USING THE H-CUBE® MINI PLUS IN THE CONTINUOUS FLOW SYNTHESIS OF DRUGS – HOW FLOW CHEMISTRY CAN FACILITATE API RESEARCH

INTRODUCTION

Designing highly efficient, scalable and safe methods of synthesis is undoubtedly one of the most important areas of the pharmaceutical industry. Although many synthetic routes solely focus on traditional batch procedures, these come with their own limitations. Continuous flow technologies could open new horizons for the industry with their inherent benefits in all aspects of chemical reaction design and execution.

In this application note we feature 3 remarkable pieces of research to highlight new ways of implementing continuous flow methodologies in pharma using ThalesNano instruments.

Kylie A. Vincent and her colleagues demonstrated highly promising biocatalytic prospects of H_2 driven reduction of Riboflavin using charcoal supported hydrogenase filled catalyst cartridges and an H-Cube® Mini reactor for on-demand hydrogen generation[1].

Highly efficient, simple and rapid production of Benzocaine, a widely used anaesthetic has been achieved by Rodrigo de Souza and his team. The team used an H-Cube® Mini reactor to simplify the synthetic route to a single reaction step with a reaction time of 12 seconds, while maintaining >99% conversion and >99% selectivity[2].

Tamás Kálai and his colleagues incorporated the H-Cube® Mini Plus flow reactor to facilitate a hydrogenation step during the synthesis of a spin-labelled derivative of Varenicline for possible use as a theranostic agent[3].

INSTRUMENTATION AND SAFETY CONSIDERATIONS

The H-Cube® Mini Plus is the smallest, all in one continuous flow reactor from the H-Cube® lineup. Within its small footprint, the instrument offers on-demand hydrogen generation up to 100 bars of pressure and 30 NmL/min flow rate using deionized water electrolysis. With heating capacity of 100 °C it can supply a wide range of reactions with the desired temperature. The instrument is designed to house 30 mm long pre-packed catalyst cartridges (CatCart®). Extended residence times or higher throughputs can be achieved by using 70 mm CatCart®s. Thanks to the H-Cube® Mini Plus's simple flow path and user interface, setting up the instrument and performing reactions is not a challenge for first time experiencers of flow chemistry either. Thanks to this the instrument can be considered as both a learning and a research tool in the hands of the chemists and chemical engineers in the field.

As with all ThalesNano instruments safety is of utmost importance in the H-Cube® Mini Plus as well. Featuring built-in automated safety triggers for leakage and blockage detection the reactor will automatically and safely stop in the unlikely event of an emergency. The instrument monitors the atmospheric H_2 concentration as well and automatically stops if a H_2 leak occurs. The transparent external water reservoir makes it easy to observe the water level at all times. The CatCart® technology eliminates the user's contact with any active catalyst material. The use of sealed cartridges enhances safety when handling pyrophoric catalysts, offering a safer alternative to traditional batch methods. All batches of pre-packed CatCart®s purchased from ThalesNano are tested for physical and chemical properties prior to shipping to customers. For specialty catalyst testing apart from purchasing pre-packed CatCart®s the cartridges can be packed and closed by the users themselves using the CatCart® Packer and CatCart® Closer devices.

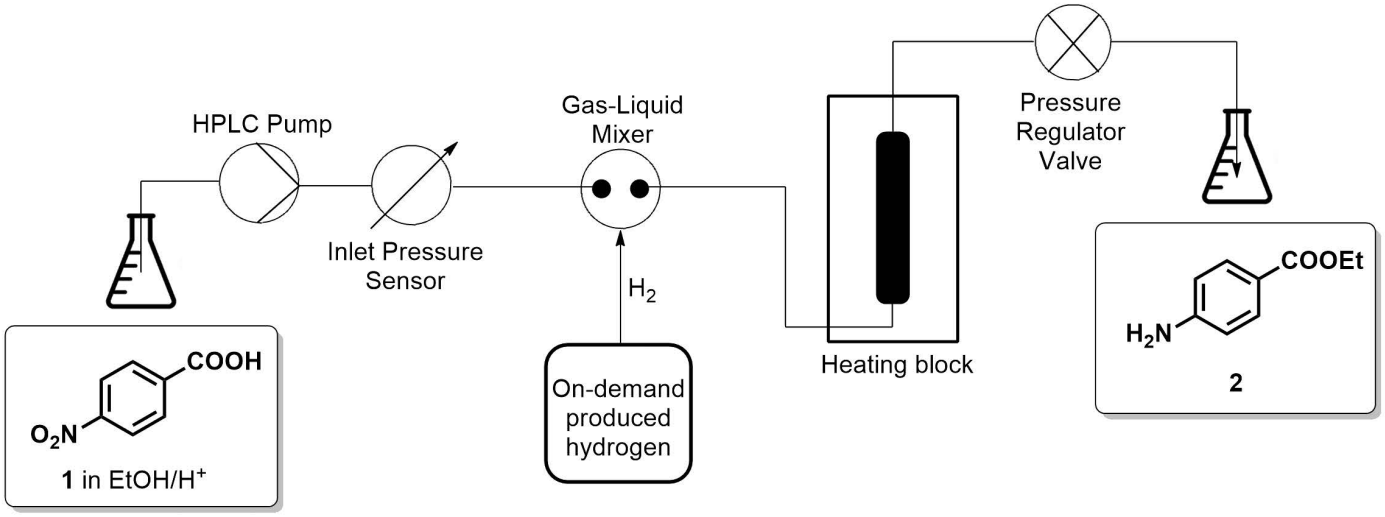
DISCUSSION AND RESULTS

Continuous-flow synthesis of benzocaine (2)[2]

Benzocaine (**2**) is commonly used across the healthcare industry as a local anaesthetic. Traditional batch synthetic methodologies have been extensively reported in the literature[2]. The batch procedure consists of multiple cooling, heating and workup steps with a reaction time in the hour range. Rodrigo de Souza and his team developed a continuous flow synthetic method that is capable of a single step rapid reaction with >99% conversion and >99% selectivity, with a catalyst residence time of 12 seconds[2].

The group exploited the benefits of flow chemistry and the H-Cube® Mini Plus such as highly precise control of reaction parameters like pressure, stoichiometry, temperature and residence time, allowing for the design of a high yielding, reproducible, scalable synthetic procedure. The system also benefits from the inherent safety aspects of flow chemistry, such as small reactor volume and improved thermal efficiency. With on-demand H_2 generation included in the H-Cube® Mini Plus, operational hazards were further minimized.

During the group's optimization process it has been realized that benzocaine (**2**) synthesis could be performed in a single step, instead of a multi-step reaction sequence, as documented in traditional methods. A solution of p-nitro benzoic acid (**1**) and sulphuric acid or trifluoroacetic acid (TFA) in ethanol was used as starting material in the H-Cube® Mini Plus with 1.0 mL/min liquid flow rate. The reactor contained a 10% Pd/C CatCart® catalyst cartridge, heated to 50 °C along with 45 bar pressurization (Scheme 1). Screening for the effect of pH for the two acids showed that TFA provides better conversion compared to sulphuric acid at the same pH. With the decrease of reaction pH, and a residence time of 12 seconds, full conversion could be achieved, along with >99% selectivity towards the desired product **2**. The reaction could be performed up to 12.0 mg/ml without loss of conversion or selectivity. The group used GC-MS to verify experimental results. The detailed experimental parameters and further findings can be found in the original research article[2].



Scheme 1: Two-step continuous flow synthesis of benzocaine (**2**)

H_2-driven reduction of Riboflavin[1]

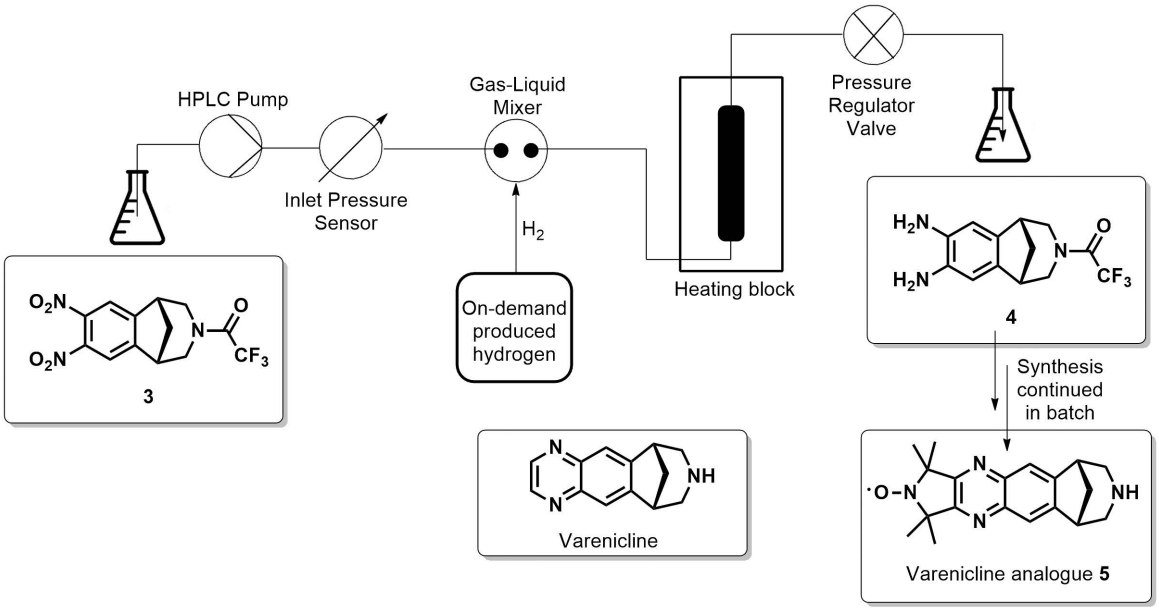
Vitamin B2 (Riboflavin) is produced in bulk on over a 9000 ton/year scale[4]. Thanks to its availability and affordability it poses a promising cost-effective opportunity to be used in pharmaceutical biocatalysis.

Kylie A. Vincent and her colleagues have investigated the biocatalytic H_2 driven reduction of riboflavin to be of consideration as a cost-effective ene-reductase cofactor for replacing expensive nicotinamide cofactors[1].

The carbon support immobilization and use of robust NiFe hydrogenase 1 (Hyd1) in flow has been reported previously[5]. Based on this, the group investigated the feasibility of extending the use of the enzyme for riboflavin reduction. A CatCart® containing the Hyd1 on activated charcoal (Mesh 100) was prepared and inserted into the H-Cube® Mini Plus flow reactor. 0.2 mM Riboflavin (pH=7, 100 mM potassium phosphate buffer) solution was passed through the flow system at 0.1 mL/min liquid flow rate and 30 mL/min H_2 flow rate, atmospheric pressure and room temperature. An in-line flow UV-Vis spectrophotometer was used for conversion monitoring. During the runs, the signal corresponding to the oxidized form of flavin has dropped to zero, indicating full single pass conversion to the reduced form. This was maintained for over a 24 hour experiment[1]. The group has also verified that there was no absorption of reactant on the carbon-support. Further detailed experimental data can be found in the original research article[1].

Facilitating the hydrogenation step in the synthesis of spin-labelled compounds[3]

Researching drug formulation limitations is a cornerstone of pharmaceutical development. Spin-labelled compounds play a key role in the understanding of macromolecular systems[3]. Varenicline is a remarkable pyrazine derivative that acts as a nicotinic receptor agonist, used in the therapy for chronic smoking addiction[6]. Kálai and colleagues presented the synthesis of a spin-labelled varenicline analogue **5**. Their synthesis route included a nitro compound (**3**) hydrogenation, facilitated by the use of the H-Cube® Mini Plus. The instrument was equipped with 20% $Pd(OH)_2$/C CatCart®. The catalytic hydrogenation reaction was performed at 6 bars, in 80 mL 1:1 (v/v) THF/MeOH mixture that contained 1.0 mmol **3**. The reaction mixture was passed through the reactor at 0.7 mL/min flow rate. The conversion was monitored by thin layer chromatography. The following reaction steps and experimental data can be found in the original research article[3].



Scheme 2: Continuous flow hydrogenation of a Varenicline analogue intermediate

SUMMARY AND CONCLUSIONS

The H-Cube® Mini Plus seamlessly integrates feature-rich capabilities, simplicity, and safety. This application note highlights three research articles showcasing the diverse real-world applications of flow chemistry using ThalesNano instruments. From enabling key steps in labelled molecule synthesis and optimizing biocatalytic processes to streamlining batch procedures for active ingredient production, the H-Cube® Mini Plus stands as a versatile and powerful synthetic platform for researchers and industry professionals alike.

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