Received: 20 December 2016 | Accepted: 19 May 2017
DOI: 10.1002/mas.21539

REVIEW ARTICLE

WILEY MassSpectrometryReviews

On-line reaction monitoring by mass spectrometry, modern approaches for the analysis of chemical reactions

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The application of on-line mass spectrometry for direct analysis of chemical and other types of process continues to grow in importance and impact. The ability of the technique to characterize many aspects of a chemical reaction such as product and impurity formation, along with reactant consumption in a single experiment is key to its adoption and development. Innovations in ionization techniques and mass spectrometry instrumentation are enabling this adoption. An increasing range of ambient ionization techniques make on-line mass spectrometry applicable to a large range of chemistries. The academic development and commercialization of small footprint portable/transportable mass spectrometers is providing technology that can be positioned with any process under investigation. These developments, coupled with research into new ways of sampling representatively from both the condensed and gaseous phases, are positioning mass spectrometry as an essential technology for on-line process optimization, understanding and intelligent control. It is recognized that quantitative capability of mass spectrometry in this application can cause some resistance to its adoption, but research activities to tackle this limitation are on-going.

KEYWORDS
Ambient ionisation, Chemical reaction intermediates, Time resolved on-line chemical reaction monitoring, Quantitation

1 | INTRODUCTION

Off-line gas chromatography-mass spectrometry (GC/MS) has historically been used for the monitoring of chemical reactions; however since the invention of thermospray, and subsequently electrospray, liquid chromatography-mass spectrometry (LC/MS) has become the technique of choice for off-line reaction monitoring. Though both of these techniques can produce significant knowledge, there are many advantages to on-line analysis. In 2005 Daniele Fabris summarized many of the key mass spectrometric approaches for the investigation of dynamic processes in the condensed phase.[1] The review discussed off-line sampling, quantitation, and kinetics (both off-line and on-line) and the characterization of impurities and intermediates. Of particular interest to us, and of great relevance to our own research in this area, was the discussion of continuous on-line monitoring using mass spectrometry (MS). It was noted that this approach has a number of advantages over off-line sampling and analysis. These include the ability to characterize unstable reaction products that may not survive sample manipulation. Fabris made reference to the approaches by Dell'Orco and Brum who continuously monitored by sampling small volumes of reaction mixture into a constant

Abbreviations: APCI, atmospheric pressure chemical ionization; API, active pharmaceutical ingredient; API, atmospheric pressure ionization; ASAP, atmospheric samples analysis probe; DAPCI, desorption atmospheric pressure chemical ionization; DART, direct analysis in real time; DESI, desorption Electrospray; DLSMS, direct liquid sampling mass spectrometry; EASI, easy ambient sonic-spray ionization; EDTA, ethylenediaminetetraacetic acid; EESI, extractive electrospray; ESI, electrospray; EI, electron ionization; FAIMS, high-field asymmetric waveform ion mobility spectroscopy; FAPA, flowing atmospheric-pressure afterglow; FIA, flow injection analysis; GC, gas chromatography; HPLC, high performance liquid chromatography; iESI, inductive electrospray; IM-MS, ion-mobility mass spectrometry; LTP, low-Temperature Plasma probe; MIMS, Membrane Inlet Mass Spectrometry; MIR, mid-infrared spectrometry; OLEMS, on-line electrochemical mass spectrometry; PATI, paper assisted thermal ionization; PSI, pressurized Sample Infusion; PTR, proton Transfer Reaction; RAFT, reversible addition-fragmentation chain transfer; SNOBFIT, stable noisy optimization by branch and fit; UASI, ultrasound-assisted spray ionization; V-EASI, venturi easy ambient sonic-spray ionization.

flow of a carrier solution compatible with MS.[2,3] This approach has influenced our own research, which builds on these studies by employing a number of new generation commercially available small footprint mass spectrometers.[4–6] Such devices are advantageous for the development of on-line MS, as they allow the mass analyzer to be easily co-located with the reaction system or process that is to be studied.

This review builds on that from Fabris in 2005, by describing the further development of MS approaches for the direct investigation of a variety of dynamic processes, with a particular emphasis on the application of small footprint and transportable mass spectrometers. The introduction of many new ambient ionization techniques has aroused much interest for reaction monitoring; and these will be discussed for both off-line and on-line monitoring. In this review we focus on reactions to form a co-valent bond and not ion-molecule association reactions or protein—drug/protein-protein interactions. Monitoring using off-line chromatography interfaced to mass spectrometry or other off-line techniques (eg, matrix assisted laser desorption-ionization (MALDI)) are also out of scope for this review.

2 | INSTRUMENTAL AND SAMPLING DEVELOPMENTS

2.1 | On-line electrospray ionization of the condensed phase

As an established technique, electrospray ionization (ESI) has received significant attention for on-line MS applications when coupled to a range of reactor designs. Schroder has reviewed a number of examples of the application of ESI-MS for mechanistic studies in catalysis research.[7] Importantly, Schröder highlights the link between solution chemistry and what is observed in the gas phase by ESI; but also alludes to the challenges of such an approach. These include the potential effects of concentration range, solvation, pH, and electrochemical processes (that occur during droplet evaporation) on the resulting MS data and the subsequent correlation to the bulk chemistry that is being studied. A further challenge when carrying out on-line ESI-MS is the transfer of condensed phase samples from a reaction or process system to the mass spectrometer for analysis. A variety of approaches have been developed in an attempt to overcome this issue.

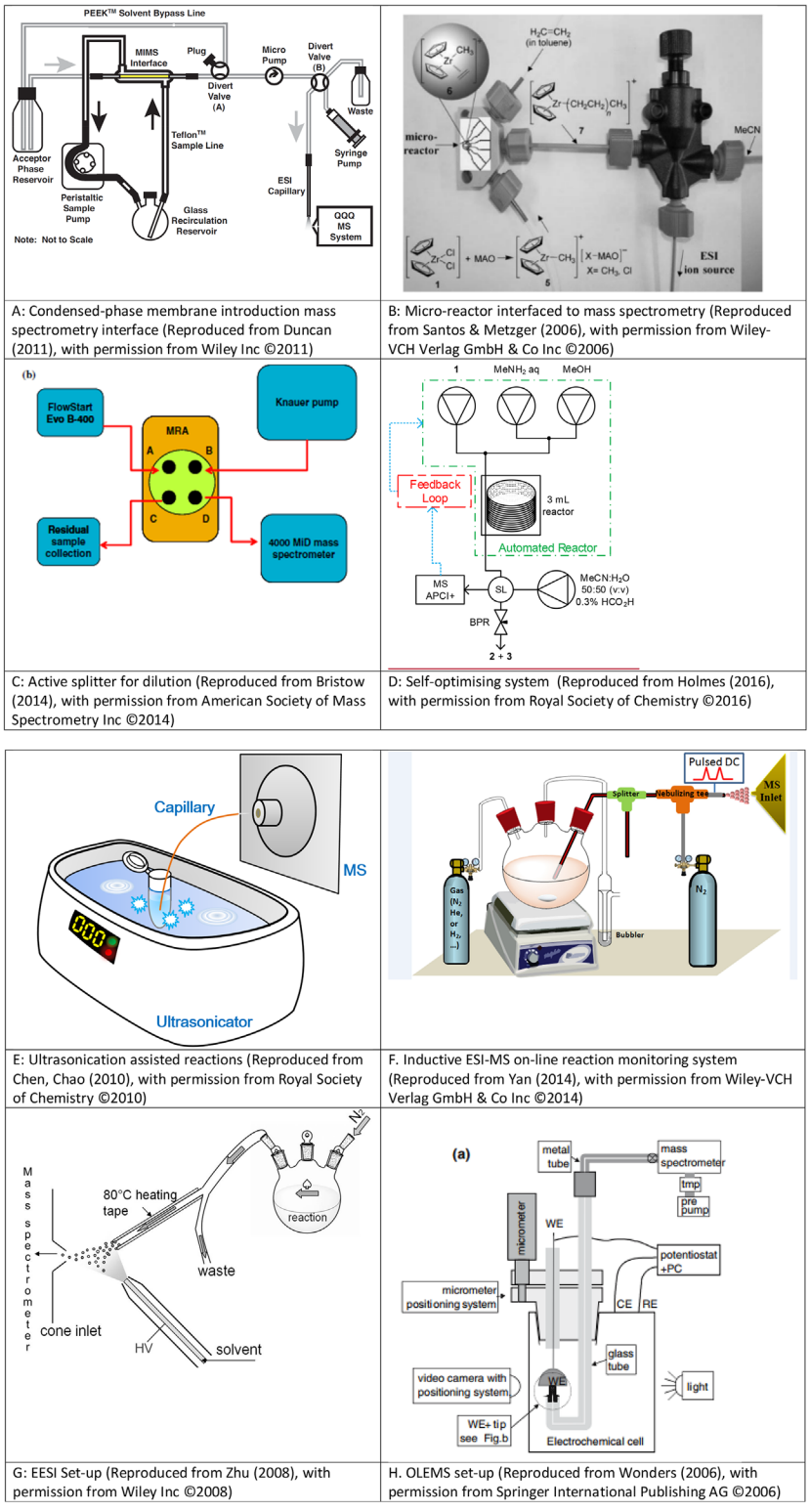
A number of groups have used micro-reactors for ease of interfacing to on-line ESI; here two liquid streams are combined and the reaction initiated.[8–13] The simplicity of a typical experimental set-up is illustrated in Fig. 1B, where two flows of reactants are combined in the reactor with the output passing to the ESI-MS system.[14] In his account on the area, Santos[15] has also discussed the information on reaction mechanism that is provided by ESI-MS and highlighted this with a number of examples including the on-line coupling of a microreactor to an ESI source to screen a Ziegler-Natta polymerization. Continuous on-line monitoring of reversible addition-fragmentation chain transfer (RAFT) polymerization and single unit monomer insertions have been monitored on-line and in real time by combining ESI-MS and a microreactor.[16]

The adoption of continuous flow chemical reactors has accelerated over the past decade for reasons including (a) suitability for dangerous reagents; (b) extremes of temperature; (c) ease of scale-up; (d) production on demand to precise quantities required; and (e) improved throughput. Therefore, the direct analysis of products and impurities formed in flow chemistry is essential and has been investigated more widely using a variety of approaches. Ley and co-workers used a switching valve to sample aliquots from a reaction stream produced by a flow chemistry reaction, passing them into a make-up flow to the mass spectrometer.[17] Bristow et al adopted a similar approach, but used an active splitter (mass rate attenuator) to sample and dilute the output from a flow reactor as shown in Fig. 1C.[4] Bourne and co-workers used a four port micro-volume (0.06 μL) sample valve to sample from a continuous reaction (Fig. 1D). Atmospheric pressure chemical ionization (APCI) was employed in the study as it was shown to produce lower baseline noise and could be used at higher flow rates when compared to ESI. The APCI-MS data were shown to be equivalent to those from HPLC/UV.[18] These applications are described in more detail in the section 4.2.

Many other innovative sampling approaches have also been developed. Chen and co-workers directly sampled a reactant solution using an ultrasonicator (Fig. 1E). This approach provides sufficient energy to transport the solution through a capillary and produces a sonic spray into the mass spectrometer[19,20] which is of particular use for ultrasound-assisted reactions. Paz-Schmidt et al used a high pressure of CO_2 to enable a reaction to proceed and also to drive the reactants through a capillary, where it is mixed with an electrospray solvent, before being analyzed by the mass spectrometer.[21] One of the simplest sampling systems was described by Hsu et al, where gravity was employed to transport reactants from a sample well (approximately 5 μL volume), through a capillary to an ESI source without the requirements for a pump for the sample or a nebulizer gas for the ESI.[22] This was used to monitor both chemical and biochemical reactions; these included formation of chelation complexes, formation of reaction intermediates, and also protein conformation changes.

Cooks and co-workers have described the application of inductive electrospray ionization (iESI) for on-line reaction monitoring and mechanistic studies.[23] In this method, a positive potential is pulsed repeatedly to produce transient strong fields in the spray solution, resulting in the emission of charged droplets in bursts. The experimental set-up for inductive electrospray is shown in Fig. 1F. The reaction solution is transferred to the spray tip by a capillary under positive gas pressure (helium). One of the key advantages of this approach is the tolerance to matrix and salt effects. The experimental configuration was used to characterize three reactions of pharmaceutical relevance; reductive amination, Negishi cross coupling and a heterogeneous Pd/C catalyzed hydrogenolysis. The system was used to continuously sample aliquots from the reactor allowing dynamic changes in the system to be followed (Fig. 2).

In further studies, the Cooks group have described the application of iESI to study the synthetic route of anagliptin. As a bench-top linear ion trap mass spectrometer was employed for the study, on-line tandem mass spectrometry (MS/MS) was used to characterize chemical species in the reaction mixture (impurities and by-products).[24] Meher and Chen adopted a similar approach, but used the high electric field of the mass spectrometer to polarize droplets of reactant solutions and generate an electrospray from the droplet.[25]



A: Condensed-phase membrane introduction mass spectrometry interface (Reproduced from Duncan (2011), with permission from Wiley Inc ©2011)

B: Micro-reactor interfaced to mass spectrometry (Reproduced from Santos & Metzger (2006), with permission from Wiley-VCH Verlag GmbH & Co Inc ©2006)

C: Active splitter for dilution (Reproduced from Bristow (2014), with permission from American Society of Mass Spectrometry Inc ©2014)

D: Self-optimising system (Reproduced from Holmes (2016), with permission from Royal Society of Chemistry ©2016)

E: Ultrasonication assisted reactions (Reproduced from Chen, Chao (2010), with permission from Royal Society of Chemistry ©2010)

F. Inductive ESI-MS on-line reaction monitoring system (Reproduced from Yan (2014), with permission from Wiley-VCH Verlag GmbH & Co Inc ©2014)

G: EESI Set-up (Reproduced from Zhu (2008), with permission from Wiley Inc ©2008)

H. OLEMS set-up (Reproduced from Wonders (2006), with permission from Springer International Publishing AG ©2006)

FIGURE 1 Examples of sampling techniques for on-line mass spectrometry

Yang and colleagues have fabricated a novel in-situ nanoelectrospray device for high throughput screening of enzymes and real-time monitoring of enzyme catalyzed reactions.[26] A capillary is simply inserted into a liquid system, and with the high voltage applied, analytes in the liquid reaction system are transferred from the solution and ionized. Analytes are extracted from the liquid by capillary action; these have included saccharides, amino acids, alkaloids, peptides, and proteins. A high throughput version of the experiment has been developed with several narrow mouthed bottles held in a water bath and individual spray capillaries in each. The water bath is mounted on a rotation stage and simple rotation of the set-up brings each capillary to the MS inlet.

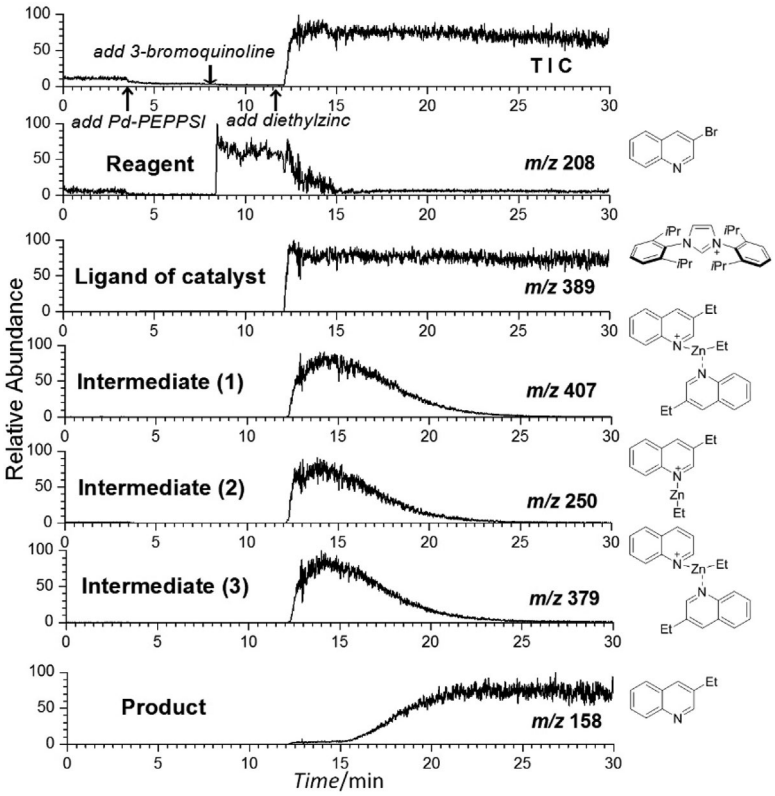


FIGURE 2 Reaction profiles recorded using inductive electrospray-MS on-line reaction monitoring (Reproduced from,[23] with permission from Wiley-VCH Verlag GmbH & Co Inc ©2014)

McIndoe and coworkers report the use of PSI for on-line monitoring of reactions.[27] PSI involves placing the reaction vessel under a slight overpressure (1-3 psi) of a reaction compatible gas to force the sample through an outlet teed into a diluting solvent. This setup was shown to be effective for the monitoring of an acid catalyzed Pbf deprotection of an amino acid, with the technique able to monitor starting material and products over the length of the reaction, with the exception of a short delay after $t = 0$ for sample loading.

Using the PSI apparatus, McIndoe and coworkers have conducted studies of a number of other reactions, with a particular focus on air and moisture sensitive reactions, to which the PSI setup is particularly suited. This has also been used in conjunction with FTIR for the monitoring of reactions involving catalysts, the focus of much of the McIndoe group's work. The hydroacylation of 1-octyne and 2-(methylthio)benzaldehyde with a cationic rhodium catalyst is presented,[28] with PSI used to track the precatalyst, resting state, impurities, an intermediate and catalyst decomposition products. The wide dynamic range of the technique shows itself particularly amenable to the monitoring of complex reactions such as those employing catalysts.

2.2 | MIMS

As discussed by Fabris and reviewed by other groups, MIMS has previously been used for studying reactions, but was limited by the applicability of electron ionization (EI) and chemical ionization (CI) to volatile analytes.[1,29,30] Fabris commented that the applicability of MIMS was also limited by the slow response times as the analytes permeate through the membrane, such that an incomplete picture of the components in the reaction may be obtained. Despite these limitations MIMS has been shown to be of particular use for monitoring a number of systems including fermentation processes.[31,32]

To broaden the applicability of MIMS, the use of a condensed phase acceptor (capturing the sample in an ESI compatible solvent system) has been investigated as it allows alternative ionization techniques to be applied. Duncan et al have used a condensed-phase membrane inlet mass spectrometry (CP-MIMS) interface, with methanol as the acceptor phase for in-situ reaction monitoring of the chlorination of phenol using electrospray ionization.[33,34] The experimental configuration is shown in Fig. 1A.

2.3 | Evolved gas analysis

Most research has been focused on monitoring the liquid (condensed) phase of a reaction, however an understanding of any evolved gases can be critical for process understanding and process safety. Pollien et al used a proton transfer reaction (PTR) mass spectrometer to monitor acrylamide being formed in the headspace of a Maillard reaction.[35] Luchner et al report the interfacing of a PTR mass spectrometer to a bioreactor, for analysis of volatile organics in off-gases, for bioprocessing monitoring and control.[36] In their paper reviewing industry perspectives on process analytical technologies for application in active pharmaceutical ingredient development,[37] have

discussed the application of MS to monitor CO_2 formed during an amine deprotection reaction, in order to understand and optimize a chemical process. In the review, the application of MS is considered in the context of other tools used as process analytical technology.

In an interesting approach,[38] have described the combination of a specially designed thermal vaporizer and a process magnetic sector mass spectrometer (Thermo Electron Prima 600S). This was applied to monitor the esterification of butan-1-ol and acetic anhydride. The system was used to continuously monitor the batch reaction at 150 mL and 1 L scale. Liquid is transferred from the reactor to the vaporizer with the mass spectrometer monitoring the resulting gas phase. Concentration profiles were generated for reactants and products from the MS data (Fig. 3).

The volatile products formed from electrochemical reactions have been monitored by an on-line electrochemical mass spectrometer (OLEMS), which uses a small inlet tip connected to the electrochemical cell to allow only a small amount of gas to enter the mass spectrometer such that differential pumping is not required compared to previous systems. The experimental set-up is shown in Fig. 1H.[39] The volatile products from electrochemical reactions have been studied using OLEMS include reduction of NO,[40] reduction of CO_2[41] and methanol oxidation.[42] Due to the speed of analysis, the OLEMS technique allows rapid screening of electrochemical method parameters.

3 | AMBIENT IONIZATION

The application of ambient ionization techniques to reaction monitoring has attracted much attention, however many of these techniques are most applicable to off-line analysis due to the difficulty in presenting a representative sample of the reaction to the source on-line. The many ionization techniques used in this application area have been reviewed.[43,44] As the ambient ionization techniques are very extensive, a list of definitions can be found in the table of acronyms included in this manuscript.

Cooks has also published an extensive review that describes the use of various ambient ionization techniques (including DESI, ESSI, DAPCI) to study reactions in confined volumes.[45] Ambient ionization was applied to the monitoring of a vast range of small scale reactions. It is noted that reagents can also be included in the spray solvents to allow rapid derivatization to accompany ionization. The review also demonstrates that organic reactions often occur on an accelerated timescale in ambient MS. Though the emphasis of the review was chemical analysis, the isolation of the products of reactions for small scale synthesis was also discussed. A specific example of the application of preparative electrospray, a Claisen-Schmidt condensation, can be found in a publication by Müller et al.[46]

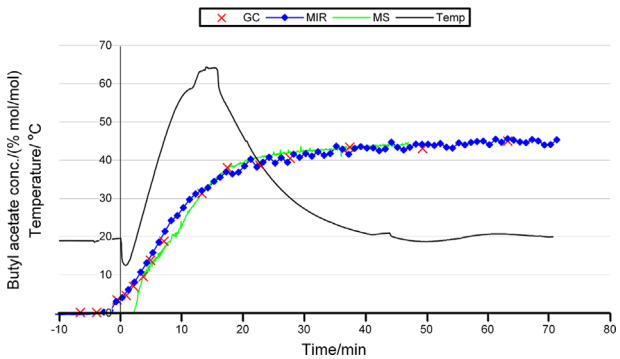


FIGURE 3 On-line monitoring of butyl acetate concentration from the esterification of butan-1-ol and acetic anhydride, comparison between off-line GC analysis, in-line MIR, and on-line DLSMS (Reproduced from,[38] with permission from Elsevier B.V ©2014)On

Rapid chemistry in the confines of an electrospray droplet, is a potentially powerful tool for synthetic route evaluation. However, a potential limitation of the use of electrospray droplets as accelerated reaction systems is that the reaction profile may have a limited relationship to the same reaction at a much larger scale. This limitation and the advantages of rapid synthesis development and the efficiency of a combined reactor and analytical system have been investigated and discussed.[47] In this study the question of whether the droplet reactor can be used to predict chemical reactivity in flow chemistry (microfluidic) systems has been discussed in detail and evaluated using several reactions. The authors clearly recognize that it may not be possible to transfer fully optimized reaction conditions from the droplet reactor to microfluidic scale. Rather they view the approach as a rapid test of possible synthetic pathways, predictive small scale synthesis.

3.1 | Off-line analysis

The use of DART for reaction monitoring and its applicability to drug discovery was initially demonstrated for off-line analysis by dipping a melting point tube into a reaction solution and analyzing.[48] The further application of DART to heterogeneous samples, which are difficult to analyze without extensive sample preparation, has been shown by Cho et al. They studied batch slurry reactions and used manual deposition of liquid samples onto capillaries followed by automated sample introduction.[49] This technique produced RSDs of 6-30%, however differences in spectra were observed for fully dissolved analytes and for samples that were dried onto the capillaries.

A FAPA source has been used to monitor the formation of methacathinone from pseudoephedrine by analysis of crude reaction mixture applied to a glass slide to determine the efficacy of two different reaction schemes.[50] Petucci and Diffendal demonstrated the use of ASAP for drug molecules and highlighted that several compounds produced molecular ions by ASAP but not by ESI or APCI. An advantage of ASAP was much faster data generation when compared to the established solids probe experiment on a GC/MS system; the applicability to reaction monitoring was shown for the study of an *N*-methylation of an indole reaction.[51]

3.2 | On-line analysis

Several investigators have reported the use of LTP to generate and characterize highly reactive species on-line. These reactions have been

shown to be particularly amenable to on-line monitoring by MS, as the plasma may be directly analyzed without any additional ion source. Na et al[52] demonstrated this by monitoring the Birch reduction of benzene, in which the headspace of benzene was passed through an LTP and into an MS instrument for analysis.

Benassi et al[53] investigated gas-phase reactions, such as imine formation and Eberlin reaction. Ma et al also achieved direct analysis by directing the plasma at a reactant solution surface which desorbs and ionises the reactants before they are sampled into the mass spectrometer.[54]

EASI uses a sonic spray of droplets to generate ions, with the Venturi effect, used to transfer a liquid sample to the spray region where the ionization occurs.[55,56] demonstrated the use of this technique for the monitoring of the Morita-Baylis-Hillman reaction, showing capability to monitor a number of intermediates. It was, however noted that solutions used should be as dilute as possible, as the technique suffered from source contamination, an issue which also limited the spray into the instrument to 3 s per minute.

Zenobi and co-workers have demonstrated the use of EESI on a quadrupole time-of-flight (Q-TOF) mass spectrometer for a well-established pharmaceutical process reaction (Michael addition). They have also demonstrated a widely used acetylation reaction in the presence of a nucleophilic catalyst, 4-dimethylaminopyridine (4-DMAP).[57] The experimental approach is illustrated in Fig. 1G. EESI-MS provided real-time information, allowing the determination of the optimum time for terminating the reaction based on the relative intensities of the precursors and products. In addition, MS/MS analysis provided on-line validation of proposed reaction intermediates. Figure 4A illustrates the approach by showing mass spectra recorded for the Michael addition at three different time points, with Fig. 4B showing the individual ion responses of various reactants and products over time.

McCullough et al studied base hydrolysis of ethyl salicylate using EESI.[58] A key concern in modern pharmaceutical production is the presence of potentially genotoxic impurities (PGI) during synthesis. These are often difficult to monitor due to their often very low concentration (ppm to ppb) relative to the active pharmaceutical ingredient. The suitability of EESI for real-time quantitative monitoring of these types of low-level impurity was assessed using terfenadine solutions with codeine spiked in at low levels (1-10 ppm relative to terfenadine) as a model PGI.

Though EESI's potential has been demonstrated, Ma et al commented that these approaches analyze only the headspace above the reaction and therefore the spectra recorded may not be representative of the bulk solution.[43] The analysis of viscous liquids by EESI has subsequently been demonstrated and overcame these issues using micro jetting, to produce microdroplets, which are representative of the bulk solution.[59] Marquez et al used the desolvating droplets in the EESI to study transient radical species in an electron-transfer catalyzed dimerization reaction.[60]

Hsieh et al[61] have developed a simple technique called contactless API where one end of a short capillary is placed in a reaction vial, and the solution flows through the capillary via capillary action to the end acting as a spray emitter placed in the high electric field (−3 kV) of the mass spectrometer. This technique was used to study Zemplen deprotection reactions.

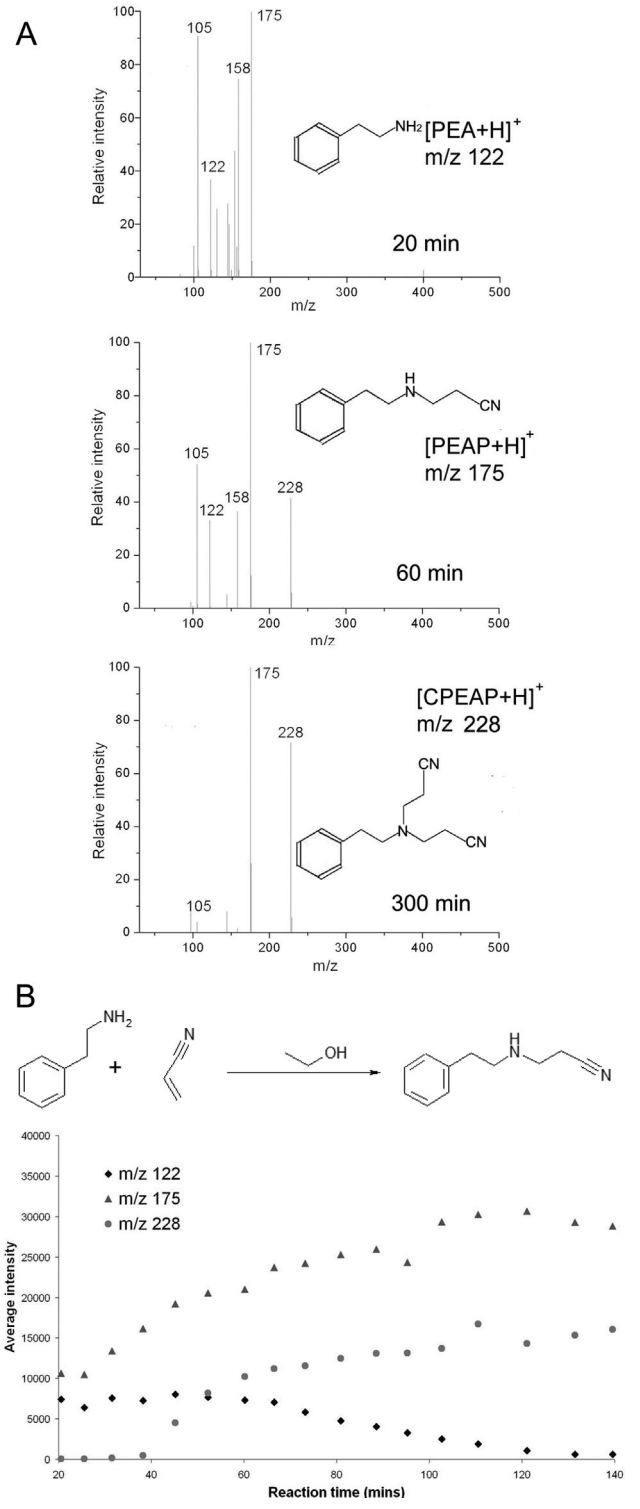


FIGURE 4 On-line EESI of a Michael addition reaction (A) mass spectra at 20, 60, and 300 min (B) traces of average signal intensity for starting material, product and side product (Reproduced from,[57] with permission from Wiley Inc ©2005)

The transfer of a reaction mixture onto a paper substrate has also received attention. Huang and co-workers used PATI to study reaction intermediates where two reactants are deposited onto filter paper positioned on a heated probe which can be used to provide heat for reaction initiation.[62] Liu et al used a steady flow of sub-microlitre droplets, generated through a capillary tube and directed onto the paper. This was then used to electrospray into the mass spectrometer to monitor the reactions.[63] The method was exemplified by monitoring the amine-aldehyde condensation reaction of butylamine and benzaldehyde. Cheng and co-workers [64] used an novel approach of irradiating the surface of an organic reactant solution mixed with carbon powders using a pulsed UV laser. The energy is adsorbed by the carbon and transferred to the solution resulting in desorption, the analyte is then ionized by an electrospray plume.

3.3 | Microfluidics

The use of microfluidics for chemical synthesis offers the potential for fast reaction times and low reagent usage. Traditionally, microfluidics have been used with off-line analysis, but work by Fritzsche et al on organocatalysis studying the Mannich reaction[65] and Kirby and Wheeler monitoring the Morita-Baylis-Hillman reaction[66] used a microfluidic platform interfaced to nanoelectrospray for on-line analysis.

4 | MASS SPECTROMETRY INSTRUMENTAL DEVELOPMENTS

4.1 | Ion mobility spectrometry-mass spectrometry (IM-MS)

The examples discussed thus far have involved presenting the complex reaction sample to the mass spectrometer without pre-separation of the components. Such an approach may lead to a lack of specificity, ion suppression and these are arguably weaknesses of direct on-line analysis. Therefore, as no pre-separation of individual components is achieved, the subsequent separation of the ions formed from the components by ion mobility prior to mass analysis could provide a more complete view of a reaction system.

IM-MS has been investigated by a number of groups to provide greater specificity for real time reaction monitoring. Harry et al and Roscioli et al independently demonstrated ion-mobility mass spectrometry (IM-MS) for pharmaceutical process understanding.[67,68] Roscioli et al applied IM-MS as a method for monitoring pharmaceutical reactions in real time and studied a reductive amination reaction that is commonly used in the synthesis of amines for drug discovery. This use of IMS in conjunction with MS provided information about the reaction mechanism that would not have been possible with MS alone.

The study by Harry et al of the use of IM-MS for reaction monitoring was applied to the direct, real-time analysis of the products formed when 7-fluoro-6-hydroxy-2-methylindole is deprotonated by sodium hydroxide. The results demonstrated that IM-MS can be employed as a rapid means to analyze a chemical reaction, offering the potential for real time reaction monitoring. The IM-MS approach has been was shown to enhance selective visualization for the analytes compared with MS alone, as a direct result of the orthogonal mobility and mass-to-charge separation (Fig. 5).

More recently, the combination of stable isotope labeling with high throughput direct infusion IM-MS was demonstrated for the qualitative and quantitative monitoring of biocatalytic reactions.[69] Advantages of the IM-MS approach included reduced analysis times, enhanced sensitivity, µL-level assay volumes and also greatly reduced the chemical noise. The capability of IM-MS was demonstrated by application to both lipase and monooxygenase enzymes, including multi-substrate screening. The quantitative potential was demonstrated for the detection of an amide product with linearity achieved between 10 nM and 10 µM.

4.2 | Miniaturized and portable mass spectrometers

Miniaturized, lightweight, and portable atmospheric pressure ionization (API) mass spectrometers further facilitate on-line MS for process analysis, by allowing the system to be positioned with any process under investigation. Snyder et al from Purdue University have recently reviewed the design challenges, development and applications of the now extensive range of miniature and field mass spectrometers (37 described in the

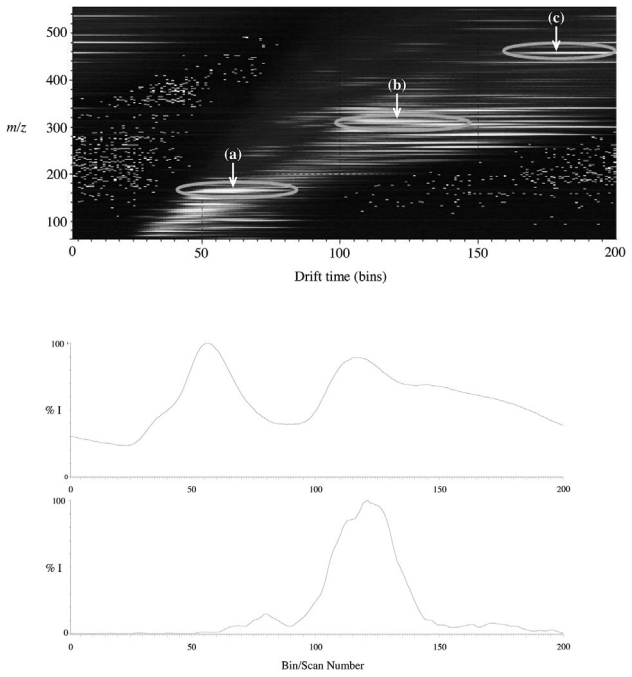


FIGURE 5 On-line IM-MS of the deprotonation of 7-fluoro-6-hydroxy-2-methylindole by sodium hydroxide. 2D plot of drift time versus m/z 1 hr after addition of aqueous sodium hydroxide ((A) is indole monomer, (B) is O-linked dimer and (C) is O-linked trimer. Lower plots are (A) ion mobility spectra of the reaction mixture and (B) selected ion mobility response for O-linked dimer (Reproduced from,[67] with permission from the Royal Society of Chemistry ©2011)

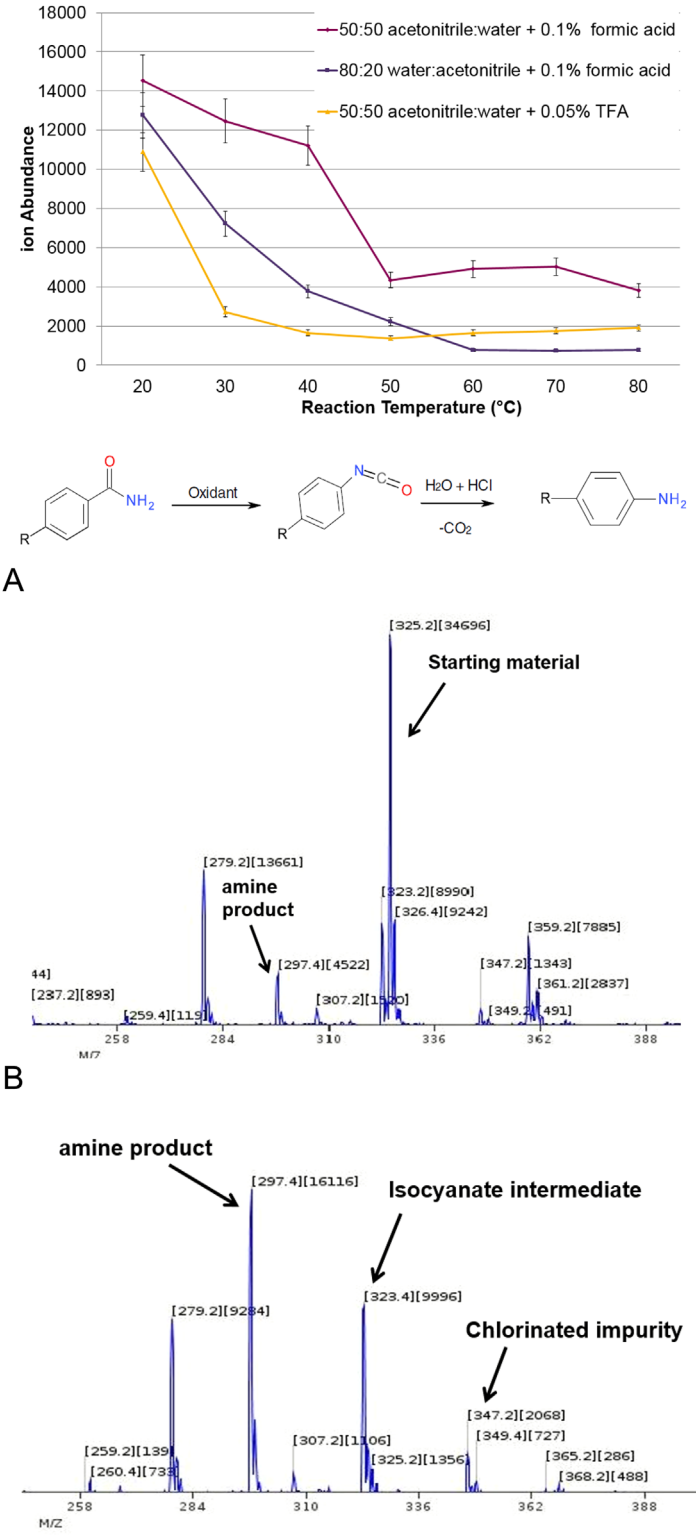


FIGURE 6 On-line MS of a Hofmann rearrangement reaction. Top figure is plot of change in ion abundance of starting material as a function of reaction temperature using three different make-up flows. Bottom figure are mass spectra showing formation of product and impurities at (A) 20°C and (B) 60 °C (Reproduced from,[4] with permission from the American Society for Mass Spectrometry Inc ©2014)

review). Many of these that have been developed and demonstrated within academia. However, the review also includes a number of commercially available small footprint instruments of the type being increasingly used for reaction monitoring in an industrial setting.[70]

The potential of such devices for reaction monitoring has been demonstrated in two recent publications. In the first, a device was coupled to a Uniqsis Flow Syn continuous flow chemistry reactor.[17] In the second, Bristow et al[4] monitored the progress of a continuous flow

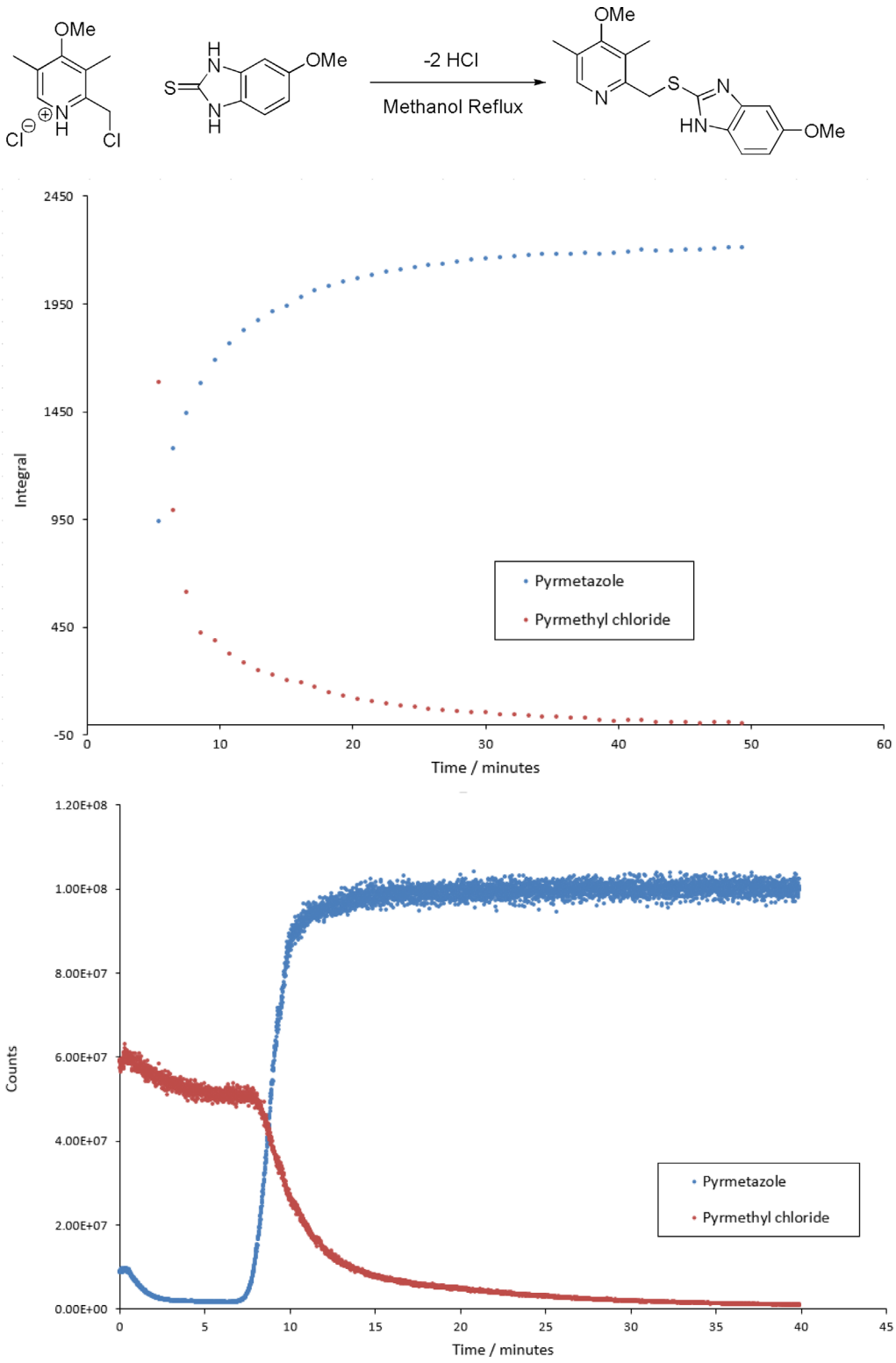


FIGURE 7 On-line NMR-MS. Top trace is response from on-line NMR, bottom trace is response from on-line mass spectrometry (Reproduced from,[4] with permission from Wiley Inc ©2016)

Hofmann rearrangement reaction. In this study, reactant dilution was achieved by transfer of small aliquots of sample solution into a sampling make-up flow, followed by electrospray ionization. The setup was able to detect at least seven compounds in the reaction mixture when used on-line. This study identified that optimization of the make-up flow composition and sample dilution factor are essential when evaluating a reaction, as they can have a pronounced effect on the observed MS response and subsequent reaction understanding (Fig. 6).

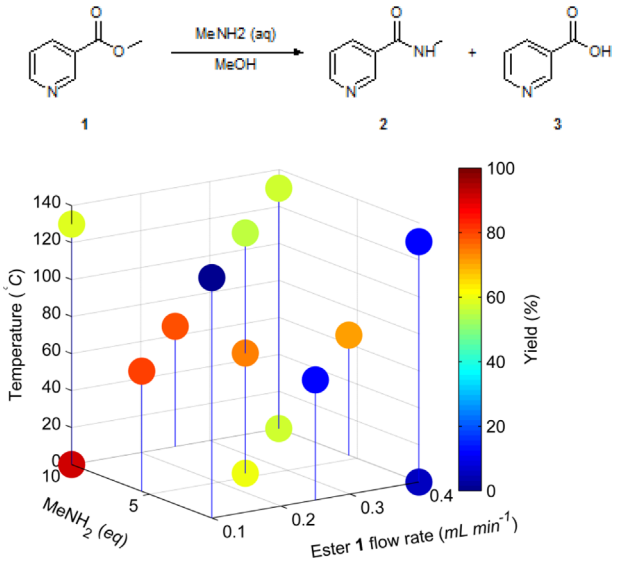


FIGURE 8 On-line MS of a self-optimising flow reactor (A) the reaction studied and (B) the graphical output of the self-optimisation (Reproduced from,[18] with permission from the Royal Society of Chemistry ©2016)

It is likely that the capability of miniaturized mass spectrometers will continue to be developed, enabling even more in-depth mass spectrometry experiments (tandem mass spectrometry for structural characterization and quantitation) to be carried out on dynamic processes in real time. Such potential has been demonstrated by researchers at Microsaic Systems in their publication describing a miniature triple quadruple mass spectrometer,[71] along with other ground breaking examples of miniaturization of mass spectrometers in a variety of application areas.[72–74]

One significant challenge for on-line MS is the ability to monitor a chemical synthesis across a very broad process concentration range. For example this could be as wide as 0.01-70.0 mg/mL. The lower end of this concentration range is not a major concern due to the inherent MS sensitivity and hence low level reactants and products will be easily detectable. However, a major challenge comes from the very high sample concentrations which would quickly result in detector saturation and therefore limit the quantitative relationship as a function of concentration. Further issues with quantitative on-line reaction monitoring arise due to the potential for suppression and enhancement effects that can be observed as no chromatographic separation is used. This could also have a detrimental effect on the ability to detect low level impurities, which can be limited by ion suppression effects.

However, there is progress on this technical challenge and examples of reaction monitoring at high concentration are found in the literature. Zhu et al used a FIA approach, with a post-injection splitter flowing into an APCI source, to analyze reactions at the molar concentration level.[75] Yan et al have described a multistage dilution system capable of application to concentrated reaction mixtures (between 0.1 and 3.9 mmol; up to 30 mg/mL) in combination with iESI.[24] Also, recognizing the potential quantitative limitations for on-line mass spectrometry for monitoring a reaction over a full industrial pharmaceutical chemical process range (0.01-70.0 mg/mL), Bristow and colleagues investigated the combination of a 500 MHz ^{1}H NMR and a small footprint mass spectrometer to monitor a batch reaction on-line. The mass spectrometer was coupled into the flow path of an on-line reaction monitoring NMR. Reaction mixture was pumped from a 100 mL vessel to an NMR flow tube before returning to the vessel. Small aliquots were diverted into a sampling make-up flow using an active flow splitter and passed to the mass spectrometer, with a typical 1000× dilution (Fig. 7).

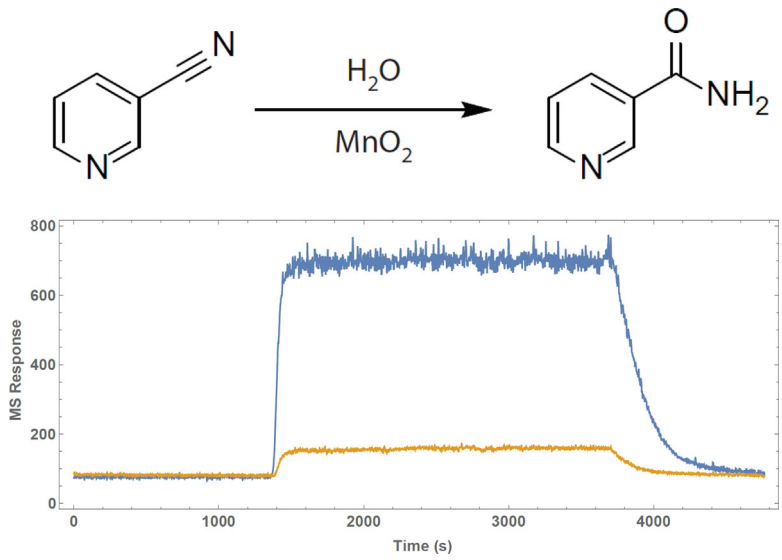


FIGURE 9 Automated optimisation of a synthetic procedure that employs online mass spectrometry (A) the reaction studied and (B) the output from on-line MS (blue line corresponds to starting material, while the orange line represents the product) (Reproduced from,[76] with permission from The American Chemical Society ©2016)

TABLE 1 Selected reactions studied by mass spectrometry

Reaction	Inlet method	Ionization technique	Reference
Tröger's base formation	Direct infusion from reaction in syringe	Electrospray	81
Morita-Baylis-Hillman	Venturi effect based direct sampling	V-EASI	55
Morita-Baylis-Hillman	On-chip reaction	Microfluidics/ nano-electrospray	64
Morita-Baylis-Hillman	Microsyringe pump direct infusion	Electrospray	82
Morita-Baylis-Hillman	Offline manual sampling	Electrospray	83
aza-Morita-Baylis-Hillman	Direct infusion from syringe driven flow reactor		84
Hofmann re-arrangement	Direct sampling with dilution by Mass Rate Attenuator	Electrospray	4
Isocyanate derivatization	Chip based reactor	Nanospray	85
Birch reduction	Reaction occurs in plasma	LTP	52
Maillard	Headspace gas by overpressure with dilution	PTR	35
Stille	Direct infusion from reaction in syringe	Electrospray	86
Protease-catalyzed	Super-fine dipped needle	Electrospray	87
Aldol	Infusion from microreactor with dilution *via* mixing tee	Electrospray	88
Mannich-type methylenation	Microsyringe pump direct infusion	Electrospray	89
Hydroformylation	Autoclave coupled to inlet, pressure of autoclave drives flow	Electrospray	90
Hydrodehalogenation	PSI	Electrospray	91
Methoxy and acetoxylation	Direct infusion from reaction in syringe	Electrospray	92
Chlorination of alcohols	Coupled microreator	Electrospray	93
Ugi	Direct infusion from reaction in syringe	Electrospray	94
Eschweiler-Clarke	DESI	DESI	95
Methacathinone from pseudoephedrine	Samples placed on glass slides and directly analysed	FAPA	50
Generation of benzyne *via* diazotisation of anthranilic acid, and its subsequent cycloaddition to furan.	Switching valve from flow with dilution *via* tee	Electrospray	17
N-methylation of an indole	Samples placed on tip of capillary tube	ASAP	51
Eberlin	Carrier gas flowed over reagent into plasma chamber	LTP	53
Imine formation	Carrier gas flowed over reagent into plasma chamber	LTP	53
Base hydrolysis	Mixture nebulised and sampled with venture pump.	EESI	58
Base hydrolysis	Flow of solvent across tee connected to tee in sample. Pumped thereafter with further dilution.	ESI	3
Michael addition	Carrier gas flowed over reaction mixture	EESI	57
Acetylation	Carrier gas flowed over reaction mixture	EESI	57
Conversion of fructose to 5-hydroxymethylfurfural	Gas jetted into solvent, overpressure carries mixture into source	EESI	59
Diels-Alder	Direct flow from microreactor	Electrospray	14
Ziegler-Natta polymerization	Direct flow from microreactor	Electrospray	8
Enzymatic reactions	Direct flow from microreactor	Electrospray	11
Zemplen	Ultrasonication and direct capillary from reaction mixture	UASI	19
Dehydration	Pressure driven with dilution	Electrospray	21
Acetylation	Low temperature plasma probe on surface desorbs analytes	LTP	54

(Continues)

TABLE 1 (Continued)

Reaction	Inlet method	Ionization technique	Reference
Condensation (Schiff base formation)	Low temperature plasma probe on surface desorbs analytes	LTP	54
Esterification	Low temperature plasma probe on surface desorbs analytes	LTP	54
Michael addition	Syringe pump *via* automatic injector into makeup flow	APCI	75
Eschweiler-Clarke methylation	Reaction conducted on filter paper on heated probe	PATI	62
Olefin deoxygenation	Reaction conducted on filter paper on heated probe	PATI	62
Amine-aldehyde condensation	Gravity flow capillary tube onto paper, voltage across paper induces spray	Paperspray	63
Addition-elimination	Gravity sampling through narrow capillary	Electrospray	22
Negishi cross- coupling	Positive gas pressure forcing solution through capillary	Inductive electrospray	23
Reductive amination	Positive gas pressure forcing solution through capillary	Inductive electrospray	23
Pd/C catalysed aldehyde hydrogenolysis	Positive gas pressure forcing solution through capillary	Inductive electrospray	23
Isocyanate reaction with amine to form urea	Pulley based gradual pulling of sample through source.	DART	49
Heterogeneous hydration of 3-cyanopyridine to the corresponding amide	Sampling from flow via mass rate attenuator	Electrospray	76
Claisen-Schmidt condensation	Direct spray of reaction mixture into instrument with syringe pump	Electrospray	45
Esterification	Direct pump from reaction vessel into thermal vaporiser	EI	37
RAFT polymerisation	Direct flow from chip based microreactor	Electrospray	16
Deprotonation by sodium hydroxide	Direct infusion from reaction in syringe	Electrospray (IM-MS)	67
Nucleophilic substitution	Pumped from reaction mixture, dilution by mass rate attenuator	Electrospray	6
Reaction of methyl nicotinate with aqueous methylamine	Sampling valve switching from reaction flow into makeup flow	APCI	18
Mannich	Direct spray from chip base microreactor	Microfluidics/ nano-electrospray	65
Lipase-catalysed oxidation	Samples centrifuged then directly injected.	Electrospray (IM-MS)	69
Ketone-Sulfite reaction	Rotating ball MIMS	EI	96
Photolysis of C-I bond in Methanol	Multi pump sampling loop with dilution	ESI	97
Knoevangel condensation	Multi-pump direct sampling with quench, dilution and HCl addition	ESI	2

Advantages of the combination were observed. ^{1}H NMR was ideal for quantitation of high level components, whereas MS showed a greater capability for detecting those at low level. In preliminary experiments MS produced a limited linear relationship with concentration (0.02% to 2% relative concentration, 0.01-1.25 mg/mL), due to signal saturation at the higher concentrations. NMR was unable to detect components below 0.1% relative to concentration maximum. However, there still remained the challenge of extending the linear dynamic range of MS, while maintaining the ability to detect low level impurities. Optimization of sample transfer (and dilution) to the mass spectrometer extended the linearity to 10% relative to the concentration maximum (7 mg/mL). Therefore, the combination of on-line NMR and MS allowed both qualitative and quantitative analysis of reaction components over the full process range. It is recognized, however, that further technical

developments are required to provide dilutions in excess of 10,000× that are required for MS response linearity over full process range.

5 | THE FUTURE

5.1 | Real time reaction control and optimization

A vision of the future is one where automated control and optimization is achieved using analytical technology including MS. Richard Bourne and colleagues have described an automated reactor for chemical synthesis that uses on-line MS for reaction monitoring, product quantification and real time optimization.[18] Fully automated optimization of the reaction was carried out using the SNOBFIT algorithm and a design of experiment statistical approach. The output of the experiment is a multi-dimensional model which can be used to select the optimum conditions via a number of criteria including reaction yield, flow rates, and temperature (Fig. 8).

Steve Ley and colleagues have also developed a system that allows the automation and optimization of synthetic procedures and employs on-line MS to monitor the reaction.[76] The internet based control system (named LeyLab) can be operated by any researcher to monitor and control their chemical reactions and was demonstrated for a number of reactions including heterogeneous hydration of 3-cyanopyridine to the corresponding amide (Fig. 9).

6 | CONCLUSIONS

In the last 10 years, there has been an upsurge in interest in on-line reaction monitoring as illustrated by the number of reactions shown in Table 1. The range of ionization modes has greatly increased driven by the development of a multitude of ambient ionization techniques. Mass spectrometers have become smaller making it easier to position them with a reaction system or process. The reduced cost of these smaller mass spectrometers also makes it easier for them to be integrated into the standard laboratory workflow. The development of self-optimizing systems may greatly speed up the time taken to understand and optimize a reactions, saving the synthetic chemist many hours that result from the need to repeat reactions or carry out off-line analysis.

Although much progress has been made, many challenges remain. These include (a) representative sampling from, and the analysis of, heterogeneous systems; (b) the issue of specificity that can result from direct analysis of a complex reaction system and also the potential ion suppression; and (c) the clear requirement to extend the dynamic range of on-line MS for quantitative analysis of high concentration reaction mixtures.

Finally, it should be noted that the developments in on-line monitoring with MS has also led to its application in other areas such as dissolution,[5,61] environmental monitoring,[77] and cell metabolism studies.[78] The recent developments in direct volatile organics analysis (such as in breath) are likely to be applicable to off-gas analysis with the potential to monitor both the condensed and aqueous phase of a reaction for a more complete understanding of the chemistry.[79,80]

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How to cite this article: Ray A, Bristow T, Whitmore C, Mosely J. On-line reaction monitoring by mass spectrometry, modern approaches for the analysis of chemical reactions. *Mass Spec Rev*. 2018;37:565–579.
https://doi.org/10.1002/mas.21539