Online Quantitative Mass Spectrometry for the Rapid Adaptive Optimization of Automated Flow Reactors**

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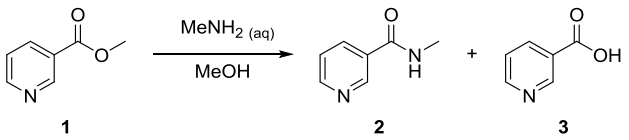
An automated continuous reactor for the synthesis of organic compounds, which uses online mass spectrometry (MS) for reaction monitoring and product quantification, is presented. Quantitative and rapid MS monitoring was developed and calibrated using HPLC. The amidation of methyl nicotinate with aqueous $MeNH_2$ was optimized using design of experiments and a self-optimization algorithm approach to produce >94% yield.

Flow reactors are increasing in popularity for the synthesis of organic compounds. Their advantages over batch reactors include higher reproducibility; safer operating conditions (particularly at increased temperatures and pressures); ease of automation; and facile integration of analysis. Therefore great success has been achieved for the optimization of chemical systems using continuous reactors.[1] Automated flow reactors typically combine online analysis with a feedback loop or PC interface to carry out reactions without any further human interference.[2] This technology has been used for the optimization of reactions using evolutionary algorithms (self-optimization),[3] design of experiments (DoE)[4] and kinetic parameters.[4b, 5]

Process analytical technologies (PAT) for automated flow reactors include UV-Vis,[3a] IR[1e, 3f, 3h, 5-6], Raman[7] and NMR spectroscopy;[3g] Gas Chromatography[3c-e] and High Performance Liquid Chromatography (HPLC).[3b] Spectroscopy benefits from rapid analytical method times, which can be used as real-time feedback to assess the steady state of a continuous reactor.[3f] However, vibrational spectroscopy generates complex spectra, which may require extensive deconvolution, and can be difficult to calibrate for multi-component systems. NMR spectroscopy is typically easier to analyze and provides more structural information than IR. The resolution and sensitivity of miniaturized low-field bench-top NMR spectrometers, which due to their small size can be used for inline analysis, means that subtle chemical transformations may not be detected and accurate quantification of low level impurities may prove difficult.[3g] Chromatography generates data that is easy to analyze and can provide structural information if combined with Mass Spectrometry (MS) detection. However the long method times significantly decrease throughput.

To overcome the issues in analysis duration, demanding calibration and sensitivity in these PAT techniques in this communication we explore the use of online MS to enable rapid quantification (<1 min analysis duration). Online MS has been used to monitor reactions carried out in continuous reactors but thus far has been limited to the identification of compounds[8] or qualitative analysis of composition.[9] MS can provide structural information and product composition, all in real-time due to its short method times. Therefore it could be the ideal analytical technique for optimizing an automated flow reactor as it can determine steady state and then calculate a product yield with minimal data manipulation.

This hypothesis was tested by carrying out a self-optimization and DoE, to optimize the synthesis of *N'*-methyl nicotinamide **2** by reacting methyl nicotinate **1** with aqueous methylamine in methanol (Scheme 1). **1** can also hydrolyze to form niacin **3**. This reaction was selected due to the presence of an easily ionizable pyridine nitrogen, loss of selectivity due to the presence of water in the aqueous methylamine and the requirement of high loadings of methylamine which may cause suppression effects. Overcoming such suppression effects is an important factor if direct MS is used for quantitative analysis.



Scheme 1. The reaction of methyl nicotinate **1** with aqueous methylamine to form the desired *N'*-methyl nicotinamde **2** and the impurity niacin **3**.

The ester and amine solutions were pumped using dual piston LC pumps, with an additional pump of solvent to clean the reactor between experiments and prevent accumulation of analyte in the mass spectrometer. Reagent feeds were mixed in tee-pieces before entering a tubular reactor (Cambridge Reactor Design, Polar Bear Flow Synthesizer) with active heating and cooling, significantly reducing the time required to perform subsequent experiments at different temperatures. Upon exiting the reactor, aliquots of reaction mixture were introduced to the mobile phase of the MS using a 4 port microvolume (0.06 µL)

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sample valve. The reactor was maintained under fixed pressure using a back pressure regulator. Pump flow rates; reactor temperature and sample intervals were controlled by a custom written MatLab program, see Figure 1.

The spectrometer used was an Advion Expression CMS operating in positive APCI (Atmospheric Pressure Chemical Ionization) mode. APCI was selected over Electrospray Ionization (ESI) due to a reduction in baseline noise and being able to handle a larger mobile phase flow rate.

The yield of each component was calculated by internal normalization of the [M+H] adducts. The internally normalized areas were corrected for the isotope abundance as the [M+1+H] isotope of **2** could be confused with the [M+H] adduct of **1**. Calibration curves for **1** to **3** were calculated for HPLC and it was possible to quantify accurately the MS to the calibrated HPLC using experiments in a central composite face centred (CCF) plot, with very good fit (R^2 0.997 – see ESI for full details of calibration).

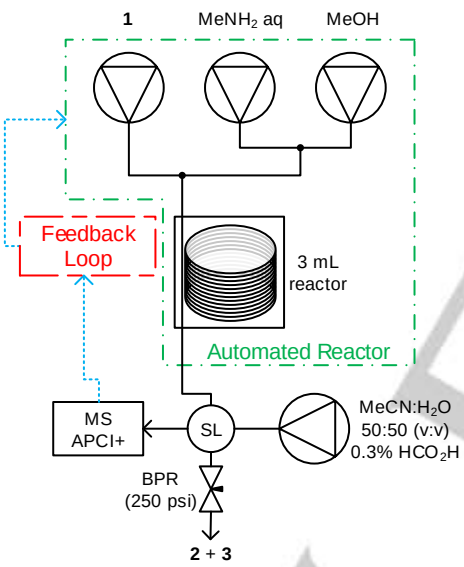

Figure 1. Reactor set-up. Reagents were pumped using Jasco PU980 pumps and were mixed in Swagelok tee-pieces. A Polar Bear Flow Synthesizer was used for heating and cooling of the tubular reactor. Aliquots of reaction mixture were delivered to the MS mobile phase using a VICI Valco 4 port sample loop (SL). The reaction was maintained under fixed back pressure using an Upchurch Scientific back pressure regulator (BPR). PTFE tubing (1/16" OD, 1/32" ID) provided by Polyflon was used throughout the reactor. Swagelok unions and fittings were used throughout apart from the sample loop (VICI) and BPR (Upchurch). An Agilent 1100 G1311A quaternary pump provided the mobile phase to the Advion Expression CMS. The automated reactor was controlled by a custom written MatLab program.

Fully automated optimizations were carried out using the SNOBFIT algorithm and a DoE statistical design (see ESI for full details) using the reactor in Figure 1 and the boundary limits shown in Table 1. SNOBFIT is a branch and fit algorithm that fits polynomials to experimental points and can find multiple optima.[10] For each experiment, the reactor is set to the desired

Table 1. Optimization limits for the self-optimization and DoE. Ester **1** concentration 1.46 mol L^{-1}, $MeNH_2$ concentration 5.77 mol L^{-1}.

Limit	Ester **1** flow rate (mL min^{-1})	$MeNH_2$ molar eq	Temperature (°C)
Lower	0.100	1	0
Upper	0.400	10	130

temperature and methanol is pumped at 0.5 mL min^{-1} and the other pumps at 0.02 mL min^{-1} to minimize reagent usage during temperature changes. When the reactor reaches the set temperature the reagent pumps are set to their desired flow rates and allowed to pump for one residence time. During this time, the MS is directly sampled at 40 s intervals using a 5:2 flow splitter to further reduce sample concentration. We believe that the nanolitre injection volumes, combined with the flow splitter and APCI ionization technique reduce the sample concentration within the MS detector to the linear range allowing accurate quantification. After 1.1 reactor volumes of fluid are pumped, a steady state function monitors the last three samples and when variation of the amide % yield was less a deviation of ± 0.75% the system is deemed to be at steady state. The composition of the fluid is then recorded and the next experiment conditions are set and the process above repeated. Detection of steady state with near real-time monitoring reduces material usage and more accurate quantification than single data point analysis.

The change in the responses of **1**-**3** for the first 4 experiments in the self-optimization is shown in Figure 2. Optimum conditions were reached in 21 experiments, which corresponded to less than 12 hours of experiment time. The optimum conditions generate **2** in 94 % yield (Ester **1** flow rate 0.1 mL min^{-1}, $MeNH_2$ 10 eq, 10.6 °C, Figure 3).

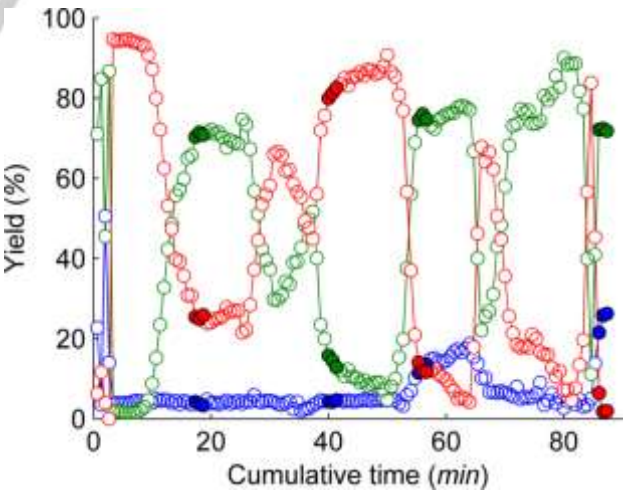

Figure 2. MS plot for the first 4 experiments in the self-optimization where red is **1**, green is **2** and blue is **3**. The filled points show the last three points where steady state was reached.

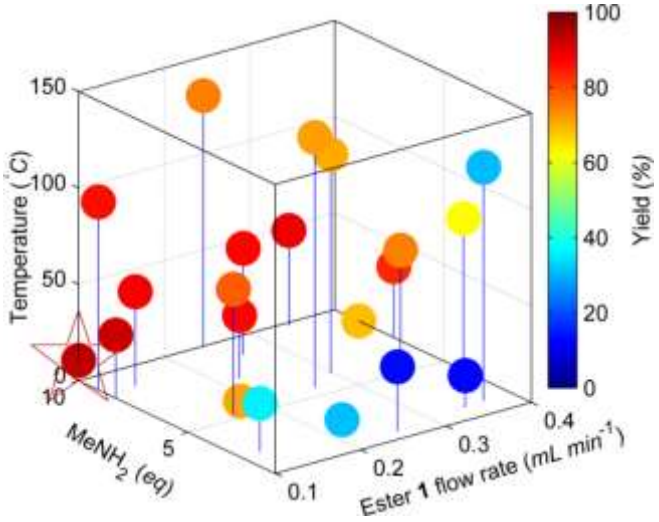

Figure 3. Optimization plot for the SNOBFIT self-optimization of amide 2. Optimum point highlighted by the star, ester **1** flow rate 0.1 mL min^{-1}, $MeNH_2$ 10 eq, 10.6 °C.

A DoE statistical design was constructed using a central composite faced (CCF) design. The CCF design enables curvature of the response surface to be modeled statistically. The reaction conditions were ranked into blocks of ascending temperature and then randomized within these blocks. Traditionally, statistical experiments require full randomization to eliminate systematic errors that can create bias in the results.[11] However, we have found that waiting for heating and cooling of the reactor is the biggest contributor to the total optimization time, and that randomization did not lead to any difference in experimental results. Therefore it was decided that a higher intensification of experiments could be achieved with ascending ordering of temperature.

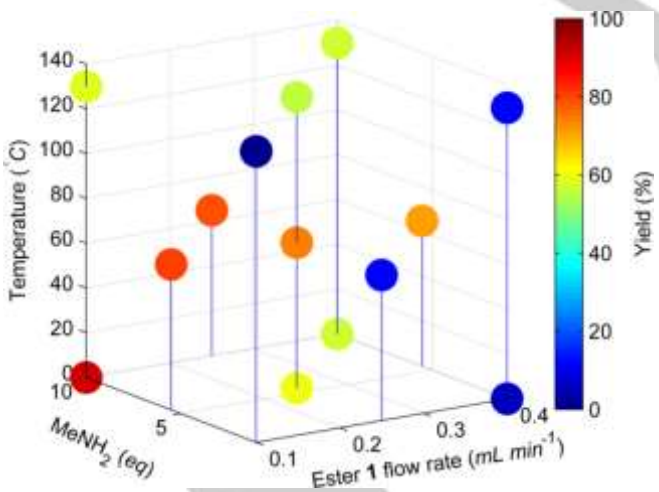

Figure 4. 3-D plot showing the yield of **2** for each experimental data point in the CCF DoE.

Models for the composition of compounds **1**-**3** were generated by generating a saturated model including all square and interaction terms and then manually removing any non-significant terms.[12] The yield of **2** for each data point is shown in Figure 4, and further model information can be found in the supplementary information. These models were generated using experiments conducted over a period of 5.5 hours with excellent fit and predictability (R^2 = 0.999 and Q^2 = 0.977). An optimum for **2** was predicted by minimizing **1** and **3** and maximizing **2**, which predicted conditions to generate **2** in 96 % yield (Ester **1** flow 0.1 mL min^{-1}, $MeNH_2$ 9.7 eq, 7 °C, Figure 5).

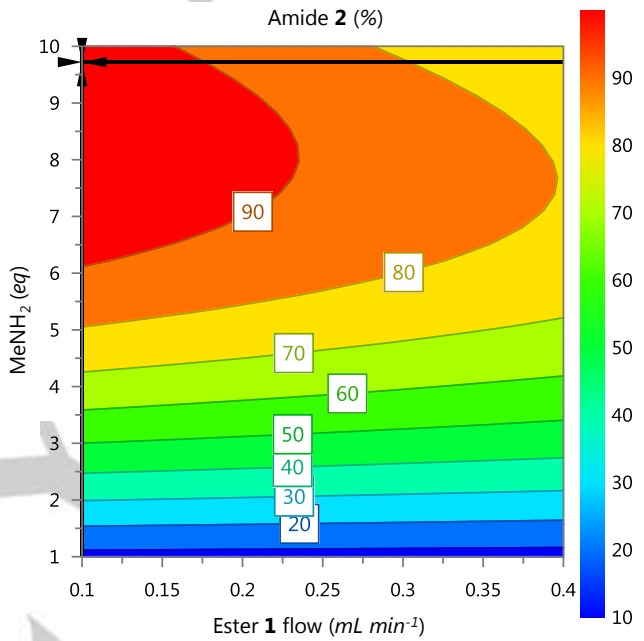

Figure 5. Contour plot for the optimum conditions derived from the CCF model, generated in MODDE. Temperature fixed at 7 °C, optimum point highlighted by the crosshair.

Online MS has been shown to optimize a model reaction using an automated continuous reactor. It was possible to calibrate the MS signal to HPLC using linear relative response values, with minimal effort in data manipulation. It was also possible to distinguish between product adducts and isotope patterns. The MS was subsequently used to determine steady state and calculate the yield in two separate optimizations. The optimum conditions achieved match very well showing the high reproducibility using this approach (Table 2) and either approach could be used to optimize the reaction system, It is important to consider that SNOBFIT experimentally verifies the optimum as part of the algorithm process giving higher confidence but took significantly longer (12 hours vs. 5.5 hours) than the structured DoE optimization as these experiments were ordered to minimize reactor temperature changes. However it should be noted that a fully randomized statistical design would take considerably longer.

Table 2. Comparison of the optimum conditions obtained through the CCF (predicted) and self-optimization (experimental)

Optimization	Ester **1** flow rate (mL min^{-1})	$MeNH_2$ molar eq	Temperature (°C)	Amide **2** yield (%)
DoE Predicted	0.100	9.7	7	96 (predicted)
Experimental SNOBFIT	0.100	10	10.6	94 (experimental)

In addition, statistical modeling of the SNOBFIT data could also be performed to generate similar response surface models to the DoE model due to good coverage of the reaction space. It is also possible to verify model performance by inputting the SNOBFIT dataset into the DoE model. For example the optimal SNOBFIT data point from Table 2 was predicted to have a yield of 96% by the DoE model.

MS has the potential to be a powerful process analytical technology. Discrete separation and product quantification can be achieved with minimal method development, and significantly reduced method times when compared to chromatography. Therefore rapid analysis with detailed molecular characterization information can be obtained. This has been exploited to enable rapid optimization using both a black-box algorithm and statistical optimization of an automated flow reactor and we aim to extend the scope to more complex chemistries using compounds that are difficult to analyze using other techniques.

Experimental Section

Experiments were carried out using the reactor described in Figure 1. RS232 serial communication send commands from the Jasco PU980 pumps and VICI sample loop; Ethernet communication from the Polar Bear reactor and Agilent pump; and USB communication from the Advion MS. MS control and monitoring was achieved using Advion Mass Express; Agilent pump using Agilent Chemstation, and the rest of the reactor using a custom written MatLab program. Conditions for the DoE were calculated by running a script based on the 'ccdesign' MatLab function. Analysis of the DoE was carried out using commercially available DoE software MODDE. Conditions for the self-optimization were generated from within user-defined limits of the SNOBFIT algorithm and based on the results of existing experiment yields.

Solution reservoirs for the pumps were prepared by dissolving the methyl nicotinate (50 g, 36.5 mmol) in methanol (200 mL); and methylamine solution (40% wt aq, 200 mL, 5.15 mol) in distilled water (200 mL). Ester solution concentration = 1.46 mol L^{-1}, methylamine solution = 5.77 mol L^{-1}. The reactor was primed by pumping from the pump reservoirs at 1 mL min^{-1} until product was detected by MS.

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Keywords: Flow Chemistry, Mass Spectrometry, Reaction Optimization, Amides, Self-Optimization

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[12] DoE models were generated, analysed and optimized using MODDE v10.11.11 (Umetrics).

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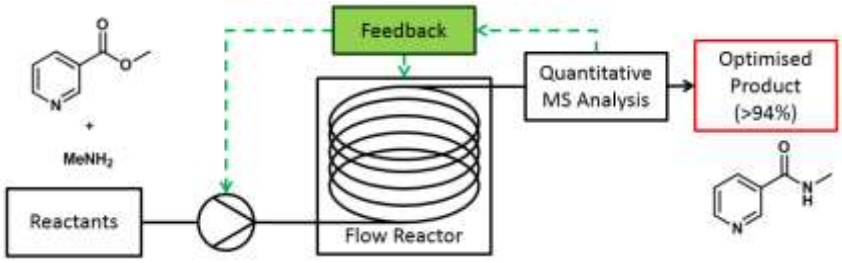

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Title

Automated Optimization: Quantitative mass spectrometry is used to rapidly optimize a reaction using both an algorithm and statistical design and a fully automated reactor system