Supporting Information

Selective Separation of Amines from Continuous Processes using Automated pH Controlled Extraction.

Luke A. Power[a], Adam D. Clayton[a], William R. Reynolds[a], David R. J. Hose[b], Caroline Ainsworth[b], Thomas W. Chamberlain[a], Bao N. Nguyen[a], Richard A. Bourne[a], Nikil Kapur[a] and John A. Blacker[a*].

a. Institute of Process Research and Development, School of Chemistry, University of Leeds, Leeds, LS2 9JT, UK.

b. Chemical Development, Pharmaceutical Technology and Development, Operations, AstraZeneca, Macclesfield, SK10 2NA, UK.

Table of Contents

1. System Configuration

1.1. pH Meter Design

All components for the pH meter were purchased from Atlas Scientific[1], Whitebox Labs[2], Arduino[3] or Hanna instruments[4]. The pH meter was composed of an Arduino uno (**1**) with two whiteboxlab tentacle minis stacked on top (**2**). Each tentacle mini held an Atlas Scientific ezo pH (**3**) and Atlas Scientific ezo RTD temperature (**4**) embedded chips. This gave a total of two pH and two temperature probes. pH probes used were the HI-1413B surface probe bought from Hanna instruments. The temperature probes used were the PT-1000 Temperature probes purchased from Atlas Scientific.

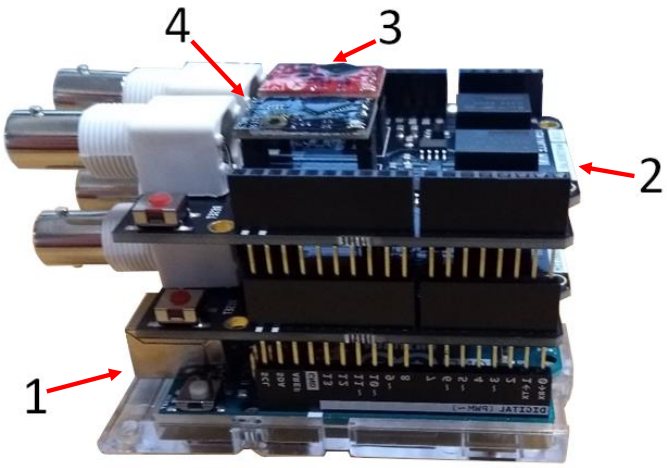

*Fig S1. pH meter composed of an Arduino uno **(1)**, two whiteboxlabs tentacle minis **(2)**, two Atlas Scientific ezo pH embedded chips (**3**) and two Atlas Scientific ezo RTD temperature embedded chips (**4**).*

The chipsets were set to I2C communication mode and an Arduino script was written using the Arduino IDE to read the pH and temperature at each position. The effect of temperature on the pH was compensated for using the equation:

$$pH_{corrected} = 7.0 + (pH - 7.0) \times \frac{T}{T_0} \quad (1)$$

Where pH is the pH read by the probe, T is the temperature in Kelvin and T_0 is standard temperature 298.15 K. The temperature and corrected pH values were printed to the serial port. This allowed for the meter using a computer script, enabling live data monitoring and logging.

1.2. fReactor Probe Holder

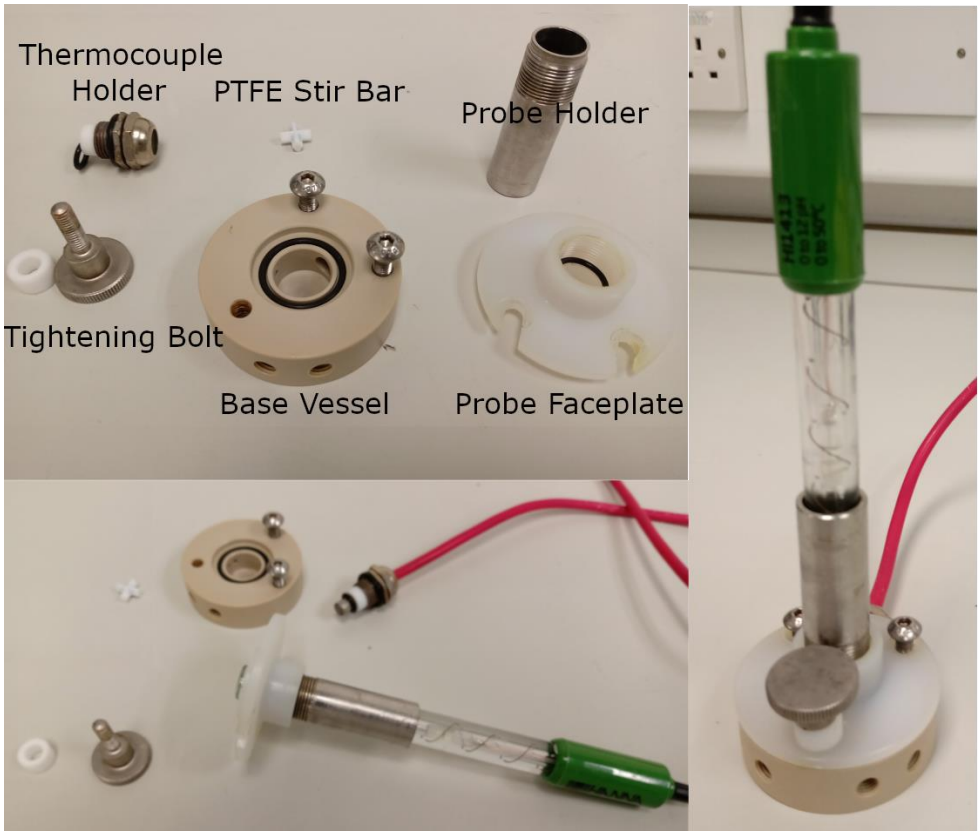

Fig S2. Parts and assembly of the pH probe and thermocouple fReactor. Upper Left: The parts used for the housing. Lower Left: The pH probe and thermocouple fitted into their housings along with the rest of the components for assembly. Right: Assembled inline pH and temperature sensor.

To incorporate the pH and temperature probe into the flow system a fReactor was altered with a new port added at the back to house the thermocouple using the fitting that came with the thermocouple from Atlas Scientific. The usual glass face was exchanged for a probe holder faceplate to hold the pH probe and create a seal. This consisted of a PTFE faceplate covering with an o-ring. This allowed the probe to sit in the fReactor and was tightened into place with the probe holder screws.

While running this allowed for continuous inline monitoring of both the temperature and pH at various points in the reactor. A set of sample points of the two pH positions can be seen in Fig S3.

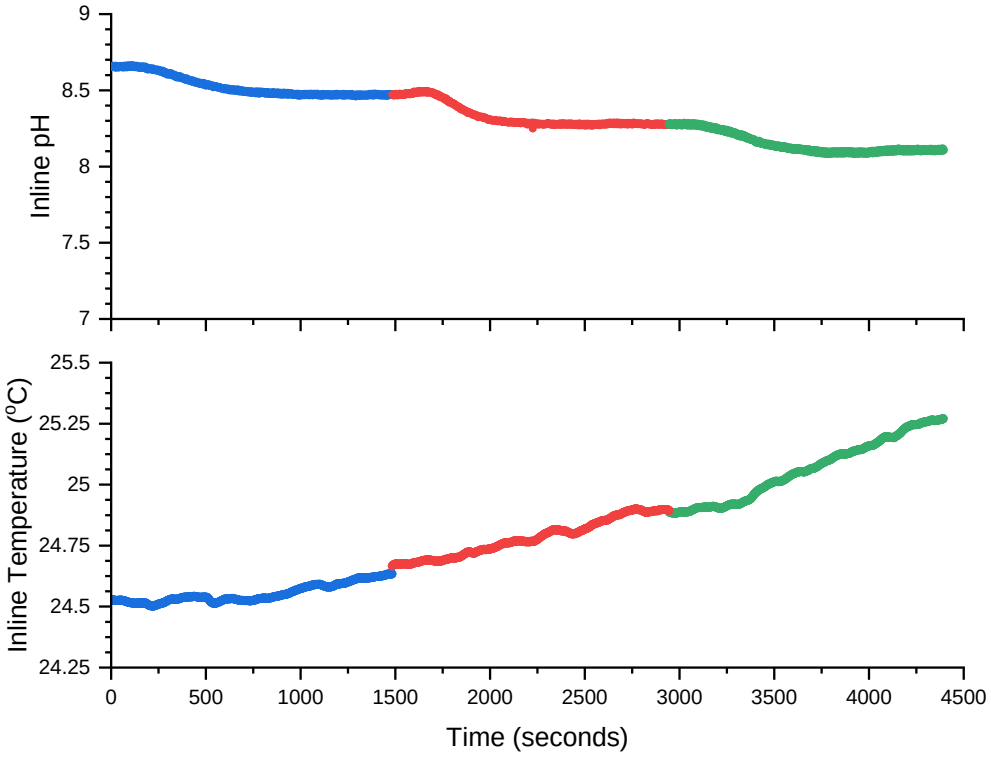

Fig S3. Inline recording across three successive experiments (each colour is a separate experiment). Upper: The pH values observed after the membrane separator. Lower: Temperature recording observed after the membrane separator.

1.3. System Configuration

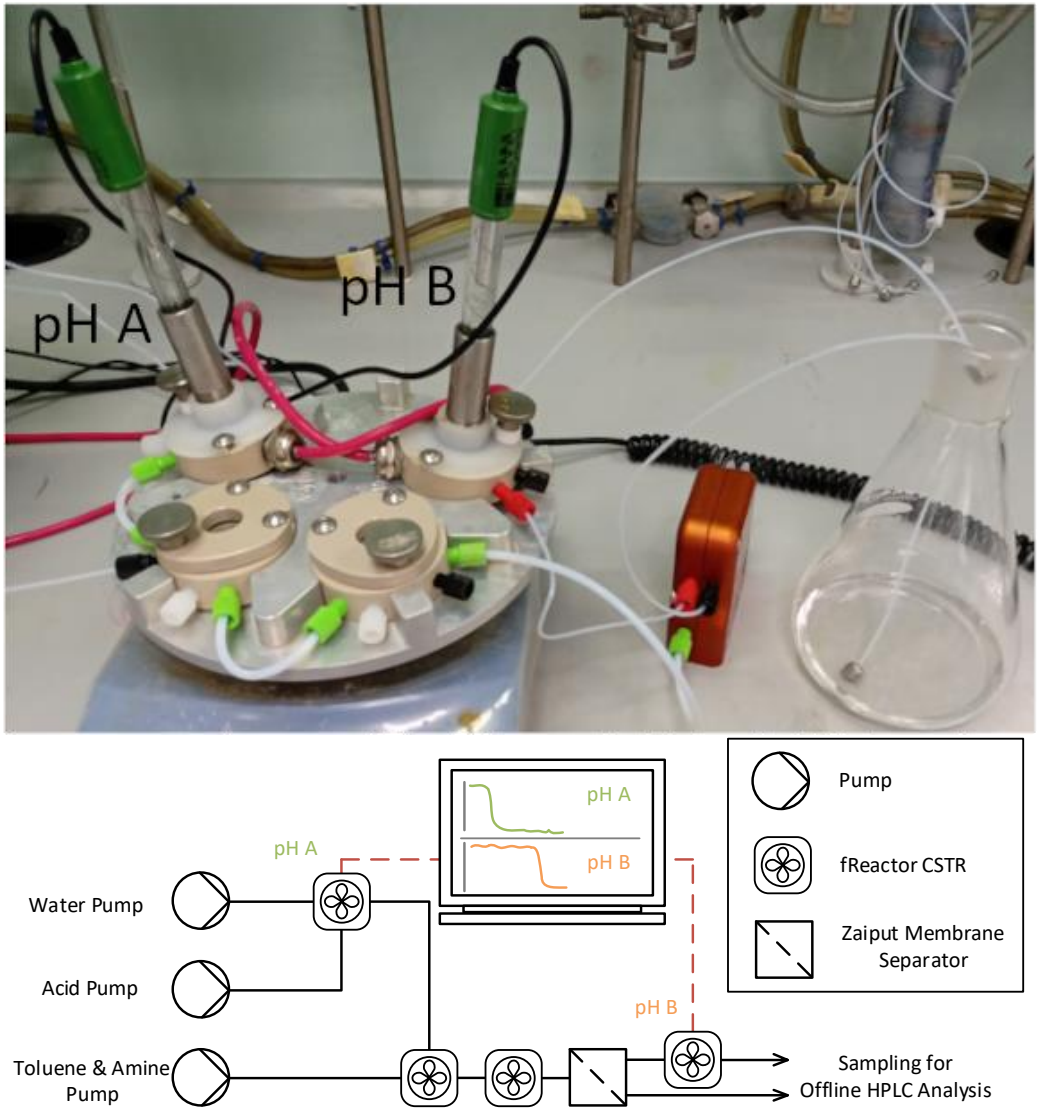

Fig S4. Image of the continuous monitoring reactive extraction system used with the scheme.

The general system across all experiments was composed of:

Table S1. List of components used for the general flow system.

Quantity	Equipment
2	Standard fReactor
2	fReactor with probe holder faceplate.
1	Zaiput Membrane Separator
≈50 cm	1/8” tubing
9	1/4” 28-UNF Nut
13	1/8” ferrule
≈25 cm	1/16” tubing
13	1/16” ferrule
6	1/4” 28-UNF plug nut

The system was setup in the manner detailed in Fig S4. An initial fReactor with a faceplate mount had one port closed with a plug nut. Two ports were left to attach to each aqueous pump and a final was attached to the first standard fReactor. This fReactor again had a single plug nut with one port attached to this aqueous stream and another attached to the stream coming from the organic pump. The final port was the outlet that led to the second standard fReactor.

The second standard fReactor had two ports closed with one open port for the inlet and the other for the outlet stream. This outlet carried the multiphasic mixture to the Zaiput membrane separator. The organic outlet was left for sampling and the aqueous outlet was attached to the second probe holder fReactor. Two ports of this were closed. The outlet was left for sampling.

Three hydrophobic membranes were screened across varying flowrates and volume ratios to determine the loading or breakthrough point. This was done through visual monitoring, but also the residual pressure difference after the separator was monitored with a manometer attached between each outlet. All the membranes functioned well under the initial working conditions (total flowrate = 2 mL/min; Volume Ratio = 1). The largest pore size (2 µm) membrane performed worst at extreme flowrates and volume ratios, but little difference was observed with the 0.9 µm and 0.45 µm membranes. The optimal membrane was determined to be a 0.9 µm hydrophobic membrane (purchased from Zaiput Flow Technologies under the name OB-900-S10).

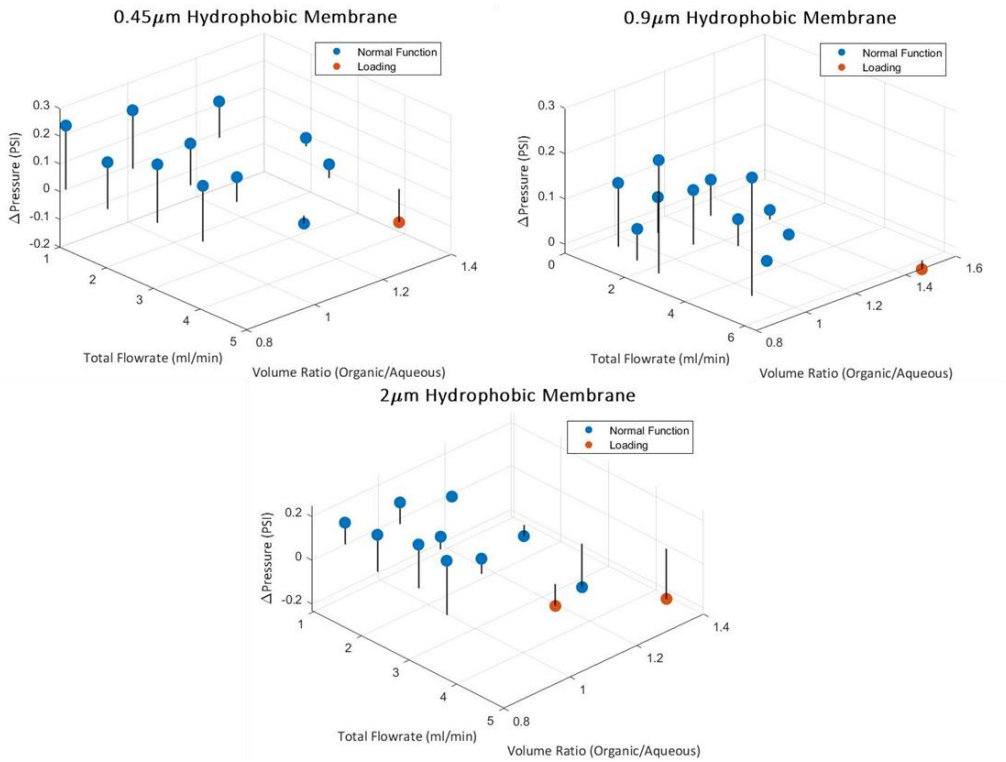

Fig S5. Screening of toluene and water across varying flowrates and volume ratios with a 0.45 µm (Upper Left), 0.9 µm (Upper Right) and 2 µm (Bottom) hydrophobic membranes.

A residence time distribution (Fig S6) was carried out by pulse injecting hydrochloric acid (0.05 mL; 4 M) and allowing it to move through the multiphasic system. Both the aqueous and toluene streams were set to 1 mL/min each. This was monitored at the aqueous outlet by the final pH probe and the pH was converted to proton concentration. 99.9% of all protons were seen to have left after 1500 seconds.

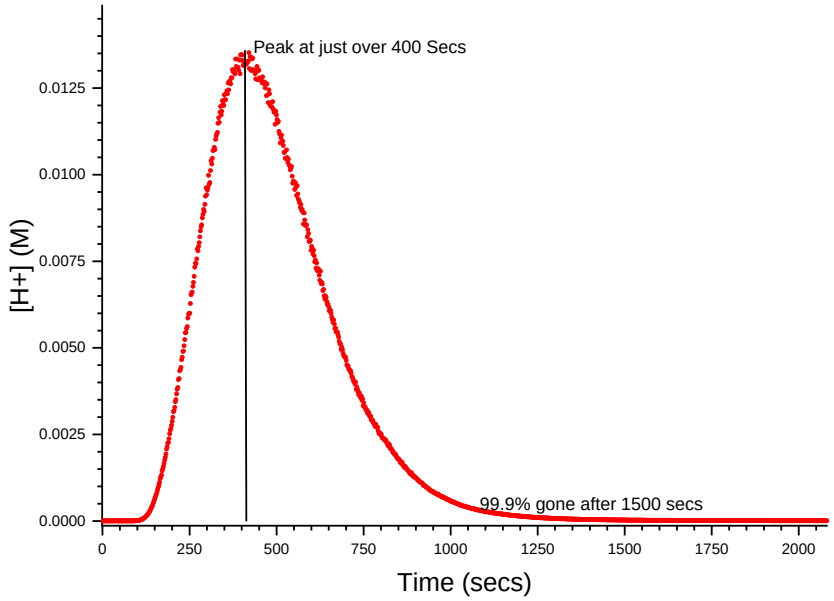

Fig S6. Residence time distribution of the multiphasic system observed by extrapolation of the pH after separation.

1.4. Additional Off-line Considerations

For the off-line experiments Harvard pump 1 syringe pumps along with 50 mL SGE gas-tight syringes were used. 32-gauge luer lock needles were cut with a pipe cutter and fitted with a Swagelock 1/16" nut and ferrule. This allowed connection to 1/16" PTFE tubing with a Swagelock union ferrule and nut. The other side of this tubing was attached to the fReactor by a 1/4" 28-UNF nut and ferrule.

1.5. Additional Considerations for On-line

When looking to include communicable pumps along with on-line HPLC a few changes had to be made. A Matlab interface previously used within the group already possessed the ability to communicate with pumps and take samples for HPLC analysis. To make the equipment fully functional for these extractions, a set of drivers were written to communicate with the pH meter. This in turn allowed it to be read and logged to file along with the pump flowrates.

To allow for extended periods of running uninterrupted, the Harvard pumps discussed in ESI section 1.4 were exchanged for a Jasco PU-980 HPLC pump to feed the toluene and amine mixture to the reactors and two Syrdos syringe pumps to feed the concentrated and dilute acid streams. The pumps were connected to the fReactors using 1/4" 28-UNF nuts as mentioned previously.

The outlet was of each stream was connected to a VICI Valco EUDA-CI4W sample loop (4-port) with 0.06 µL rotor volume. Minor fluctuations in sample loop volume between the two loops caused some issues with loading for the Zaiput membrane separator. This was resolved by including Idex micro-splitter valves (P-470) before the sampling valves to allow for fine adjustments for this increase in pressure difference.

2. Procedures

2.1. General Considerations

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich, Fisher Scientific or Fluorochem Ltd., and were used without further purification. All solvents were of HPLC grade and water used was 18.2 mΩ deionised water. For HPLC analysis an Agilent 1100 series HPLC was used comprising of a degasser (G1379S), quaternary pump (G1311A), column compartment (G1316A) and Diode array detector (DAD) (G1315A). Sampling was carried out for off-line samples using an Agilent 1100 series autosampler (G1313A) and for the on-line sampling, two VICI Valco EUDA-CI4W sample loop (4-port) with a 60 µL rotor volume were integrated into the HPLC flowpath where an autosampling unit would be.

2.2. Off-line Experimental Procedure

Reservoir solutions were prepared by mixing the desired reagents with solvent under ambient conditions. Organic reservoir solution was prepared with α-methyl-benzylamine (100 g; 0.825 mol; 0.825 M), and *N*-benzyl-α-methyl-benzylamine (10 g; 0.047 mol; 0.047 M) and made to 1000 mL with toluene. The water reservoir was prepared with deionised water alone and the acid reservoir was prepared with hydrochloric acid (37% w/w; 42 mL; 1.0 M) and made to 500 mL with deionised water.

The system was setup as mentioned in sections 1.3 and 1.4. A 50 mL SGE gastight Luer lock syringe was primed with each reservoir solution and was attached to the corresponding fReactor inlet. The organic phase syringe was kept constant at 1 mL/min and the aqueous phase syringes were varied depending on the acid concentration required in Table S4 to sum to 1 mL/min. The hotplate was set at 1200 rpm. Samples and pH readings were taken after steady state was reached after 1500 seconds, while also ensure the pH after extraction had reaches a constant.

2.3. On-line Experimental Procedure

Reservoir solutions were prepared by mixing the desired reagents with solvent under ambient conditions. Organic reservoir solution was prepared with α-methyl-benzylamine (100 g; 0.825 mol; 0.825 M), *N*-benzyl-α-methyl-benzylamine (10 g; 0.047 mol; 0.047 M), and biphenyl (3 g; 0.019 mol; 0.019 M) and made to 1000 mL with toluene. The dilute acid reservoir was prepared with hydrochloric acid (37% w/w; 21 mL; 0.5 M), and benzamindine hydrochloride (2 g; 0.013 mol; 0.026 M) and made to 500 mL with deionised water. The concentrated acid reservoir was prepared with hydrochloric acid (37% w/w; 42 mL; 1.0 M), and benzamindine hydrochloride (2 g; 0.013 mol; 0.026 M) and made to 500 mL with deionised water.

The system was setup as mentioned in ESI sections 1.3 and 1.5. The pumps were primed with their corresponding solutions and flowrates were set for the experiment as calculated from the acid concentration and volume ratio on Table S5 in section 4.2 to sum to 2 mL/min. The hotplate was set at 1200 rpm. Once steady state was reached the organic sample loop was triggered. After 5 minutes the aqueous sample loop was triggered.

3. HPLC Analysis

3.1. Off-line HPLC Analysis

To analyse the organic and aqueous phase a reverse phase HPLC method was used. The method for this is detailed in Table S2.

Table S2. HPLC method used for off-line experiment analysis.

Column	Agilent Eclipse Plus C18 25 cm x 4.6 mm, 5 µm
Solvent A	0.1% TFA in Deionised Water.
Solvent B	0.1% TFA in Acetonitrile.
Flowrate (mL/min)	1.0
Column Temperature (°C)	40
Wavelengths used (nm)	210; 260

Flow Gradient	
Time (min)	0.1% TFA in Acetonitrile (%)
0.0	5.0
10.0	5.0
12.5	80.0
13.0	80.0
13.1	5.0
16.5	5.0

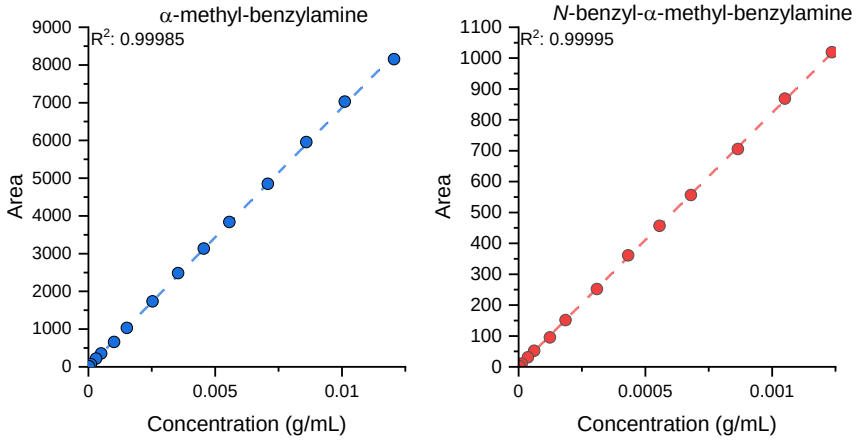

Fig S7. Calibration for α-methyl-benzylamine and N-benzyl-α-methyl-benzylamine.

3.2. On-line HPLC Analysis

The HPLC method for on-line analysis was designed with the two sampling loops in mind. Initially the organic sample loop would trigger and the HPLC run would begin. After 5 minutes the aqueous sample loop would trigger. The method was developed to repeat a 5-minute gradient to allow for this second injection to also appear on the same chromatogram.

Table S3. HPLC method used for on-line experiment analysis.

Column	Ascentis Express C18 5 cm x 4.6 mm, 2.7 µm
Solvent A	0.1% TFA in Deionised Water.
Solvent B	0.1% TFA in Acetonitrile.
Flowrate (ml/min)	1.5

Column Temperature (°C)	20
Wavelengths used (nm)	210; 260

Flow Gradient	
Time (min)	0.1% TFA in Acetonitrile (%)
0.0	2.0
0.7	2.0
4.0	95.0
4.1	2.0
5.7	2.0
9.1	95.0
9.2	2.0
10.5	2.0

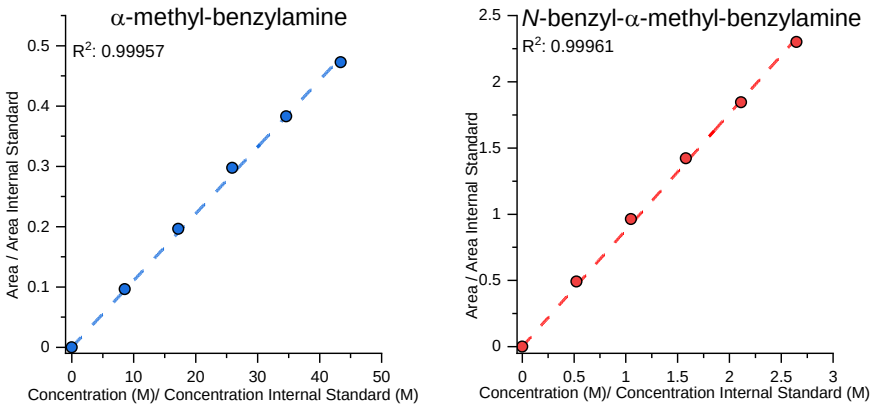

Fig S8. Organic phase calibration for α-methyl-benzylamine and N-benzyl-α-methyl-benzylamine.

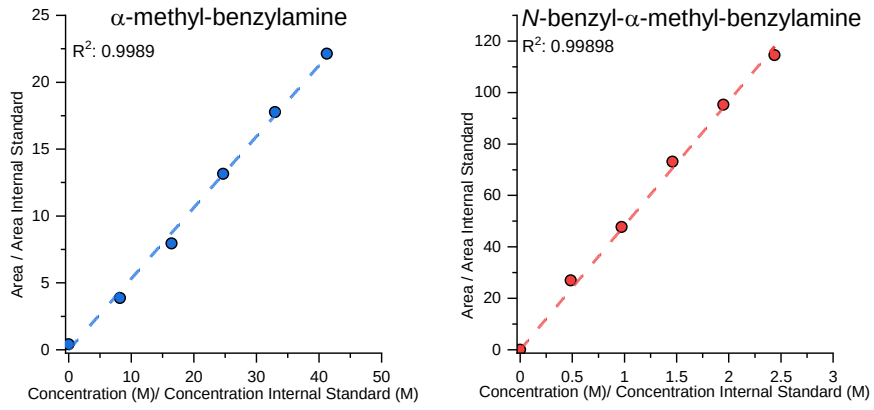

Fig S9. Aqueous phase calibration for α-methyl-benzylamine and N-benzyl-α-methyl-benzylamine.

4. Reactive Extraction Results

4.1. Off-line Data

Table S4. Results of the off-line extraction experiments.

Expected Acid Concentration (M)	Observed Acid Concentration (M)	pH After Extraction	ΔExtraction Efficiency	Separation Factor
0.000	0.000	10.972	0.080	14.696
0.084	0.107	9.154	0.219	46.930
0.168	0.192	8.630	0.412	115.975
0.252	0.277	8.334	0.556	218.938
0.336	0.341	8.413	0.496	169.431
0.420	0.513	8.197	0.626	234.057
0.504	0.555	8.003	0.681	380.181
0.588	0.614	7.785	0.781	277.987
0.672	0.719	7.568	0.848	1989.927
0.756	0.798	7.229	0.917	1596.596
0.840	0.872	7.289	0.912	2205.896
0.000	0.000	11.142	0.082	14.919
0.140	0.148	9.052	0.248	58.474
0.280	0.264	8.628	0.447	148.994
0.560	0.546	7.689	0.848	902.927
0.700	0.715	4.429	0.263	20.049
0.840	0.826	0.635	0.004	1.152
0.280	0.447	8.573	0.450	135.964
0.420	0.543	8.204	0.636	296.592
0.700	0.750	5.213	0.354	28.227
0.280	0.228	8.489	0.528	182.995
0.350	0.307	8.173	0.712	416.586
0.280	0.247	8.656	0.454	137.515
0.350	0.311	8.471	0.513	176.115
0.420	0.361	8.276	0.587	247.074
0.490	0.447	8.110	0.685	367.704
0.560	0.512	7.947	0.760	1246.017
0.630	0.595	7.682	0.827	1042.956
0.700	0.644	7.294	0.919	2632.115
0.700	0.649	7.288	0.921	3118.344
0.700	0.648	7.287	0.917	1972.825
0.770	0.799	5.551	0.799	3884.061
0.770	0.817	5.562	0.808	3942.295
0.770	0.839	5.566	0.817	4310.504
0.840	0.877	0.283	0.057	38.971
0.840	0.869	0.269	0.060	37.140
0.84	0.867	0.26675	0.05316	51.055

To validate that the initial pH was accurate at determining the acid concentration it was compared to the concentration calculated from the inlet flowrates. This was compared with the calculated acid

concentration from the probe giving a strong linear correlation with an R^2 of 0.992 and a slope of 0.975 +/- 0.014.

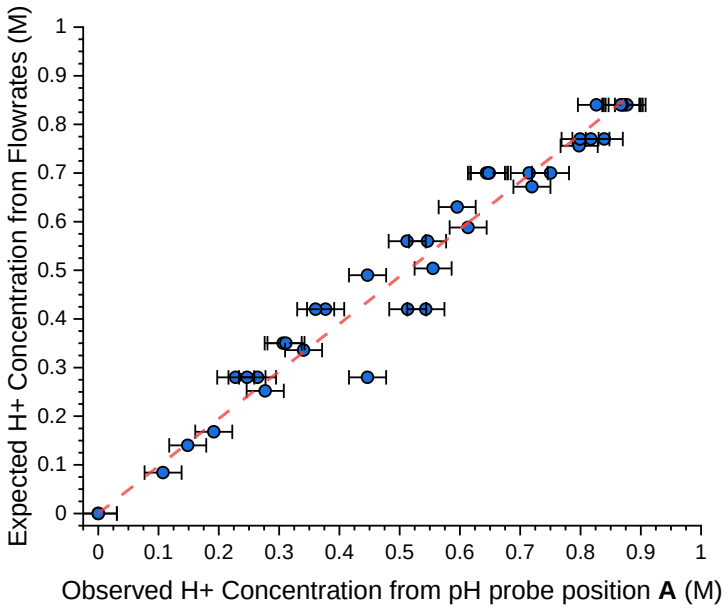

Fig S10. Expected acid concentration vs the observed for the off-line experiments.

A decrease in point-to-point variance within the system at the optimal extraction values was found when using the ΔExtraction Efficiency over the more frequently used separation factor or logarithmic expression of this. The region of ΔExtraction Efficiency greater than 0.7 yielded a near exponential increase in the separation factor, but also significant variance. The 5 points clustered around pH 7.3 have a mean ΔExtraction Efficiency of 0.917 and standard deviation of 0.0036 (0.36% of the mean value), whereas the separation factor has a mean of 2305.156 and standard deviation of 589.604 (25.6% of the mean value). In addition, the $\log_{10}$(Separation Factor) highlighted very similar trend in response to the ΔExtraction Efficiency so overall gives quite comparable results.

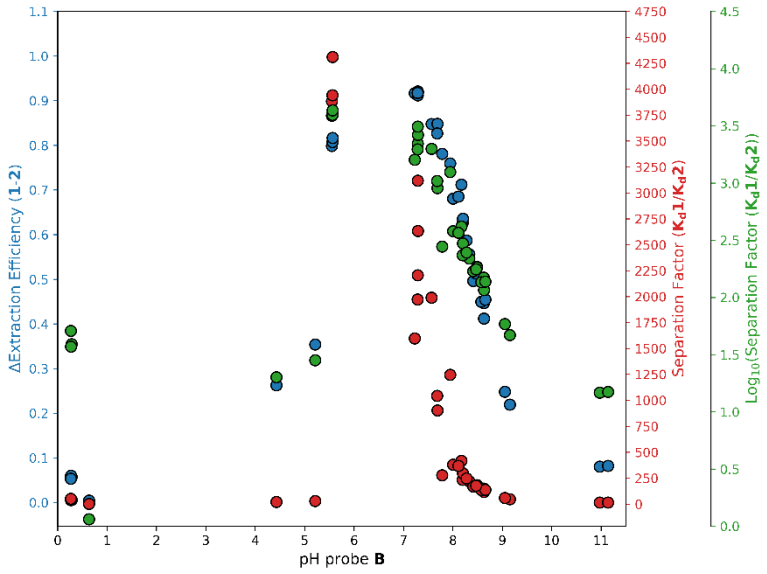

Fig S11. Comparison of data analysis methods between ΔExtraction Efficiency, Separation factor and Log_{10}(Separation Factor).

4.2. DOE Design

To explore the wider space a DOE was generated using Modde (Version 1.2)[5]. Additional points to cover the previously observed optimal were selected and incorporated. This was done to highlight the steepness of this type of surface and better display the responses from the extraction in terms of phase transfer and pH. This optimal space was calculated from the initial optimal observed in batch of 0.8 M at a volume ratio (organic: aqueous) of 1. The optimal was extrapolated by incorporating the volume ratio as increases in volume ratio would increase the amount of amine in the organic inlet relative to the volume of the aqueous phase that could extract it.

$$\Delta EE_{opt\ @\ Vr} = \Delta EE_{opt}\ x\ Vr \quad (2)$$

The Vr is the volume ratio and ΔEE_{opt} is the approximate optimal of 0.8 M observed during the linear off-line screening. This led to a series of 14 experiments covering the space displayed in Fig S12.

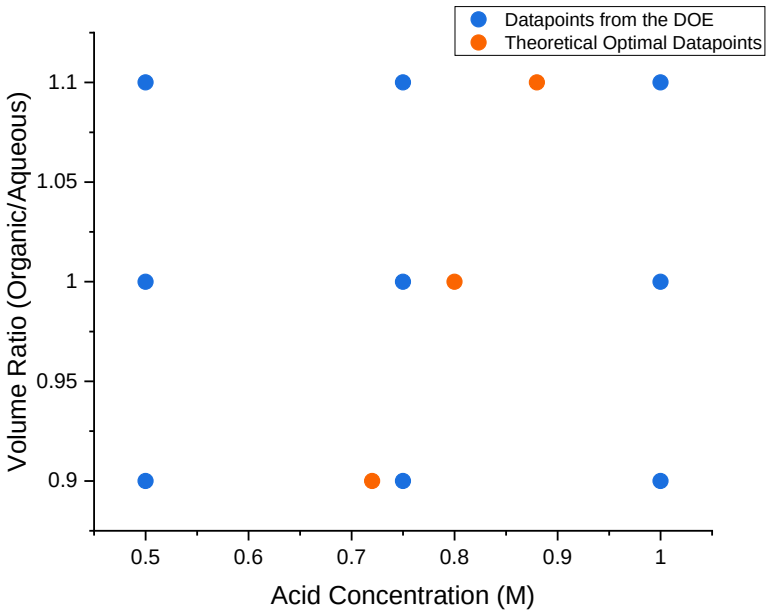

Fig S12. Experimental datapoints from the DOE and theorised optimal from initial experiments used in the on-line space exploration DOE.

Table S5. Experimental Results observed from the on-line DOE experiments.

Observed Acid Concentration (M)	Volume Ratio (Organic/Aqueous)	pH after extraction	Δ Extraction Efficiency	Separation Factor
1.00	0.9	0.708	0.184	2.254
0.87	1	7.016	0.851	3449.818
1.01	1	0.809	0.018	1.141
0.56	0.9	8.422	0.422	10.858
1.00	1.1	1.257	0.290	6.227
0.90	1.1	6.801	0.745	47.648
0.53	1	8.346	0.374	10.606
0.81	1.1	7.914	0.675	24.857
0.76	0.9	6.443	0.568	37.087
0.80	0.9	4.487	0.718	5.0378

0.55	1.1	8.512	0.302	7.414
0.79	1	7.597	0.574	34.443

The observed acid concentration was compared to the expected from the pumps. This was carried out by initially titrating both the acid feeds. The concentrated acid feed was determined to be 1.012 M ± 0.001 M and the dilute acid was determined to be 0.549 M ± 0.002 M. The pump ratios were calculated to give the true expected acid concentration, and this was compared to the observed from the initial pH. All data gave an accuracy of between 95 to 105%.

Table S6. Table of accuracy of the acid concentration for the on-line DOE experiments.

Acid Concentration Expected (M)	Acid Concentration Observed (M)	Accuracy (%)
1.01	1.00	101.2
0.82	0.84	97.8
1.01	1.00	101.4
0.54	0.56	96.3
1.01	1.00	100.9
0.90	0.88	102.4
0.78	0.78	99.5
0.78	0.77	100.6
0.54	0.53	100.9
0.78	0.81	95.0
0.78	0.78	99.0
0.75	0.76	99.0
0.78	0.80	97.4
0.54	0.55	98.9

The replicate centre points for this correlated well across all analysis. The root mean squared errors of the observed acid concentration, pH after extraction and the Δ Extraction efficiency were 0.011 M, 0.013, and 0.041 respectively.

The final data can be seen in Fig S13 which illustrates the combinational effect volume ratio and acid concentration have on the Δ Extraction efficiency and Fig S14 that highlights the negligible impact volume ratio has when looking at the extraction in terms of the final pH.

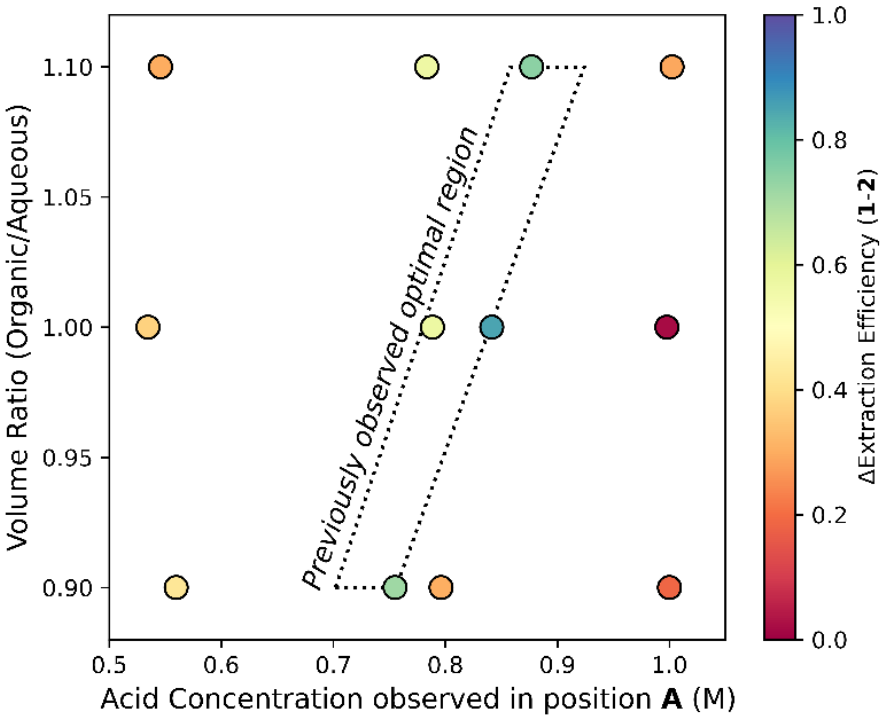

Fig S13. DOE data comparing the observed inlet acid concentration and volume ratio with the optimal extraction(colour).

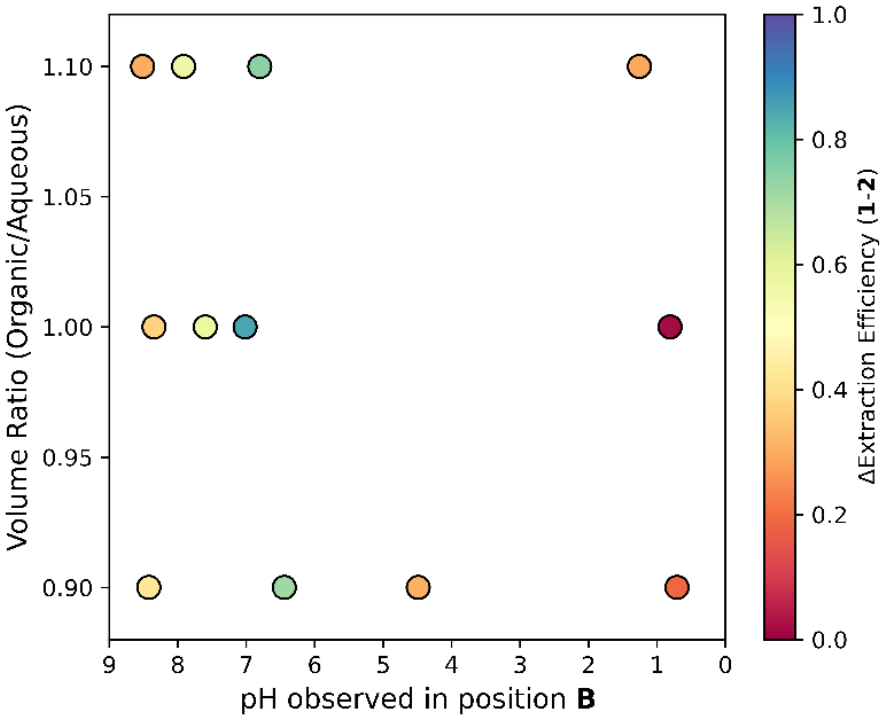

Fig S14. DOE data comparing the outlet pH and volume ratio with the optimal extraction (colour).

5. References

1. Atlas Scientific, https://atlas-scientific.com/, (accessed 14 December 2020).
2. Whitebox Labs, https://www.whiteboxes.ch/, (accessed 14 December 2020).
3. Arduino, https://www.arduino.cc/, (accessed 14 December 2020).
4. Hanna Instruments, https://www.hannainstruments.co.uk/, (accessed 14 December 2020).
5. Sartorius, Modde, https://www.sartorius.com/en/products/process-analytical-technology/data-analytics-software/doe-software/modde, (accessed 14 December 2020).