



## Multivariate analysis of a nucleophilic aromatic substitution by real-time on-line flow reaction monitoring using a miniaturized mass spectrometer

### INTRODUCTION

The Microsaic 4000 MiD<sup>®</sup> is a miniaturised single quadrupole mass spectrometer (MS) designed with the chemist in mind. By utilising micro-electro-mechanical systems (MEMS) technology we have been able to miniaturise the key components of a MS which has allowed us to produce the smallest mass spectrometer on the market. The unit has been designed with the vacuum system, electronics and computer all inside the one box. This means the instrument can be installed in places where no other mass spectrometer can be normally deployed. For instance, the product can be put into a fume hood next to a flow reactor where it is needed. The system is portable with modular “plug and play” components for maximum application flexibility.

For on-line monitoring of reactions in flow, MS can be used to analyse in real-time the chemical composition of the flow stream. This specific work-flow provides data to monitor starting materials, identify the presence of reactive transients and impurities, determine steady state conditions and optimize reaction yield.

The use of automated flow systems combined with on-line MS analysis enables rapid screening and efficient optimisation for process development is fully scalable from laboratory, pilot plant to manufacturing plant. This produces a massive saving in the time and materials required if compared to common approaches. Specifically, we investigated the optimization of the nucleophilic aromatic substitution of 2,4-difluoro-nitrobenzene with morpholine over a wide variety of stoichiometric ratios, residence times and temperatures to demonstrate all the benefits of using on-line flow reaction monitoring.

### EXPERIMENTAL

The continuous flow optimization of the nucleophilic aromatic substitution has been carried out by coupling the Microsaic 4000 MiD<sup>®</sup> mass spectrometer to the CDR Polar Bear *Plus* flow chemistry system through an interface module (Figure 1).

The time taken to install the Microsaic 4000 MiD<sup>®</sup> and connect to the flow reactor to acquire meaningful data was less than 1 hour. Using a similar set up with a conventional MS system would take anything from 2 to 5 days.

The continuous flow optimization of the nucleophilic aromatic substitution was monitored by the MS using the settings reported in Table 1.

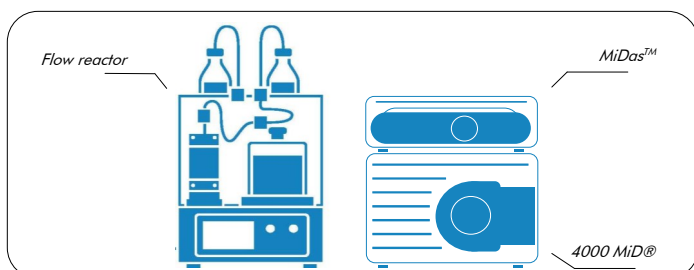
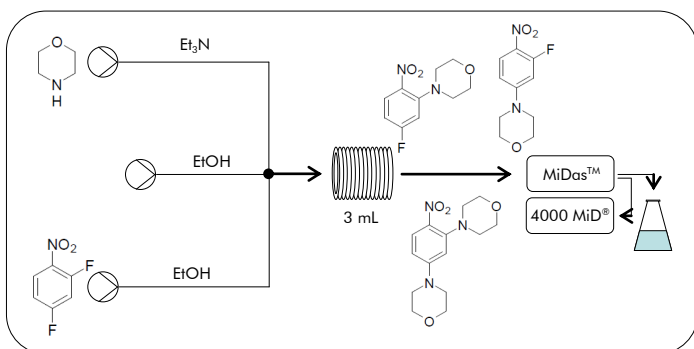


Figure 1. Schematic representation of Microsaic 4000 MiD<sup>®</sup> coupled via MiDas<sup>™</sup> (interface module) to CDR Polar Bear *Plus* flow chemistry system.

Scan mode	Full scan
Mass Range	$m/z$ 50 – 800
Scan Rate	1 scan/sec
Step size	$m/z$ 0.2
Ion polarity	Positive (ESI)
Tip voltage	850 V
Gas flow	2500 mL/min
Vacuum interface	50 V
Tube lens	10 V
Plate lens	5 V
Ion guide	1 V

Table 1. Microsaic 4000 MiD<sup>®</sup> settings for on-line flow reaction monitoring.

The reaction of 2,4-difluoro-nitrobenzene and morpholine was carried out in ethanol and triethylamine (Scheme 1) over a wide range of stoichiometric ratios, residence times and temperatures using the instrument set up reported in Table 2.



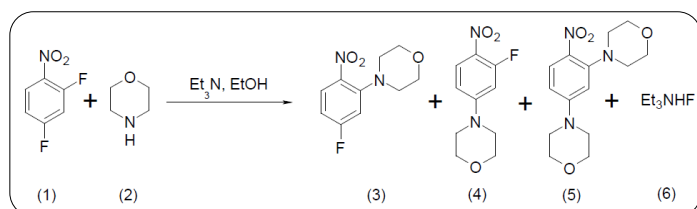
Scheme 1. Flow route for the nucleophilic aromatic substitution.

Reactor	PFA coiled tube
Reactor size	3 mL
Pressurization module	1 bar
Reagent A	2,4-difluoro-nitrobenzene (3.04 M) in ethanol (11.44 M)
Reagent B	Morpholine (4.17 M) in triethylamine (4.58 M)
Reagent C	Ethanol
Total flow rate (pump A+B+C)	Ramped from 1 to 6 mL/min over 20 min
Pump A,B and C flow rates	Dynamically adjusted to achieve desired reagent ratios and residence times
Residence time	Ramped from 0.5 to 3 min over 20 min
Stoichiometric ratios A:B	1:1, 1:1.5, 1:2 and 1:3
Reactor temperatures	60, 80, 100 and 120 °C
Make-up pump solvent	Methanol, 0.1% formic acid
Make-up pump flow rate	1 mL/min
Split ratio	3000:1

**Table 2.** Flow reactor-MS set up for the chemical transformation of 2,4-difluoro-nitrobenzene with morpholine.

## RESULTS AND DISCUSSION

The reaction expected is shown in the Figure 2, where 2,4-difluoro-nitrobenzene (1) reacts with the nucleophile morpholine (2) to afford substitution products (3,4 and 5) by an addition-elimination mechanism. The reaction is conducted in triethylamine to neutralize the hydrofluoric acid generated as side product by forming triethylamide hydrofluoride (6).

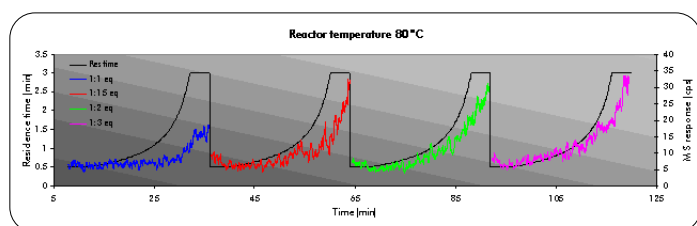


**Figure 2.** Reaction scheme for nucleophilic aromatic substitution of 2,4 difluoro-nitrobenzene with morpholine.

The desired flow experimental conditions were inputted into a custom control program. The system used was solvent-free which means that the reagents' stoichiometric ratios and the residence times of the different components vary as the algorithm changes the flow rates of the reactants.

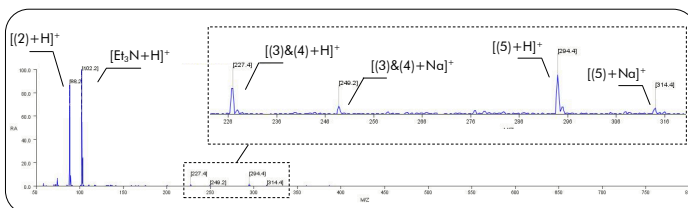
The multivariate analysis of the reaction was carried out adopting a 4 x 4 grid of different 2,4-difluoro-nitrobenzene:morpholine stoichiometric ratios (1:1, 1:1.5, 1:2, 1:3) and reactor temperatures (60, 80, 100, 120 °C) ramping the total reagents residence time from 0.5 to 3 min over 20 min. This approach resulted in approximately 15,000 conditions monitored in real-time by analysing the reactor mixture on-line using the Microsaic 4000 MiD<sup>®</sup> mass spectrometer.

Figure 3 shows the extracted ion chromatogram (EIC) of a reaction product monitored in real-time under different stoichiometric ratios and residence times at a specific reactor



**Figure 3.** EIC of 2,4 dimorpholino-nitrobenzene ( $m/z$  294.4) monitored ramping the residence time from 0.5 to 3 min in 20 min for stoichiometric ratios 2,4-difluoro-nitrobenzene:morpholine 1:1, 1:1.5, 1:2 and 1:3 at 80 °C.

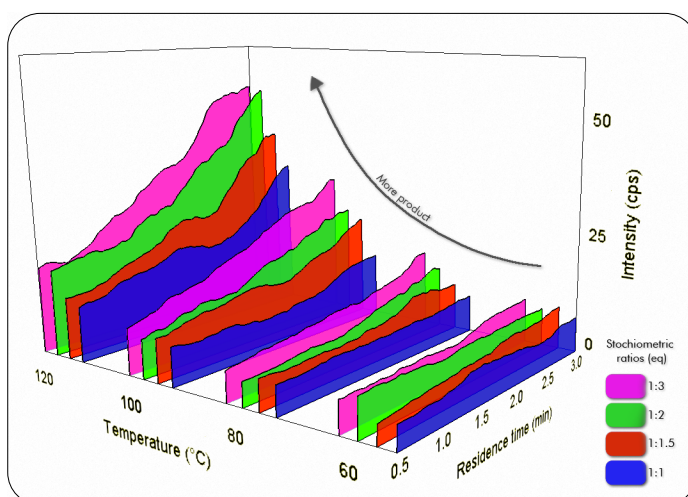
Figure 4 shows the mass spectrum of both reagents and products ions at a reaction temperature of 80 °C.



**Figure 4.** Mass spectrum of protonated morpholine (2) at  $m/z$  88.2, triethylamine at  $m/z$  102.2, 4-fluoro-2-morpholino-nitrobenzene (3) and 2-fluoro-4-morpholino-nitrobenzene (4) at  $m/z$  227.4, 2,4 dimorpholino-nitrobenzene (5) at  $m/z$  294.4 monitoring the reaction at 80 °C. Typical sodium adducts of reaction products are also shown.

To optimize the studied chemical transformation, the effect of the various reaction parameters was evaluated plotting the EICs of 2,4 dimorpholino-nitrobenzene ( $m/z$  294.4) against residence times, stoichiometric ratios and temperatures in a waterfall 3D graph. Figure 5 shows the effect on the product intensity and reaction yield of increasing the residence time, stoichiometric ratio and reactor temperature.

The completion of the whole set of tests took 16 hrs, a fraction of the time and chemicals needed if conventional off-line analysis was used.



**Figure 5.** Waterfall 3D graph for the extracted ion chromatograms of 2,4 dimorpholino-nitrobenzene at  $m/z$  294.4 as a function of the residence time, stoichiometric ratio and reaction temperature.

## CONCLUSIONS

A nucleophilic aromatic substitution was studied using a multivariate analysis approach by means of an automated continuous flow reactor with real-time on-line MS analysis. Using the Microsaic 4000 MiD<sup>®</sup> mass spectrometer for on-line reaction monitoring enabled yield optimization evaluating a wide range of reaction conditions, resulting in a reduction of materials and time required compared to routine off-line approaches.

With the results presented in this application note, Microsaic Systems has successfully deployed its miniaturised mass spectrometer in a multivariate analysis for rapid and low cost reaction yield optimization by using on-line flow reaction monitoring.