pt	ACN	4	0.1	83	33
Tolbutamide	C/Pt	ACN:H_2O	6	0.05	19	N/A

Summary:

These results show that simulation of the CYP450 in vivo metabolism of drug molecules has been demonstrated utilising a microfluidic electrochemical cell in scales of 10 mg to 100 mg/hr, which is higher than that of typical electroanalytical techniques by several orders of magnitude[6].

The technology allows for the production of compound quantities suitable for full structural elucidation by NMR with the scale of isolated drug metabolites generated to allow further bioassays and toxicology studies. This electrosynthetic methodology is not intended to replace biosynthetic methods (as the in vivo CYP450 oxidation profiles may differ). It is envisaged that this method will work 'hand-in-hand' with current methods and will reduce route development and synthesis time of putative drug metabolites, engender rapid synthesis of new potential drug candidates and analogues and opens new chemical space for both Medicinal chemists and Synthetic chemists to explore.

References:

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