Research Article

Continuous flow reaction monitoring using an on-line miniature mass spectrometer

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RATIONALE: A recently developed miniature electrospray ionisation mass spectrometer has been coupled to a preparative flow chemistry system in order to monitor reactive intermediates and competing reaction paths, screen starting materials, and optimise reaction conditions. Although ideally suited to the application, mass spectrometers have rarely been used in this way, as traditional instruments are too bulky to be conveniently coupled to flow chemistry platforms.

METHODS: A six-port switching valve fitted with a 5 μL loop was used to periodically sample the flow stream leaving the reactor coil. Mass spectra corresponding to the sample loop contents were observed approximately 10 s after activating the valve. High fluidic pressure was maintained throughout to ensure that gaseous products remained in solution. As an illustrative example of how this apparatus can be employed, the generation of benzyne and its subsequent reaction with furan were investigated. Benzyne was prepared via diazotisation of anthranilic acid using *tert*-butyl nitrite.

RESULTS: Unexpectedly, the explosive diazotised intermediate was detected by the mass spectrometer at low coil temperatures or short residence times. The optimum reactor temperature and residence time for production of the desired Diels-Alder product are 50 °C and 3–5 min, respectively. There are competing reaction pathways leading to the formation of acridone and several other by-products.

CONCLUSIONS: On-line mass spectrometry allowed the flow conditions to be quickly tuned for safe operation and optimal generation of the desired product. The validity of this approach was corroborated by off-line liquid chromatography/mass spectrometry (LC/MS) analysis of flow samples. Copyright © 2012 John Wiley & Sons, Ltd.

When compounds are synthesised using conventional laboratory batch processing techniques, progress can be impeded by low reaction rates, poor reproducibility, and the considerable manual effort required to monitor reactions and isolate the product at each stage of the synthesis process. Flow chemistry is a growing technology[1–7] that can improve productivity in synthetic chemistry laboratories. Reactions are precisely and reproducibly controlled in flow systems, as the flow stream is uniformly mixed and heated or cooled. By employing back-pressure regulators, a high fluidic pressure can be achieved. Consequently, if desired, it is possible to increase the reaction rate (compared with an open batch reaction) by heating the flow stream to temperatures well above the atmospheric pressure boiling point of the solvent. The need for manual processing is minimised, as a series of reactions and purification steps can be arranged to occur in a continuous, sequential manner.[8] Using appropriate analytical techniques, it is possible to quickly optimise flow parameters and screen combinations of substrates and reagents.[9]

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Although still considered to be in its infancy, the field of flow chemistry has progressed to the extent that total syntheses of natural products have been demonstrated.[10–16]

Flow synthesis involves the serial manipulation of reactant streams using modular components such as mixers, temperature-controlled reactors, and packed columns. Peristaltic or piston pumps are generally used to maintain and meter the flow in meso-scale systems, whereas syringe pumps are used for micro-scale syntheses. Reactions are often initiated when two reactant streams are combined at a tee-piece connector, or by the use of a static mixer. The reaction proceeds as the resulting mixture flows through a reactor, which is typically fabricated as a coiled tube or serpentine channel. The reactor is held either in an oven or in contact with a thermal reservoir. In an optimised setup, the residence time, reactor temperature, and reactant stoichiometry are such that the product yield has reached a maximum when the flow exits the reactor. In recent years, the toolbox of flow processes that may be used to effect chemical transformations has become increasingly sophisticated and wide-ranging. For example, an array of immobilised reagents, catch-and-release agents, catalysts, and scavengers has been developed that allows some key reactions to be achieved efficiently and without contamination of the downstream flow.[1,17] Processes that involve reaction with a gas such as hydrogen,[18,19] ozone,[20] carbon monoxide,[21,22] carbon dioxide,[23] oxygen,[24] or ammonia[25]

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can also be undertaken in a safe and convenient manner. Numerous other advances in this growing field have been recently reported, including devices to aid mixing,[26,27] the processing of slurries,[28] and continuous processes at cryogenic temperatures.[29]

When optimising a flow synthesis, it is common practice to collect samples corresponding to a range of reaction conditions or a selection of reactants, and then submit these to a communal liquid chromatography (LC) or liquid chromatography/mass spectrometry (LC/MS) instrument for off-line analysis. An optimum set of reaction conditions or choice of reactant can then be selected from the matrix of results obtained. It is important that the reaction is quenched at the time of sampling so that the results do not reflect any continuing reactions in the collection vial. The time delay and level of operator intervention associated with analysis in this way do not lead naturally to rapid reaction optimisation, which is the desired principle of flow chemistry.

There has been some progress in the development of in-line and on-line monitoring techniques for the rapid optimisation of preparative flow syntheses. The terms 'in-line' and 'on-line' refer to methods of analysis that do not require manual transfer of samples. All the flow is continuously analysed by an in-line technique, whereas representative aliquots are periodically analysed by a technique that is described as on-line. Systems that automatically sample the flow stream and initiate on-line analysis by LC or LC/MS have been demonstrated.[10,30–32] In one example, the output flow from a continuous multi-step synthesis of grossamide was allowed to flow directly through the sampling stage of an LC/MS instrument.[10] Equipment that additionally dilutes the sample prior to analysis is now commercially available from both Syrris (Syrris Ltd., Royston, UK) and Accendo (Accendo Corporation, Tucson, AZ, USA). These systems use a six-port valve fitted with a sample loop to extract an aliquot from the flow stream.[33,34]

One attraction of modular flow chemistry equipment is that it can be easily assembled or dismantled according to the day-to-day activities of a synthetic chemistry laboratory. The analytical instruments that are most likely to see widespread use in reaction monitoring are those that can be easily integrated into this way of working. A conventional LC/MS system, complete with floor-standing pump and attendant computer, is bulky and not easily moved. Given the scarcity of space in or near fume hoods in many laboratories, the logistical difficulties associated with using such an instrument for reaction monitoring are inevitably often seen as prohibitive.

Other analytical techniques are much more convenient to use and can provide information regarding dynamic processes occurring in a flow system. In-line UV absorption cells have proved useful in detecting the extent of dispersion due to diffusion and chromatographic effects, and for triggering product collection.[35] However, this technique can lack selectivity and the compounds to be analysed must possess a suitable chromophore. A key recent development has been the introduction of IR spectrometers with in-line flow cells.[36–40] It has been shown that the appearance of products in the flow stream, and the extent of reaction as a function of time and temperature, can be followed by monitoring the intensity of bands due to specific vibrations. In one recent study,[40] IR spectroscopy was used to trigger and control the flow rate of a third pump so as to match reagent stoichiometries to the dispersed reaction plug. However, while in-line IR spectroscopy is well suited to the optimisation of established procedures, it is less useful when assessing 'unknown' reactions and flow processes, as it is difficult to uniquely identify a compound from its IR spectrum alone.

There is clearly a need for an additional monitoring technique that is both compact and selective. We have, therefore, investigated the possibility of using a recently developed miniature electrospray ionisation (ESI) mass spectrometer for on-line reaction monitoring.[41] In this work, we demonstrate how rapid interrogation of the flow stream using this instrument can be employed to guide the optimisation of reaction conditions, screen starting materials, and highlight interesting chemical intermediates. The combined apparatus is compact and readily accommodated within a fume hood. No attempt has been made to integrate any form of chromatographic separation at this stage, as this would add to the complexity of the system, decrease the rate of sampling, and potentially lead to the loss of short-lived reactive intermediates. However, it is appreciated that in some cases the ability to quantify the amounts of each component present is important and that incorporation of an LC column would therefore be appropriate as a later stage development.

There are numerous examples of the direct *in-line* coupling of mass spectrometers to flow chemistry reactors. Typically, the application involves screening of library compounds[42] or investigations of reaction mechanisms, intermediates, and kinetics.[43,44] In both cases, the purpose of the flow chemistry system is to produce sufficient material for analysis by the mass spectrometer, which by necessity consumes all the available flow material. As mass spectrometry is a destructive technique, these applications are analytical rather than preparative. There has been considerable interest in the in-line coupling of microchip reactors to mass spectrometers.[45–51] In some examples, a nanospray emitter has been fabricated as part of the microchip whereas, in others, a direct fluidic coupling has been made between the microchip and a standard ESI source.

As an illustrative example of how *on-line* analysis using a miniature mass spectrometer can be applied to preparative flow synthesis, we have investigated the generation of benzyne via diazotisation of anthranilic acid, and its subsequent cycloaddition to furan. This reaction was specifically chosen as it is complex and would lead to both reactive intermediates and gaseous by-products. As indicated in Scheme 1, treatment of anthranilic acid (**1**) with *tert*-butyl nitrite yields the zwitterion, benzenediazonium-2-carboxylate (**2**), which readily loses N_2 and CO_2 to give benzyne (**3**). This highly reactive intermediate subsequently undergoes Diels-Alder cycloaddition with furan, yielding the desired product, 1,4-endoxide-1,4-dihydronaphthalene (**4**).

This traditional method of producing benzyne is no longer popular as a batch technique as benzenediazonium-2-carboxylate presents a risk of explosion if produced in significant quantities.[52] In crystalline form it detonates violently if heated or mistreated.[53] Many other less hazardous benzyne precursors have since been reported. Currently, *o*-trimethylsilylphenyl triflate[54,55] and related reagents such as 2-(trimethylsilyl)iodobenzene[56] are preferred, owing to the mild conditions needed to unmask benzyne. Unfortunately, a multi-step synthesis of these precursors is often

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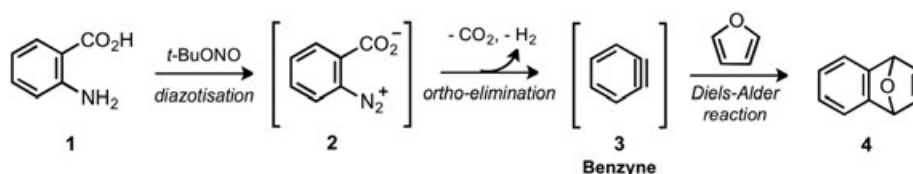
Scheme 1. Reactions leading to the formation of 1,4-endoxide-1,4-dihydronaphthalene (**4**).

required prior to use, as only *o*-trimethylsilylphenyl triflate and a few of its derivatives are currently commercially available. The motivation for revisiting the traditional approach using flow chemistry methods is twofold. First, the safety hazard is significantly reduced if benzenediazonium-2-carboxylate is both generated and promptly consumed within the flow reactor. Secondly, in contrast to the modern precursors described above, there are numerous commercially available anthranilic acid derivatives that might yield synthetically useful substituted benzyne derivatives. Figure 1 indicates how the synthesis of **4** has been undertaken using flow techniques.

EXPERIMENTAL

A 3500 MiD miniature mass spectrometer (Microsaic Systems plc, Woking, UK) was coupled to a FlowSyn continuous flow chemistry system (Uniqsis Ltd., Shepreth, UK), as shown in Fig. 2. Other commercial flow chemistry platforms, for example, those supplied by Vapourtec Ltd. (Bury St Edmunds, UK), Syrris Ltd. and Accendo Corporation, as well as bespoke systems (assembled from off-the-shelf pumps, valves, tubing, and connectors), should also be suitable for this application.

A solution of furan and *tert*-butyl nitrite in acetonitrile (both 0.24 M) and a second solution of anthranilic acid in acetonitrile (0.20 M) were separately pumped by high-pressure pumps (A and B) to a tee piece mixer (C), where they were combined. The flow stream then passed through a reactor coil (D) that was in close contact with a heater block. The FlowSyn system incorporates two six-port switching valves, which are intended for loop injections of reactants into a solvent stream prior to mixing. However, as this facility was not required for the purposes of the present experiment, one of the valves (E) was instead configured such that a sample of the solution leaving the flow reactor could be periodically passed to the mass spectrometer (F). An advantage of

intermittent rather than continuous sampling is that the rate at which the vacuum interface of the mass spectrometer becomes contaminated with involatile deposits is reduced. With the switching valve in the load position, the flow from the reactor passed through a 5 μL loop (G) fitted across two of the valve ports and thereafter into a collection vessel (H). For reasons discussed below, it is important that the 500 psi (34 bar) back-pressure regulator (I) was downstream of the sampling valve.

During the early stages of this investigation, it became apparent from the mass spectral data that benzenediazonium-2-carboxylate was being discharged when the coil temperature was lower than 50 °C. The flow system had previously been operated for some time at a coil temperature of 25 °C in the belief that all the benzenediazonium-2-carboxylate generated was being consumed. As this was clearly not the case, sodium thiosulphate was added to the collection vessel to act as a reducing agent. *Caution* – to avoid the risk of a serious explosion, *it is imperative that any benzenediazonium-2-carboxylate in the reactor effluent does not accumulate in the collection vessel.* One of the often-cited advantages of performing reactions in flow rather than batch mode is that hazardous intermediates are produced in small volumes within the reactor and consumed before the reaction products are collected. As the present example demonstrates, this advantage can only be realised if the system is properly optimised. It will be demonstrated below that on-line mass spectrometry is a convenient method of detecting problems relating to hazardous intermediates at an early stage.

The method used to prepare the sample for analysis by the mass spectrometer is loosely based on the arrangement developed by Dell'Orco and co-workers to monitor batch reactions.[57] This group recognised that the solvents and high molar concentrations typically used in preparative synthetic chemistry are not compatible with analysis by ESI-MS. Their apparatus employed a total of four pumps to withdraw solution from the reaction vessel, add methanol to quench the reaction and reduce the concentration, pump a portion
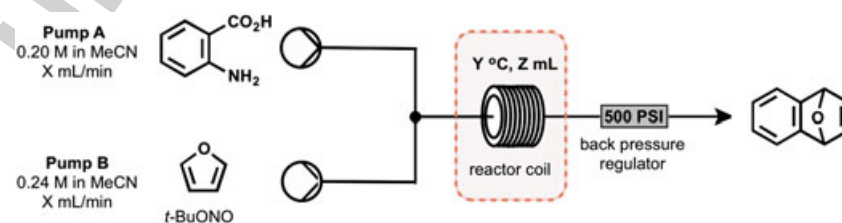
Figure 1. Details of how 1,4-endoxide-1,4-dihydronaphthalene (**4**) has been synthesised using a flow system. A solution of furan and *tert*-butyl nitrite in acetonitrile and a second solution of anthranilic acid in acetonitrile were separately pumped to a static mixer. The product formed as the combined solution passed through a reactor coil.
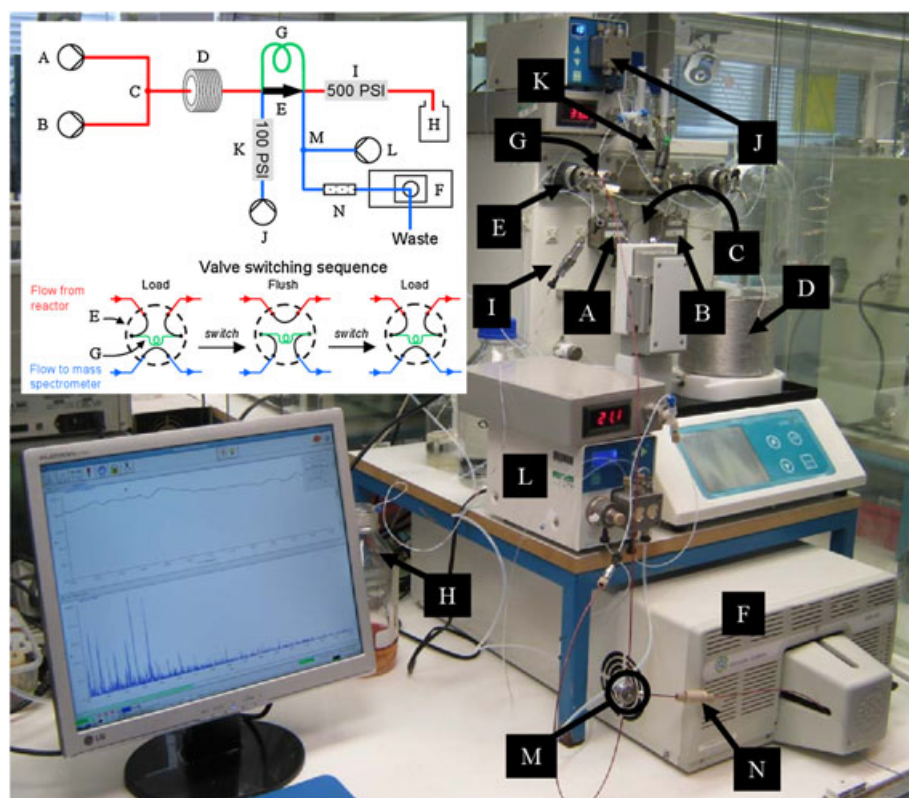
Figure 2. Microsaic 3500 MiD miniature mass spectrometer coupled to a Uniqsis FlowSyn flow chemistry system. The reactant solutions were pumped by high-pressure pumps (A and B) to a mixing tee (C, obscured in photo). The flow stream then flowed through the reactor coil (D) and sampling loop (G), which was fitted across two ports of the six-port valve (E). After passing through a back-pressure regulator (I), the solution was discharged into a collection vessel (H). When the six-port valve was switched to the inject position, a third high-pressure pump (J), which was stabilised by back-pressure regulator (K), pumped acetonitrile through the loop to flush the contents into mixing tee (M). A 50:50 (v/v) mixture of acetonitrile and water with 0.1% formic acid was pumped into the mixing tee by a fourth high-pressure pump (L) in order to further dilute the sample and modify the solution to aid the ESI process. An in-line filter (N) removed particulates before analysis of the sample by the mass spectrometer (F).

through a split, and finally add water and HCl to the solution to aid the ESI process. As the mass spectrometer and the fume hood housing the reaction vessel were in separate rooms, the delay between sampling and analysis was 4–6 min.

A simplified and more compact approach is shown in Fig. 2. With the six-port valve in the inject position, the sample was flushed from the loop and into a static stainless steel mixer tee (M) with acetonitrile supplied by a high-pressure Knauer pump (Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH, Berlin, Germany) operating at 0.1 mL/min (J). There was some tendency for this pump to exhibit an oscillatory instability at low flow rates. It was found that a 100 psi (7 bar) back-pressure regulator (K) inserted immediately downstream of the pump helped in this regard. Some dilution is expected as a result of axial dispersion during transit of the sample between the six-port valve and the mixing tee. A second high-pressure Knauer pump (L) operating at 0.9 mL/min provided a flow of solvent to the mixer tee that further diluted the sample and modified the solution to aid ESI. A 50:50 (v/v) mixture of acetonitrile and water with 0.1% formic acid was found to be suitable for this purpose. Initial investigations

using tubing of 760 μm i.d. suffered from long delays between valve activation and sample detection by the mass spectrometer. Consequently, in all subsequent work, a 1/16" PEEK capillary of 127 μm i.d. was used for the fluidic connections between the six-port valve and the mixer tee, and between the mixer tee and the mass spectrometer. At the mass spectrometer, a static split incorporated into the microspray assembly allowed a flow of approximately 0.5 μL/min to pass to the emitter. The remainder of the flow passed through the waste leg of the split.

There are some limitations to the universal applicability of the setup described above. The most significant of these is that precipitates, slurries and suspensions cannot be passed to the mass spectrometer. The microspray ion source does not tolerate particulates well and is consequently protected by an in-line filter (N), which quickly becomes congested if presented with significant levels of particulate contamination. Even if all components remain in solution while in the reactor, subsequent mixing, first with the cold flush solvent and then with the dilution solvent, might result in the formation of a precipitate (particularly if the latter has a high water content).

It is likely that, in most cases, flush and dilution solvents can be found that are compatible with both the ESI process and the requirement that all components remain in solution.

The miniature mass spectrometer is a recently developed instrument that was originally conceived as a detector for use with high-performance liquid chromatography (HPLC) systems.[41] The mass range is m/z 80–800, which may be scanned at a maximum rate of 1000 m/z units/s when the interval between points is 0.2 m/z units. Unit resolution is achieved over the full m/z range and peak positions are correct to within ±0.3 m/z units. Loop injections of reserpine at a flow rate of 1 mL/min have shown that the limit of detection (3:1 signal-to-noise (S/N) ratio, RMS definition) is 8 pg in selected ion monitoring (SIM) mode.

The main enclosure measures only $35 \times 18 \times 62$ cm and houses the system electronics, on-board computer, and the entire vacuum assembly, including all high-vacuum and backing pumps. Ions are generated at atmospheric pressure by ESI, transferred to high vacuum via a vacuum interface and ion guide, and mass analysed by a quadrupole mass filter. The instrument is much smaller than a conventional mass spectrometer because the microspray ion source, vacuum interface, ion guide, and quadrupole mass filter are all microengineered, and the gas load is judiciously managed to avoid the need for a large external rotary pump.[41,58,59] Microelectromechanical systems (MEMS) fabrication techniques are used to manufacture the miniature mass spectrometry components in wafer-scale batches using multiple photolithography, etching, and deposition steps.

Off-line LC/MS was used to corroborate some of the data relating to the optimisation of the flow parameters. LC/MS analysis was performed using an Agilent HP 1100 series liquid chromatograph (Agilent, Santa Clara, CA, USA) coupled to a Waters ZQ2000 mass spectrometer (Waters Corporation, Manchester, UK). The ESCi dual ionisation source fitted to the mass spectrometer was operated in ESI mode. A Mercury Luna 3 μm C18 (2) column (Phenomenex, Torrance, CA, USA) was used and the solvent gradient of acetonitrile and water, both with 0.1% formic acid, was set such that the organic component was 5% at 0 min, 5% at 1 min, 95% at 4 min, 95% at 5 min, 5% at 7 min, and 5% at 8 min.

RESULTS AND DISCUSSION

(i) Sampling characteristics

The total ion intensity recorded while a series of samples was taken from the reactor effluent over a period of 20 min is shown in Fig. 3. Each peak corresponds to the arrival of a sample at the mass spectrometer, which occurred approximately 10 s after activation of the switching valve. The trailing edges decay relatively slowly due to dispersion of the sample in the capillary tubing.

The peaks in Fig. 3 are each 40 s wide at half-height. Given that the total flow was 1 mL/min and the loop volume was 5 μL, it follows that the sample was diluted by a factor of approximately 130 as a result of the combined effects of dispersion and addition of the dilution solvent. As the initial concentration of the stock anthranilic acid solution was 0.20 M, an estimate of the average concentration of total analytes at the point of analysis is 1×10^{-3} M.
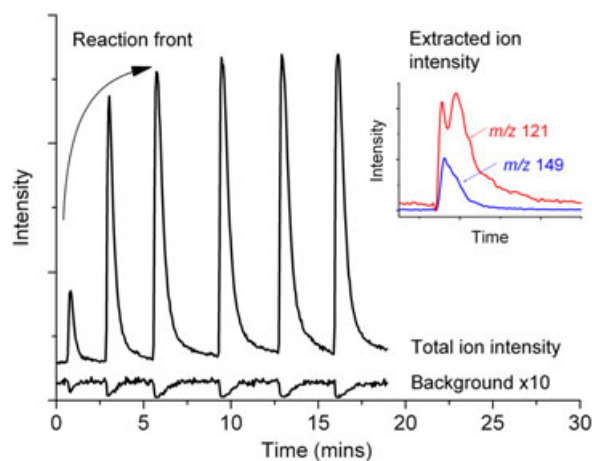

Figure 3. A typical total ion signal (upper trace) showing the arrival at the mass spectrometer of six consecutive samples taken from the reactor effluent. The first three samples intercepted the reaction front. The summed signal due to four peaks in the background spectrum (lower trace) is suppressed by the analytes in the samples. The inset graph shows the extracted ion signals (average of three samples) for m/z 121 and 149.

Although the time at which a reaction front exits a reactor coil can be calculated approximately using the known reactor coil volume and flow rate, in practice the profile is complicated by the effects of dispersion. Product profiles are particularly difficult to predict when an in-line solid-supported reagent column is incorporated into the flow path, as dispersion, diffusion, and chromatographic effects become dominant. It is evident from Fig. 3 that the first three samples intercepted the reaction front while the fourth and subsequent samples represent steady-state. This information could be used to divert the flow to a sample collector, or synchronise the addition of another reagent stream.

The lower trace in Fig. 3 shows the summed signal due to the four most prominent ions in the background spectra. Each occurrence of a sample peak in the total ion signal is mirrored by a dip in the background ion intensity. Evidently, the detection of background ions is suppressed by the relatively high concentrations of analyte from the reaction sample. Behaviour of this type is known[60] to be a consequence of the competition for charge. This presents some difficulties with respect to background subtraction, as it is not appropriate to subtract a constant background signal from the reaction sample spectra.

The possibility that bubbles might form when gaseous reaction products are released also requires consideration. Clearly, the ion current will collapse each time a gas bubble passes through the microspray emitter. A periodic loss of data and signal instability as the spray is re-established after each bubble is likely to yield unreliable results that are difficult to analyse. The formation of benzyne from benzenediazonium-2-carboxylate results in the generation of N_2 and CO_2. Both these products remained in solution while under pressure but were released in gaseous form after the 500 psi (34 bar) back-pressure regulator, as shown by the observation of gas pockets passing through the transparent downstream tubing. If the switching valve had been inserted on the low-pressure side of the back-pressure regulator, as in some arrangements,[30] gaseous and mixed phase samples would inevitably have been passed to the mass spectrometer. With

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the switching valve being upstream of the back-pressure regulator, the reactor effluent remained pressurised in the sample loop. The combined effect of the flush and dilution pumps together forcing solvent into the system at a rate of 1 mL/min ensured that high pressure was maintained in the capillary tubing. Typical pressures measured at the flush and dilution pumps were 40 bar and 20 bar, respectively. Although not measured directly, the pressure downstream of the dilution mixer tee must have steadily decreased to 1 bar, as the fluid was eventually discharged from the waste leg of the mass spectrometer flow split. Fortunately, the sample was also much less concentrated following the addition of the diluent, and a lower pressure was therefore required to maintain dissolution of the gaseous products (Henry's law). It appears that the high initial pressure and decreased concentration were sufficient to keep the gaseous products in solution at the point of analysis, as no signal fluctuations can be seen in Fig. 3 that might suggest the transit of gas bubbles.

(ii) Reaction pathways

Representative positive ion mode mass spectra acquired when the reactor coil temperature was set at 25 °C and 50 °C are shown in Fig. 4. Each spectrum represents a summation of all the individual scans corresponding to a
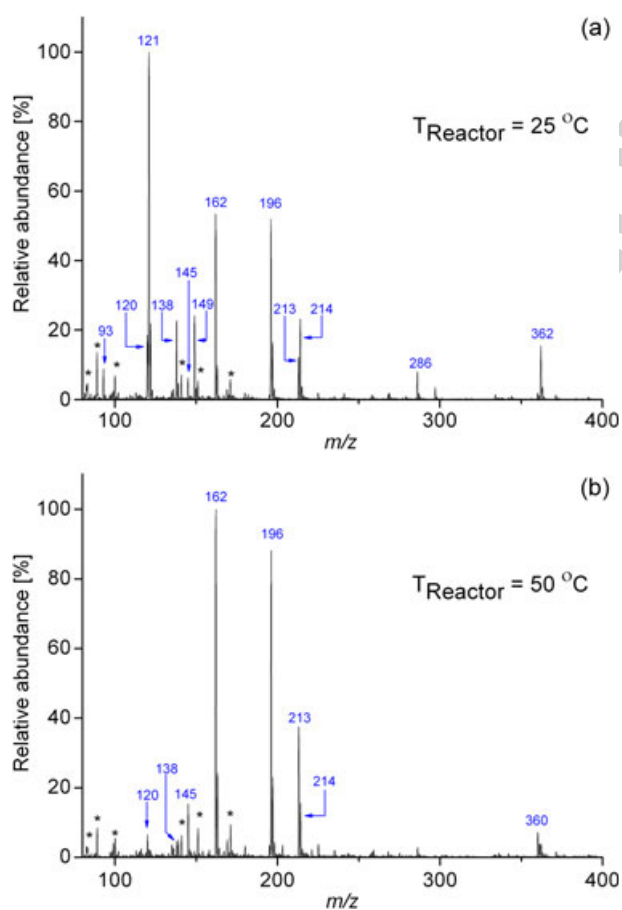
Figure 4. Representative spectra corresponding to reactor temperatures of (a) 25 °C and (b) 50 °C. The reactor residence time was 20 min in both cases. Background peaks are labelled with asterisks.

single peak in the total ion signal. No attempt has been made to subtract a background for the reason described above, but background peaks are labelled with asterisks. The $[M+H]^+$ ions resulting from unreacted anthranilic acid (**1**) and the desired product **4** can be immediately identified at m/z 138 and 145, respectively. Furthermore, allowing the reagent streams to flow through the reactor one at a time showed that the peak at m/z 120 is also due to anthranilic acid, which apparently undergoes collision-induced loss of water from the $[M+H]^+$ ion in the vacuum interface of the mass spectrometer.

In the low-temperature spectrum there is a peak at m/z 149 that can be assigned to protonated benzenediazonium-2-carboxylate (**2**). This was an unexpected result as it had been assumed that all the benzenediazonium-2-carboxylate would have been consumed during the 20 min transit of the reactor coil. The prominent peak at m/z 121 can be attributed to the loss of N_2 from benzenediazonium-2-carboxylate. Some of the intensity at m/z 121 is undoubtedly the result of collision-induced fragmentation in the interface of the mass spectrometer. It is known that aryldiazonium cations readily lose N_2 following relatively low-energy collisions.[61] However, it is evident from the inset graph in Fig. 3, which shows the extracted ion signals for both m/z 149 and 121, that this cannot be the only process involved. The m/z 121 signal has a second component that is clearly not correlated with the m/z 149 signal and therefore is not attributable to collision-induced fragmentation. Consequently, this second component must be assigned to another reaction product in the flow stream. It may be that the second component arrives later due to chromatographic effects in the capillary tubing, or that the m/z 149 signal is quenched early due to hydrolysis of the benzendiazonium-2-carboxylate in the tail of the dispersed sample. In support of these observations, investigations[62,63] of benzenediazonium-2-carboxylate reactions in solution have indicated that N_2 loss is a necessary intermediate step in the formation of observed products. Scheme 2 is a summary of the reaction pathways proposed in these reports. The formation of the reactive 2-carboxyphenyl intermediate **5** is indirectly confirmed by the presence of a peak at m/z 162 in Fig. 4, which can be attributed to products (**6** and **7**) arising from nucleophilic attack on **5** by acetonitrile. While it is unlikely that **5** survives long enough to be detected by the mass spectrometer, the m/z 121 peak can equally be assigned to rearranged products such as the β-lactone, **8**, or ketene, **9**. Compared with the monoisotopic peak at m/z 121, the peak at m/z 122 is noticeably more intense than expected on the basis of isotopic abundances. The m/z 121 signal might have been differentially attenuated to an extent by some non-linearity in the response of the pulse-counting electronics at high count rates. However, there are also differences in the extracted ion profiles for m/z 121 and 122, which suggest that an isobaric interference is responsible for some of the intensity at m/z 122.

Other peaks in Figs. 4(a) and 4(b) can be assigned according to the reactions shown in Scheme 3. Some of the products and reaction pathways are well known, whereas others are proposed tentatively. The diazotisation of anthranilic acid in aprotic media is known to yield both *N*-phenylanthranilic acid and acridone.[52,64] This is consistent with the observation of peaks at m/z 214 and 196, which may be assigned to the corresponding $[M+H]^+$ ions. In Scheme 3, benzyne

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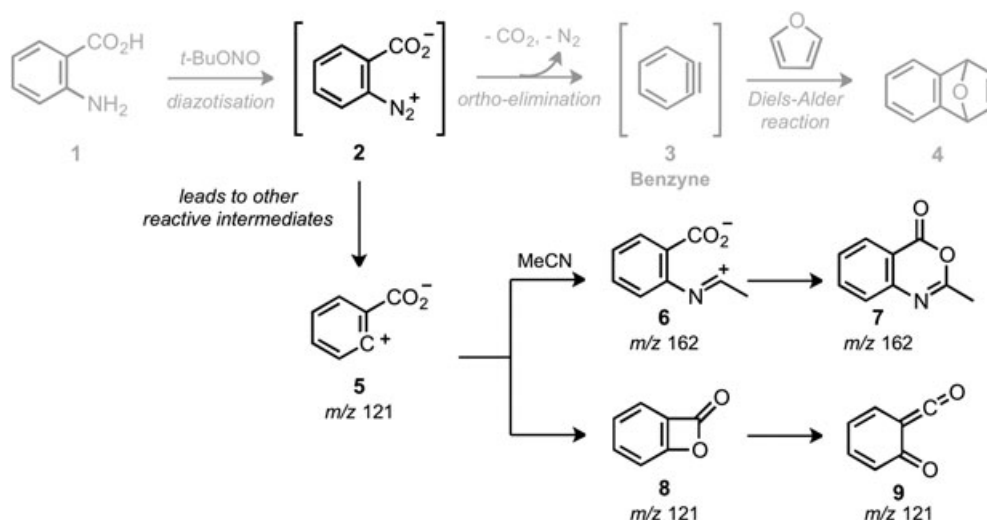
Scheme 2. Reaction pathways involving loss of N_2 from benzenediazonium-2-carboxylate in solution.[62,63] Compounds are labelled with the m/z value of the corresponding [M+H]$^+$ ion.
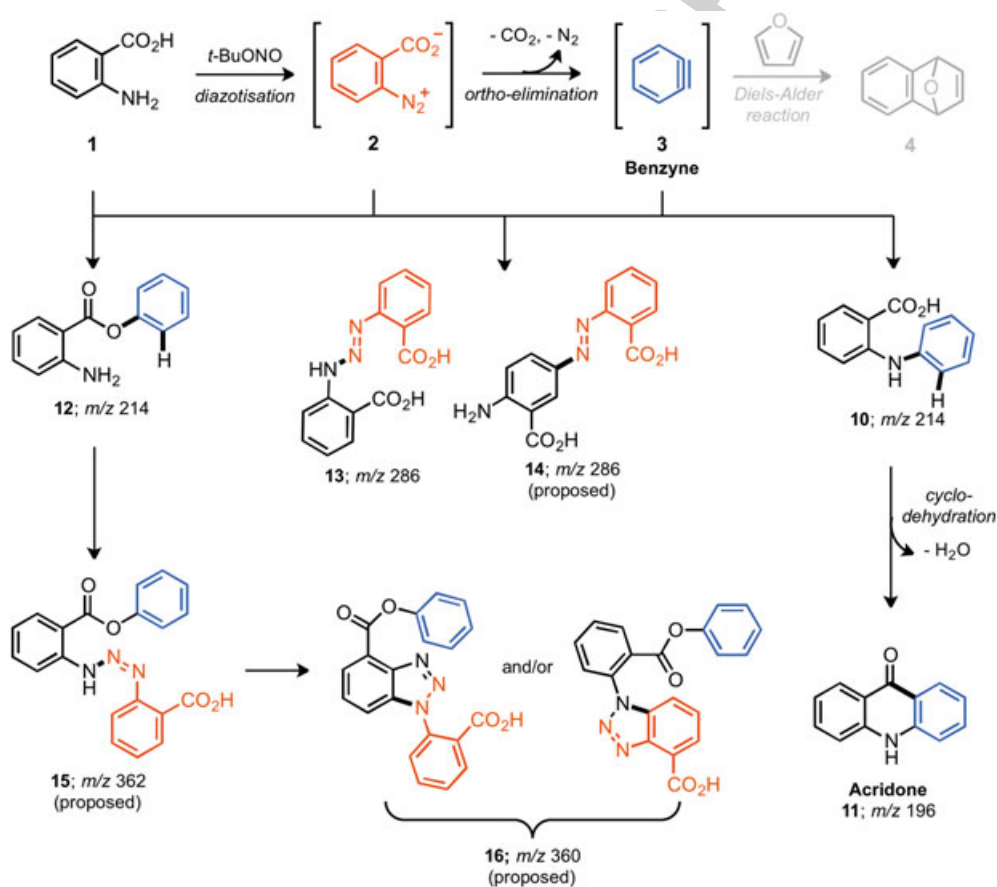
Scheme 3. Proposed reactions leading to formation of the primary by-products observed in the mass spectra. Compounds are labelled with the m/z value of the corresponding [M+H]$^+$ ion.

reacts with the amino group of anthranilic acid to yield *N*-phenylanthranilic acid (**10**), which subsequently undergoes cyclisation with loss of water to give acridone (**11**). However, it should be noted that the standard method for preparing acridone from *N*-phenylanthranilic acid requires prolonged heating in concentrated acid, i.e. harsher conditions than are present in the flow reactor.[65] As benzyne can also react with carboxyl groups,[66,67] an alternative assignment for m/z 214 is possible, namely phenyl anthranilate (**12**). It has previously been observed that a transient orange to brick-red precipitate, identified as 2,2′-dicarboxydiazoaminobenzene (**13**), can form during the diazotisation of anthranilic acid.[52,53] An alternative

diazo-coupled product (**14**) is also plausible. Hence, the small peak at *m/z* 286 can be assigned to either or both of the corresponding [M+H]$^+$ ions.

The peaks at *m/z* 362 in Fig. 4(a) and *m/z* 360 in Fig. 4(b) do not correspond to any previously reported products of anthranilic acid diazotisation. We propose that the diazo intermediate **2** reacts with phenyl anthranilate (**12**) to provide triazene **15** (*m/z* 362). Furthermore, we suggest that, at the higher temperature, triazene **15** may undergo a C–H insertion–cyclisation process with loss of H$_2$ to yield benzotriazoles such as **16** (*m/z* 360). Interestingly, such transformations have been reported, but they usually require metal catalysts and a C–X bond to undergo oxidative insertion.[68] It may be that the specific molecular architecture renders the transformation of **15** to **16** particularly facile. However, there is no evidence in the mass spectra for a similar cyclisation of the proposed triazene **13**.

The peak at *m/z* 213 also does not appear to correspond to any previously reported product. However, a study of anthranilic acid diazotisation using isoamyl nitrite in hot dichloroethane showed that the many reaction products included carbazole (**17**) and 2-nitrodiphenylamine (**18**), which were formed with yields of 2% and 4%, respectively (Scheme 4).[69] While neither of these compounds have been observed in the present study, we tentatively assign the *m/z* 213 peak to 1-nitrocarbazole (**19**) on the basis of its structural similarity. It is possible that 2-nitrodiphenylamine cyclises to 1-nitrocarbazole.

In summary, the spectra shown in Figs. 4(a) and 4(b) indicate that there are a number of reactions that compete with the cycloaddition of benzyne to furan. These include loss of N$_2$ from benzene-2-carboxylate followed by rearrangement or nucleophilic attack, various diazo coupling reactions, and the addition of benzyne to anthranilic acid. In the following section we show how peak height trends can be used to guide the optimisation of flow parameters.

(iii) Optimisation of flow parameters

Figure 5(a) shows how the intensities of selected peaks changed as the reactor temperature was increased. For each reactor temperature, a cumulative spectrum was generated by summing all the individual spectra corresponding to a peak in the total ion signal. Each data point in Fig. 5(a) represents the height of the indicated peak in the cumulative mass spectrum. The data sets have been normalised such that the signal intensities are unity at 25 °C.

The analysis of mixtures by ESI-MS requires some caution.[60,70] In general, the signal due to a particular neutral analyte is partly determined by its ability to acquire charge by
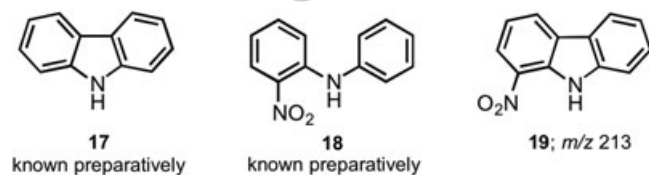
Scheme 4. Carbazole (**17**) and 2-nitrodiphenylamine (**18**) are both known by-products resulting from treatment of anthranilic acid with isoamyl nitrite. 1-Nitrocarbazole (**19**) is proposed as an assignment for the peak at *m/z* 213.
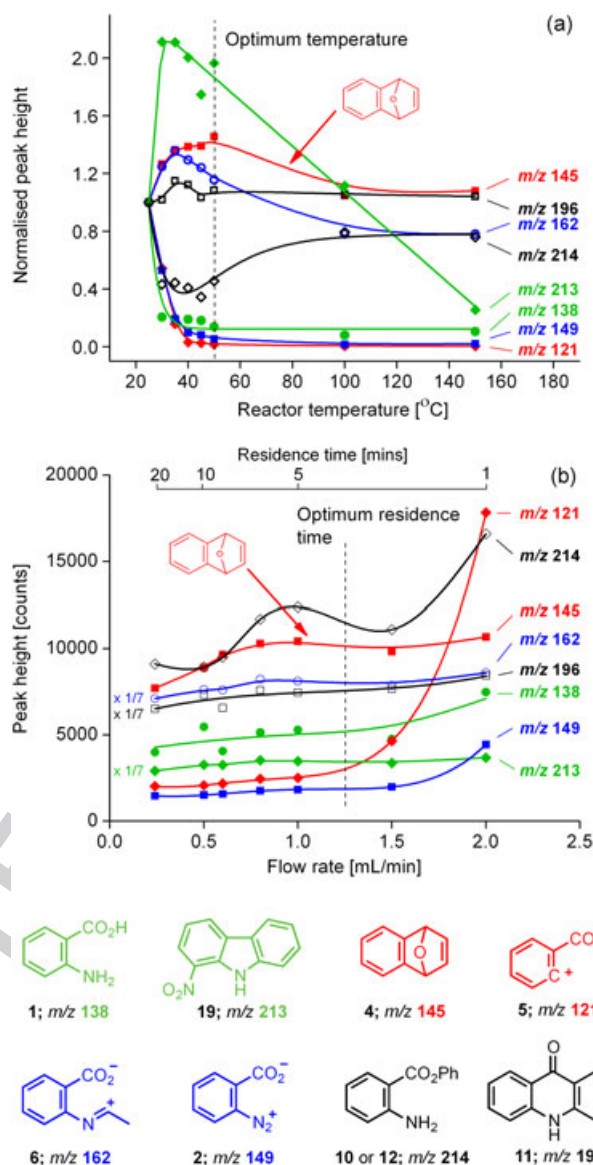
Figure 5. Response of mass spectral peak intensities to (a) reactor temperature at a residence time of 20 min and (b) flow rate at a reactor temperature of 50 °C. Some data sets in (b) have been scaled to aid presentation. The compounds in the legend are labelled with the *m/z* value of the corresponding [M+H]$^+$ ion.

protonation or adduct formation. At concentrations higher than 10^{-5} M, the analysis of multi-component mixtures is further complicated by the competition for droplet excess charge, as has already been seen in connection with Fig. 3, and the affinity of each component for the surface of sprayed droplets. Moreover, it is well known[60] that signal intensities tend to saturate between 10^{-5} and 10^{-3} M. As the samples were diluted to a concentration at the top of this range, the response of the peak heights to changes in concentration might be quite weak. Hence, the intensities of the peaks in the spectra reflect factors other than just the relative concentrations of analytes in solution. Figure 5 (a) nevertheless reveals broad trends that guide optimisation of the reactor temperature and provide an insight into the reactions occurring. Although separation of the

components of a mixture by LC is often desirable, reaction monitoring by direct ESI of reaction samples has been used previously.[45,57,71,72] Direct ESI is not necessarily the only means of interrogating the flow stream. Other means of ionisation may offer advantages with respect to response linearity, matrix effects, and issues relating to gas evolution or precipitate formation. For example, it has been shown that batch reactions can be monitored by extractive ESI of head-space vapours or reaction mixture aerosols.[73,74]

At a reactor temperature of 50 °C, the intensities of the peaks assigned to the starting material (m/z 138) and benzene-diazonium-2-carboxylate (m/z 149) are minimised, while the intensity of the peak assigned to the product (m/z 145) is maximised. The height of the acridone peak at m/z 196 remains remarkably constant despite the marked tempera-ture dependence of the m/z 214 peak, which has been assigned to the precursor from which acridone is likely to be generated. The intensity of the m/z 121 peak decreases sharply as the temperature is increased above 25 °C. This is partly a consequence of the thermal degradation of benzene-diazonium-2-carboxylate, but also reflects the loss of **5** or its rearrangement products (**8** and **9**). Interestingly, the initial increase in the intensity of the m/z 162 peak, which was attributed to a secondary product of **5**, mirrors the decrease in the m/z 121 signal.

In order to confirm that the observed increase in the m/z 145 peak height corresponds to a genuine increase in the yield of the desired product, samples corresponding to reactor tem-peratures of 25 and 50 °C were analysed by off-line LC/MS. At each reactor temperature, three samples were manually taken from the flow, diluted by a factor of six, and transferred to the autosampler of the LC/MS system. For each sample, the peak centred at a retention time of 3.7 min in the m/z 145 extracted ion chromatogram (EIC) was integrated. The averaged peak areas corresponding to reactor temperatures of 25 and 50 °C are 1.4×10^7 and 2.5×10^7 arbitrary units, respectively. Hence, the product yield is 79% greater at the higher temperature. While this a little more than the increase in the m/z 145 signal seen in Fig. 5(a), the trend observed by on-line mass spectrometry is strongly corroborated by these measurements.

Figure 5(b) shows how the peak intensities respond to the total flow rate through the reactor. The reactor temperature was set to the optimum value of 50 °C. In the experiments described thus far, the reactor volume, total flow rate, and residence time were 20 mL, 1 mL/min, and 20 min, respec-tively. In order to access short residence times without consuming large amounts of reactants, the reactor volume was reduced from 20 mL to 5 mL. Starting from a flow rate of 0.25 mL/min, which equates to the original residence time of 20 min, the intensity of the product peak at m/z 145 initially increases somewhat as the flow rate is increased. At the highest flow rate used, the m/z 149 peak is significantly more intense than at lower flow rates. Evidently, not all the benzenediazonium-2-carboxylate is consumed when the residence time is short, even at a coil temperature of 50 °C. If the discharge of benzenediazonium-2-carboxylate from the reactor is to be avoided, a residence time of approximately 3–5 min yields the highest rate of product formation. The most notable feature of Fig. 5(b), however, is the very large increase in the m/z 121 peak height at high flow rates. If preferential formation of the reactive 2-carboxyphenyl

intermediate (**5**) or its rearrangement products (**8** and **9**) was desired (for use in a subsequent reaction, for example), operation at low residence times would be appropriate.

It was suggested above that the peak at m/z 362 is due to a triazene (**15**) that can undergo cyclisation with loss of H_2 to give a benzotriazole (**16**), as evidenced by a second peak at m/z 360. These assignments are supported by Fig. 6, which shows how the peak heights at m/z 360 and 362 change with flow rate. The trends appear to be correlated, and consistent with a conversion of **15** into **16**. As the residence time increases (flow rate decreases) the extent of the conver-sion increases.

(iv) Screening of derivatised starting materials

The flow process was repeated using 2,5-dimethylfuran instead of furan. Figure 7 shows the mass spectrum obtained at a reactor temperature of 50 °C and a residence time of 5 min. The strong peak at m/z 173 can be assigned to the
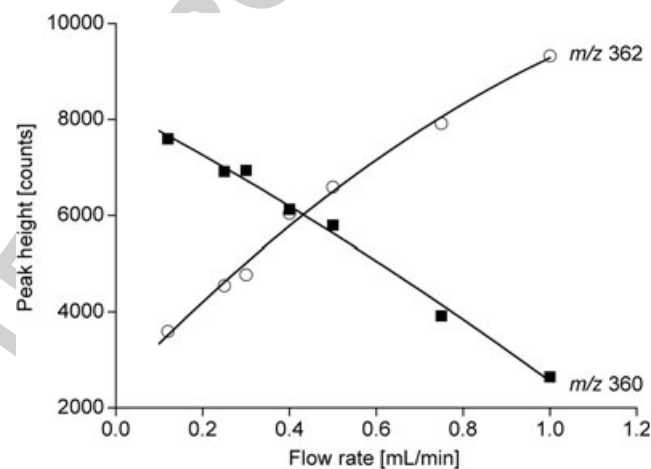
Figure 6. Response of the peak intensities of m/z 360 and 362 to flow rate at a reactor temperature of 50 °C. The trends appear correlated and support the suggested conversion of triazene **15** into benzotriazole **16**.
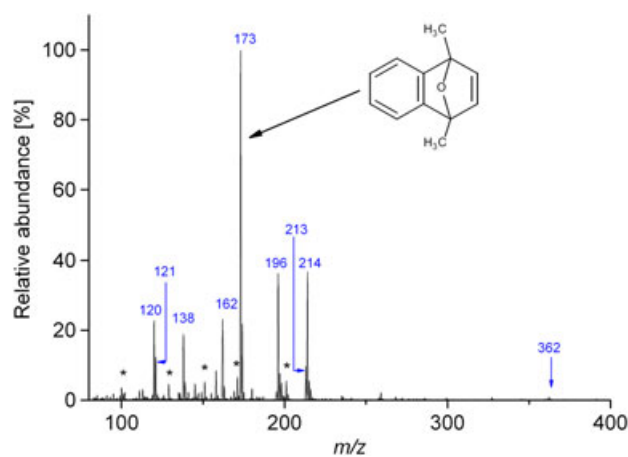
Figure 7. Mass spectrum of products resulting from the use of 2,5-dimethyl furan instead of furan. The Diels-Alder product shifts from m/z 145 to m/z 173, but other peaks are unaffected.

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expected Diels-Alder product, 1,4-endoxide-1,4-dihydro-1,4-dimethylnaphthalene. Importantly, the peaks at m/z 162, 196, 213, 214 and 362 are common to both Figs. 4(b) and 7, which dictates that these must be assigned to products that are not derived from furan or 2,5-dimethylfuran. This is entirely in support of the proposed reaction pathways and peak assignments given in Schemes 2 and 3.

CONCLUSIONS

On-line mass spectrometry provides a detailed snapshot of the materials discharged by a flow chemistry reactor system. While the absence of chromatographic separation adds a level of complexity to the interpretation of the data, the overwhelming benefit of direct and rapid analysis of samples from the flow stream is that unstable or reactive intermediates and products can be identified. Trends in peak heights as a function of residence time and reactor temperature can be used to guide the optimisation of the flow conditions. The relationship between peak height trends and actual conversion has been corroborated by off-line LC/MS analysis of flow samples. We have shown that discharge of the potentially explosive intermediate, benzenediazonium-2-carboxylate (2), from the flow system can be avoided if the reactor coil is operated at or above 50 °C. Compelling evidence for the presence of the 2-carboxyphenyl intermediate (5) and its rearranged products (8 and 9) has also been presented. Although reactive intermediates have been detected by analytical-scale in-line mass spectrometry many times previously, the results presented in the present paper suggest a potentially valuable application of on-line mass spectrometry in preparative flow synthesis. It is clear that, using this technique, a flow chemistry system can be set up to generate reactive intermediates that could then be intercepted by another reactant flow stream. Further investigations into this exciting prospect are ongoing, and we hope to exploit the outcomes in a preparative manner in due course.

Acknowledgements

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USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

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The latest version of Acrobat Reader can be downloaded for free at: <u>http://get.adobe.com/uk/reader/</u>

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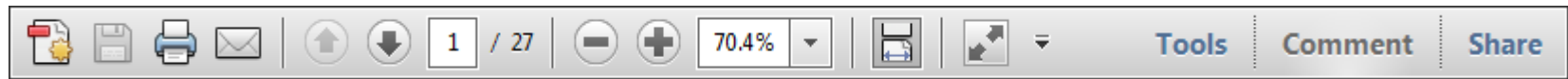
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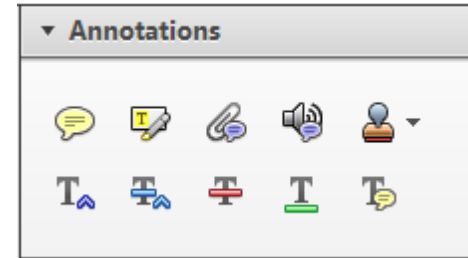
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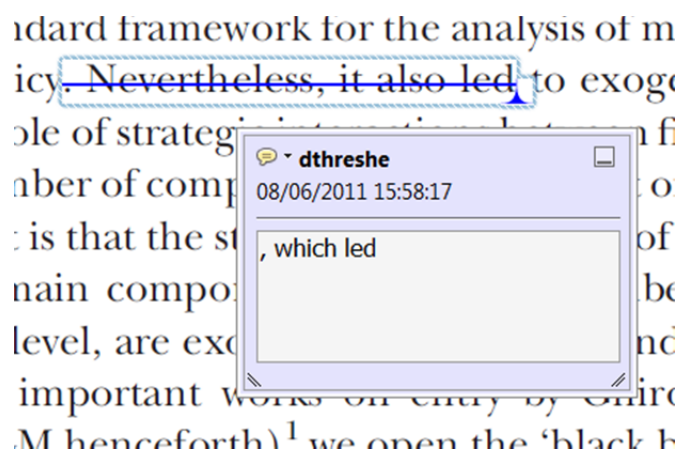
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How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

there is no room for extra profits ar
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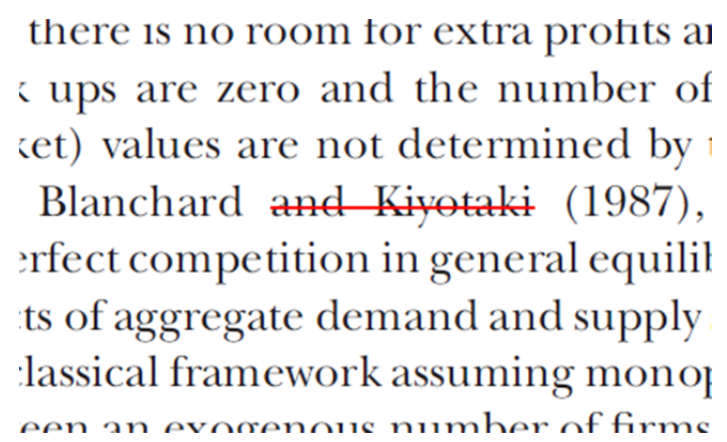
3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
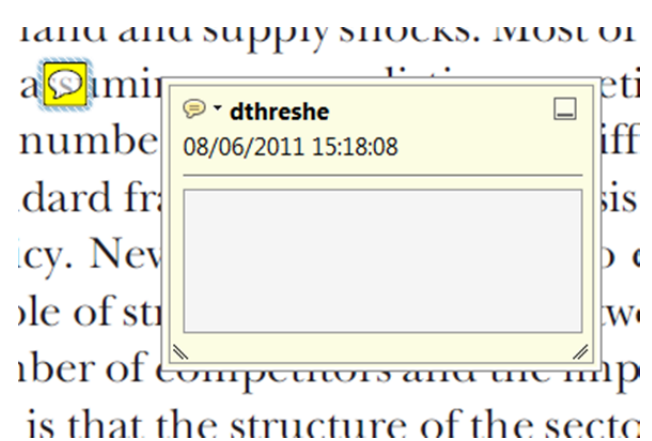- Type instruction on what should be changed regarding the text into the yellow box that appears.

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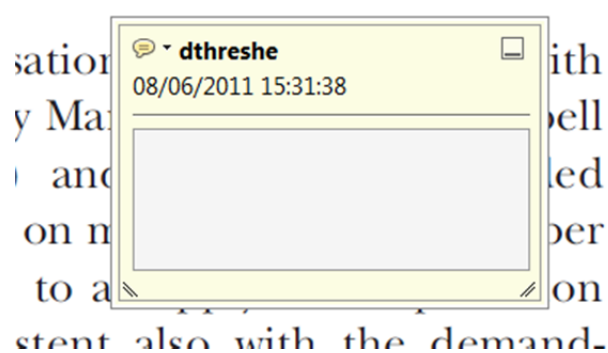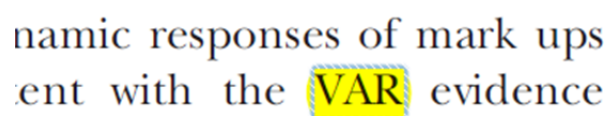
4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

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# USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

END

0.20

0.15

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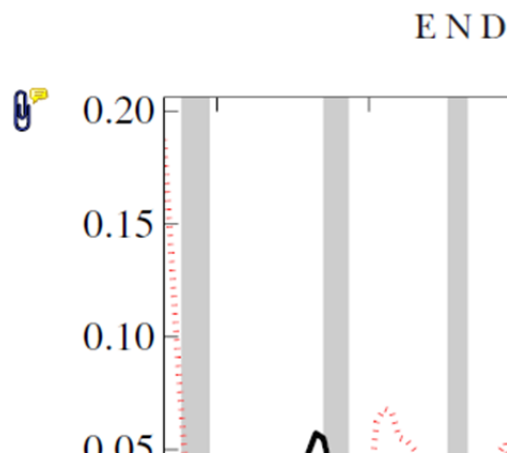
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

or the business cycle, starting with the
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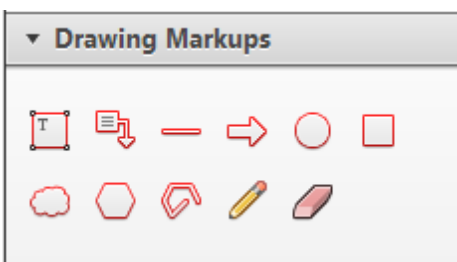
▼ Drawing Markups

7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

Mark Up

New Incorporations

-0.001

0.000

-0.001

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Profits

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08/06/2011 16:23:54

Move to the bottom of the left column of panels.
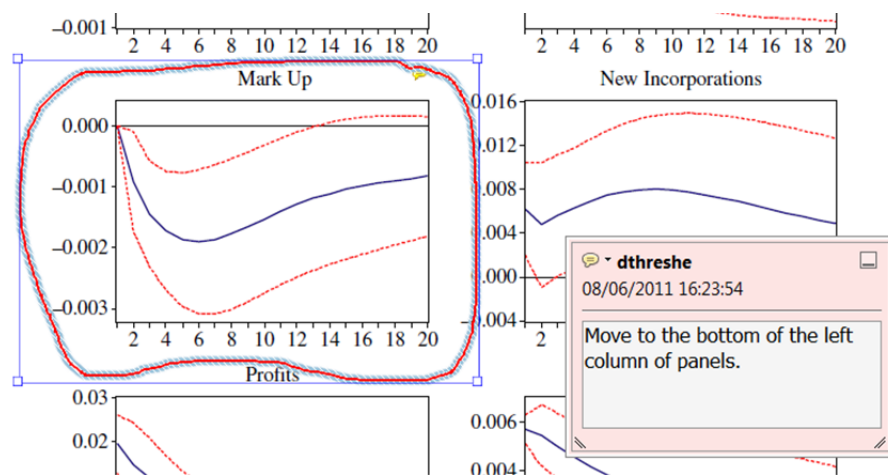
For further information on how to annotate proofs, click on the Help menu to reveal a list of further options:

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▼ Annotations

▶ Drawing Markups

▼ Comments List (14)

Find

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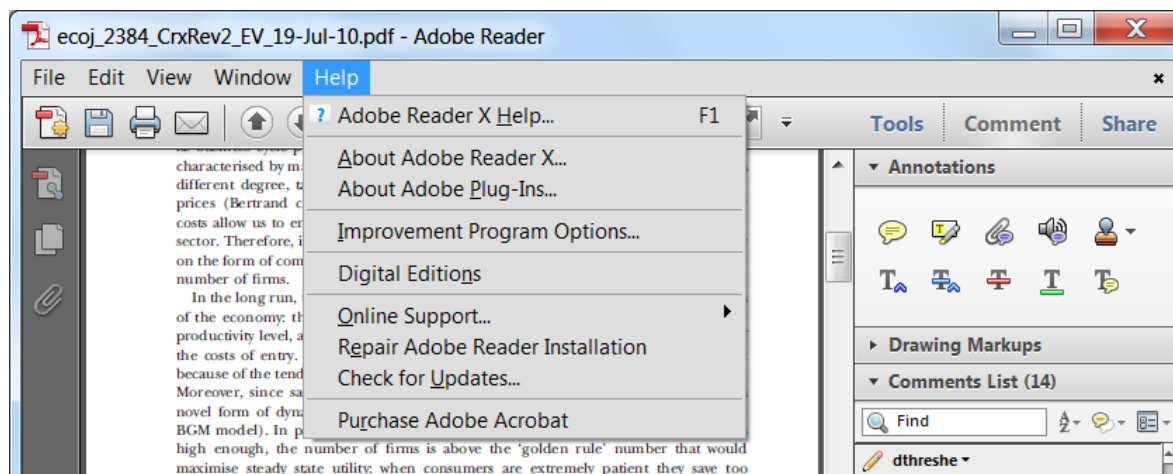
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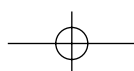
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			Manufacturing	
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