A New Mass Spectrometry Based Approach for Organic Synthesis Monitoring

Veronica Termopoli[1‡], Gregory W. Vandergrift[2,3‡], Elena Torrisi[4], Giorgio Famiglini[1], Pierangela Palma[1,2], Giovanni Zappia[4], Achille Cappiello[1,2]*, Misha Zvekic[2], Erik T. Krogh[2,3], Chris G. Gill[2,3,5,6]*

[1] LC-MS Laboratory, Department of Pure and Applied Sciences, University of Urbino Carlo Bo, Urbino, Italy

[2] Applied Environmental Research Laboratories (AERL), Chemistry Department, Vancouver Island University, Nanaimo, BC, Canada, V9R 5S5

[3] Chemistry Department, University of Victoria, Victoria, BC, Canada, V8P 5C2

[4] Biomolecular Sciences Department, University of Urbino Carlo Bo, Urbino, Italy

[5] Chemistry Department, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

[6] Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA, 98195

ABSTRACT

Current mass spectrometry-based methodologies for synthetic organic reaction monitoring largely use electrospray ionization (ESI), or other related atmospheric pressure ionization-based approaches. Monitoring of complex, heterogeneous systems may be problematic because of sampling hardware limitations, and many relevant analytes (neutrals) exhibit poor ESI performance. An alternative monitoring strategy addressing this significant impasse is condensed phase membrane introduction mass spectrometry using liquid electron ionization (CP-MIMS-LEI). In CP-MIMS, a semi-permeable silicone membrane selects hydrophobic neutral analytes, rejecting particulates and charged chemical components. Analytes partition through the membrane are then transported to the LEI interface for sequential nebulization, vaporization, and ionization. CP-MIMS and LEI are both ideal for continuous monitoring applications of hydrophobic neutral molecules. We demonstrate quantitative reaction monitoring of harsh, complex reaction mixtures (alkaline, acidic, heterogeneous) in protic and aprotic organic solvents. Also presented are solvent-membrane compatibility investigations, and *in situ*, quantitative monitoring of catalytic oxidation and alkylation reactions.

Introduction

The synthesis of organic compounds on an industrial scale is of enormous economic importance. Understanding optimum reaction conditions is key to maximizing the yields of desired products, as well as simultaneously reducing the production of inadvertent side products, which lower yields, may be harmful, and require additional separation steps. Synthetic chemists have a wide variety of analytical methodologies at their disposal to investigate and optimize reaction conditions for industrial applications. These include spectroscopic methods (e.g. FT-IR, UV-vis, fluorescence, NMR, etc.)[1-3] as well as mass spectrometry (MS)[4]. However, many of these techniques are carried out off-line, requiring subsample collection and rapid analysis, frequently with some form of quenching. This is because the reaction will continue in the subsamples until the time of measurement, potentially compromising any information obtained. Online dilutions of subsampled reaction mixtures are also frequently employed to make satisfactory measurements, reducing sensitivity for trace analytes. Kinetic data obtained in this manner is laborious and often intermittent. A number of spectroscopic techniques have been adapted for continuous online monitoring[5,6], but these tend to lack specificity for target analytes and/or the sensitivity for quantitation of trace components.

To increase both sensitivity and selectivity for quantitative online reaction monitoring, chemists have largely turned to MS based approaches, as resolution by *m/z* ratios is more selective and reliable when compared to other chemical analysis strategies (*i.e.*, monitoring chromophores). Atmospheric pressure ionization sources, particularly electrospray ionization (ESI), have been used extensively for coupling reactions to MS. As a recent example, the Cooks group has demonstrated online, multiplexed MS reaction monitoring system based upon direct sampling ESI-MS, simultaneously monitoring up to six reactions types without sample carryover[7]. The McIndoe group has also been exploring the use of online ESI-MS monitoring to investigate catalytic reactions[8-10]. Many other variants of ESI-MS have been used for reaction monitoring, including desorption ESI (DESI), extractive ESI (EESI), ESI assisted laser desorption ionization (ELDI) and paper spray (PS-MS)[4]. However, for many ESI-based techniques, care must be taken to ensure that clogging of fine sampling capillaries does not occur. This may preclude their use in complicated, heterogeneous reaction slurries or for small reaction volumes, as the monitoring itself consumes a portion of the reacting mixture.

While the Cooks group has bypassed many practical challenges of this strategy by using inductive ESI[11,12], perhaps the most significant obstacle associated with ESI related monitoring techniques is the requirement that the chemical species being monitored must be satisfactorily ionized by ESI (*e.g.* easily charged species). ESI sensitivity is further confounded by high salt concentrations and varying solvent compositions[13,14], excluding the use of these techniques from many reaction monitoring applications because of analyte and reaction mixture incompatibility. Other ambient ionization techniques, such as direct analysis in real-time (DART)[15], have been used to bypass ESI ionization complications encountered in reaction monitoring in principle. In a particularly intriguing application of DART-MS/MS, the Volmer group monitored micro-reactions in acoustically levitated droplets[16]. However, DART based MS methods often lack reliable quantitative information, and are predominantly restricted to offline, snapshot measurements[4].

As an alternative sample introduction strategy for online reaction monitoring, the method of membrane introduction mass spectrometry (MIMS) may offer a suitable solution to the identified deficiencies. There are several reviews of MIMS that explain the methodology, as well as its suitability for direct, online monitoring in complex sample mixtures[17,18]. Simply described, MIMS utilizes a semi-permeable membrane, often polydimethylsiloxane (PDMS, aka silicone), to non-exhaustively extract and directly transfer analytes (as a mixture) to a mass spectrometer, where they are resolved by their *m/z* ratios, by selective ionization, and/or by tandem mass spectrometry (MS/MS). The membrane rejects particulate materials and is hydrophobic, with perm-selectivity characteristics favoring mass transfer of non-polar analytes, rejecting highly polar and charged components, making MIMS ideally suited for the online extraction of neutral compounds from a complex reaction mixture[17,18].

There is some precedent in the literature for the use of MIMS for reaction monitoring utilizing electron ionization (EI). As a few examples, in early work, the Cooks group used a silicone membrane interface to transfer permeating analytes directly into a high vacuum EI triple quadrupole MS. While effective, the membrane interface design was also prone to memory effects and slow signal response times[19]. The Cooks group also demonstrated MIMS use for the online monitoring of the reactions of epichlorohydrin in water using a polyphenyl ether liquid membrane and a quadrupole ion trap[20]. Our group has used a flow cell interface MIMS system to follow the oxidative degradation kinetics for trace gasoline contaminants in aqueous samples[21] and also the reductive dehalogenation kinetics of organic contaminants in natural waters[22]. A commonality with these early MIMS reaction-monitoring studies was the use of a gaseous acceptor (or high vacuum) to desorb permeating analytes from the membrane, and frequently from aqueous reaction systems. While effective for smaller analytes of higher volatility, these strategies were ineffective for larger, less volatile analytes. Alternatively, permeating analytes in a MIMS measurement can be transferred from the membrane by a continuously flowed liquid (condensed) phase, and then coupled

with a variety of atmospheric pressure ionization strategies. This approach has been termed condensed phase MIMS (CP-MIMS), and has been described in several reviews[17,18].

The use of CP-MIMS has allowed researchers to utilize a variety of MS ionization strategies, including ESI, atmospheric pressure chemical ionization (APCI)[23-28] and liquid electron ionization (LEI),[29-31] to extend the application of MIMS to larger, significantly less volatile analytes. In early work, Creaser and co-workers demonstrated the use of a CP-MIMS type interface system with a hydrophobic polyvinylidene fluoride microporous sheet membrane coupled with APCI to follow the course of a synthetic reaction, implemented by sampling reaction aliquots as a function of time[32]. Additionally, we have presented the real-time monitoring of aqueous chlorination reactions,[24,27] as well as the photo-oxidative degradation[28] and adsorption of naphthenic acids in aqueous solutions[18] by CP-MIMS using ESI. The coupling of LEI with CP-MIMS is of particular interest for reaction monitoring, as it is a more universal ion source and overcomes a limitation with ESI, which typically requires polar functional group to carry a charge. Thus, CP-MIMS paired with LEI represents an ideal monitoringstrategy for synthetic organic reactions, especially for measuring neutral hydrophobic analytes that exhibit poor performance with ESI.

The LEI concept has been discussed in several recent publications[30,31]. It represents a new approach for efficiently addressing the difficult conversion of a liquid-phase into a gas-phase for EI, using a nano-scale flowrate, generating library searchable spectra. Since ionization occurs in the gas phase, LEI strongly mitigates suppression effects from co-eluting reaction components permeating through the membrane, offering reliable quantitative reaction data.

We present the newer variant of LEI[33] paired with CP-MIMS, as a strategy for direct, quantitative online monitoring directly in organic solvent reaction mixtures. Permeating neutral analytes are effectively vaporized and ionized directly by LEI, where the vaporization and ionization steps are spatially decoupled. As previously mentioned, LEI is effective for ionizing analytes with poor ESI performance, and as presented with CP-MIMS, addresses a significant gap in currently available online quantitative reaction monitoring strategies. This manuscript presents the first use of CP-MIMS coupled with LEI for quantitative online monitoring of non-aqueous synthetic organic reactions. The resulting system is robust, and facilitates direct, quantitative measurements of neutral reactant and product species in complex, highly acidic and heterogeneous organic solvent reaction mixtures.

Experimental section

Standards and Solvents

All stock analyte solutions were prepared gravimetrically. Analyte sub-stocks and online additions of analytes were done volumetrically using a mechanical micropipette. Acetophenone (99%), biphenyl (99.5%), chlorobenzene (99.5%), and phenylacetylene (98%) were obtained from Sigma Aldrich (Oakville, ON, Canada). *(R)*-α-methyl benzylamine was obtained from Sigma Aldrich (Milan, Italy). Cyclohexane, NaCl, Na_2SO_4 were obtained from Carlo Erba Reagents (Milan, Italy). Ethyl bromoacetate and triethylamine were purchased from VWR International (Milan, Italy). Acetonitrile, methanol, (HPLC grade) and ethyl acetate (99.9%) were obtained from VWR International (Edmonton, AB, Canada). Reagent alcohol (90% ethanol, 5% 2-propanol, 4.5% methanol, HPLC grade) and N,N-dimethylformamide (99.9%) were obtained from Fisher Scientific (Ottawa, ON, Canada). Reagent alcohol is referred to as ethanol for the purposes of this study. Dichloromethane (≥99.5%) was obtained from Sigma Aldrich.

All analytical standards and reactants were ACS grade or better. Details of the synthesis and purification of some of the product standards as well as suppliers for the various reagents and solvents used are given in the Supporting Information.

All standard and reaction measurements were made in magnetically stirred 20 or 40 mL glass vials with Teflon faced septum seals (EPA/VOA Type, Scientific Specialties Inc., Hanover, MD, USA) at ambient conditions (*ca* 25 °C, 101 kPa).

Instrumentation

Two EI-MS systems were employed for these studies, including a single quadrupole system (Model 5975, Agilent Technologies, Santa Clara, CA, USA) operated in selected ion monitoring mode (SIM), and a triple quadrupole tandem mass spectrometry (MS/MS) system (Model 7010, Agilent Technologies, Santa Clara, CA, USA) operated in multiple reaction monitoring mode (MRM). Both MS systems were equipped with identical CP-MIMS and LEI configurations. Details of the SIM and MRM conditions used for all analytes are given in the supporting information (Table S-1). In addition, for the MS/MS system, concomitant changes in the UV/Vis absorbance of post membrane permeant was also continuously monitored by diverting the splitter waste flow through a capillary UV-Vis detector (Spectra 100, Spectra Physics Inc., Santa Clara, CA). MS tuning parameters were checked periodically, and remained constant for the studies shown here, demonstrating robustness and ease of use.

Results and Discussion

CP-MIMS-LEI Instrumentation

The base CP-MIMS-LEI experimental system has been described elsewhere[30]. A schematic diagram of the specific configuration used for synthetic reaction monitoring is given in Figure 1. Several modifications were necessary to reduce the analyte flux transferred to the MS ion source, allowing successful monitoring of high concentrations present in synthetic reaction mixtures. These included a shorter hollow fibre polydimethylsiloxane membrane (5.0 mm long, 0.30 mm i.d., 0.64mm o.d., 170 µm thick), an increased methanol acceptor phase flowrate through the membrane lumen (100 µL/min), and drastically reducing acceptor phase flowrate to the LEI interface (*ca* 50 nL/min); all of which act to reduce the mass transfer of analyte to the MS. The sensitivity of the presented system is highly tunable, and may be adjusted according to the requirements of a given online reaction monitoring application.

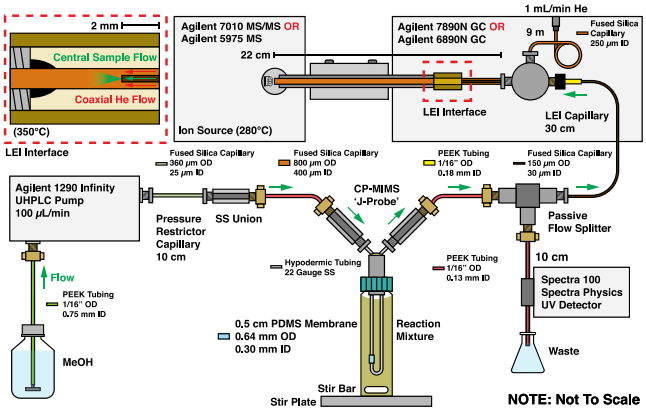

Figure 1. Schematic diagram of the modified CP-MIMS-LEI system.

Solvent-Membrane Compatibility Investigations

To date, the majority of CP-MIMS type measurements have been made in aqueous samples[23,25,29,30]. It is known that solvent-membrane solubility can significantly influence membrane permeability[34-36]. We have begun to exploit acceptor phase co-solvents with CP-MIMS by forming *in situ* polymer inclusion membranes, which improve both the sensitivity and measurement duty cycle[30,35]. In addition, recent developments utilizing modified donor phases (*e.g.* mixed organic sample solvents) with CP-MIMS have

shown promise for making direct measurements of fatty acids[37]. However, because synthetic reactions are most often conducted in non-aqueous solvents, the analytical performance of PDMS membranes in a variety of non-aqueous sample solvents was investigated.

A series of individual standards containing biphenyl or chlorobenzene in the 10 to 50 mM range were prepared in a wide range of common protic and aprotic solvents that could potentially be used for organic syntheses (acetonitrile, dichloromethane, N,N-dimethylformamide, ethanol, methanol). Analyte signals for CP-MIMS-LEI measurements for each standard were allowed to reach steady state, and were background-subtracted using the signal from the appropriate solvent blank (Table S-2 and S-3). In all cases, calibrations showed satisfactory linearity ($R^2 \geq 0.98$), suggesting broad applicability of CP-MIMS-LEI quantitative reaction monitoring in different solvent/reaction systems. The order of the relative sensitivities for both analytes (*i.e.* calibration slopes) in the different solvents was conserved., and faster $t_{10\text{-}90\%}$ signal response times also correlated with greater sensitivities. These trends may be understood by considering the intrinsic factors governing membrane transport. For a given analyte, the steady-state signal intensity for a CP-MIMS measurement is related to membrane permeability (P), which can be expressed as the product of analyte diffusivity through the membrane (D_m) and the partitioning constant of the analyte between the membrane and the sample ($K_{m\text{-}s}$, Eq.1)[38]:

(1) $P = K_{m\text{-}s}\, D_m$

The time required for an analyte signal to reach steady-state can be expressed as the $t_{10\text{-}90\%}$ response time and is inversely proportional to D_m (Eq. 2)[39].

(2) $t_{10\text{-}90\%} \propto 1 / D_m$

The differing analyte response times in different solvents indicate changes in the corresponding diffusivities (D_m), as these solvents swell membrane to varying extents and change their permeation characteristics. However, it is clear that the permeability is also affected by concomitant changes in the partitioning behavior ($K_{m\text{-}s}$) in different solvent systems.

Simultaneous Time Resolved MS and UV-Vis Detection

Because a large fraction of the (post-membrane) acceptor phase is diverted from the CP-MIMS-LEI combination via the passive flow splitter (Figure 1), this stream can also be analyzed by other continuous analyzers to provide orthogonal datasets. To demonstrate this, the diverted acceptor phase waste was passed through a capillary UV-Vis detector. Figure 2 illustrates an example of an orthogonal time series dataset obtained from MS and UV-Vis detection of a 25 mM chlorobenzene standard prepared in methanol. As expected, the data illustrates identical time resolved signals, with superior signal-to-noise for the MS trace (due to the lower sensitivity of UV-Vis absorbance measurements). The time constant associated with the rise to steady-state signal is correlated with membrane transport kinetics and depends on a variety of factors including the membrane thickness, the size of the permeant and the presence of solvent co-permeants. The $t_{10\text{-}90\%}$ signal risetime for chlorobenzene under these conditions is 0.33 s, typical for the compounds studied here. The technique is therefore well suited for monitoring reactions that take place on the time scale of minutes to hours, applicable for a wide range of synthetic organic reactions. While spectroscopic methods can be utilized to characterize a number of reactions based upon changes in their optical properties, they are inherently less selective than MS, and may therefore be challenging in complex reaction mixtures with overlapping chromophores. This highlights the advantages of MS based reaction monitoring strategies.

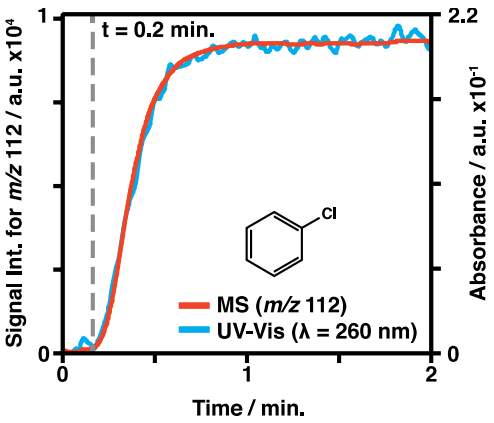

Figure 2. Comparison of simultaneous online monitoring data measured by CP-MIMS-LEI (m/z 112) and parallel UV-Vis spectrophotometry detection (λ=260 nm) for 25 mM chlorobenzene in methanol (spiked at t = 0.2 minutes).

Online Synthetic Reaction Monitoring Examples

As a first demonstration of CP-MIMS-LEI for *in situ*, continuous reaction monitoring, the catalytic oxidation of phenylacetylene to acetophenone in highly acidic methanol solvent was examined (Figure 3) using a single quadrupole MS system (SIM mode). Phenylacetylene undergoes acid-catalyzed hydrolysis, and the reaction rate is greatly increased in the presence of a chloroauric acid ($HAuCl_4$) catalyst. As shown in Figure 3, 90 mM phenylacetylene (*m/z* 102) was added to the reaction mixture (95:4:1 methanol:de-ionized water:sulfuric acid v/v, 4 mM $HAuCl_4$) at t = 0 min, followed by rapid catalytic conversion to the Markovnikov-favoured addition product, acetophenone (*m/z* 105, 100% yield) with gentle heating (~50°C). Concentrations were determined using direct calibrations made with methanol standards, and the $t_{10\text{-}90\%}$ signal rise times were ≤0.4 s (Table S-4), whereas the reaction occurred over *ca* 30 minutes. The presence of acetophenone was monitored by *m/z* 105 ($[M\text{-}CH_3]^+$), chosen since it is free of isobaric interference from phenylacetaldehyde, a possible non-Markovnikov product. No substantial amount of phenylacetaldehyde was formed under the given reaction conditions, indicated by the stoichiometric mass balance illustrated in Figure 3.

The online reaction monitoring of phenylacetylene oxidation to acetophenone illustrates the significance of MIMS-LEI pairing, as both the reactant and product are incompatible with ESI based monitoring systems. As shown by the photo insets in Figure 3, the reaction is occurring in a heterogeneous (cloudy) system, which would pose serious problems if direct capillary sampling strategies (*e.g.* ESI) were employed. The PDMS membrane provides an on-line 'clean-up' that effectively removes particulates, and charged corrosive components (*e.g.* sulfuric acid, chloroauric acid catalyst), while allowing hydrophobic molecules to permeate for continuous mass spectrometric analysis. While some oxidative damage was observed for the PDMS membrane near the completion of the reaction (due to the high concentration of sulfuric acid, ~1% v/v in methanol), the membrane and/or probe assembly are both inexpensive and easily replaced within minutes, without the need to vent the MS. The LEI interface required no maintenance during the experiments here, but the LEI capillary may also be simply replaced as needed without venting the MS.

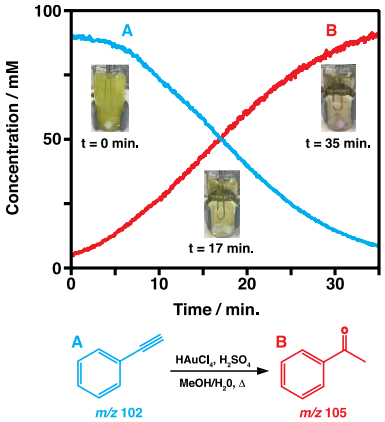

Figure 3. CP-MIMS-LEI online monitoring of the catalytic oxidation of phenylacetylene to acetophenone under highly acidic conditions.

To further demonstrate the utility of CP-MIMS-LEI for online reaction monitoring, the synthesis of alkyl glycinates in an aprotic solvent was examined. Alkyl glycinates have been used as intermediates for the preparation of α-amino acids[40], as well as building blocks in the preparation of peptidomimetics[41]. A synthesis strategy is presented in the reaction scheme given in Figure 4. While advantageous because of the mild reaction conditions, this reaction can be plagued by over-alkylation products, reducing the yield of a desired product[42]. In this context, simultaneous monitoring of the formation of various alkylation products is of significant synthetic interest.

For this demonstration, the MS/MS system was employed. To initiate the reaction, 1 mL of ethylbromoacetate (initial concentration of 330 mM) was added to the magnetically stirred reaction mixture (330 mM triethylamine, 300 mM α-methyl benzylamine in dry acetonitrile) at t = 2 min. Production of both monoalkylated (major product, 70% Yield) and dialkylated (minor product, 0.7% Yield) species was observed (Figure 4). The synthesis was conducted in acetonitrile with a basic catalyst, yielding an alkaline reaction mixture. Under these conditions, both the reactants and products are neutral, and successfully monitored by CP-MIMS-LEI. If attempted, direct sampling ESI reaction monitoring would not be satisfactory, as it would require post-sampling pH adjustments to protonate the analytes, as well as dilution steps. Figure 4 also illustrates that the ethylbromoacetate alkylation reagent is consumed at a faster rate than α-methyl benzylamine. This is likely due to formation of the dialkylated product and the presence of trace moisture in the reaction system, possibly present in the triethylamine catalyst used. Information such as that shown in Figure 4 may be used for rapid, online reaction optimization, as the real-time data allows for continuous assessment of reagent purity, consumption, and production of possible side-products.

All reagents and products for this study demonstrated linear direct calibrations over the concentration ranges presented, and the $t_{10\text{-}90\%}$ signal rise times were significantly faster than the observed reaction rates (Table S-5). Even for reactions where product crystallization occurred over the course of a measurement (Figure 5), free solution concentration changes for reactants and products were still observable in these heterogeneous slurries, further demonstrating the robustness of the CP-MIMS-LEI synthesis monitoring strategy.

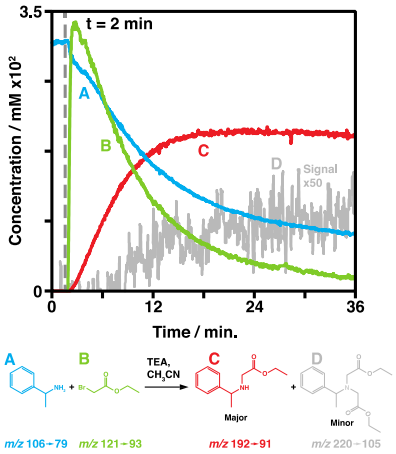

Figure 4. CP-MIMS-LEI demonstration of the quantitative online monitoring of an alkyl glycinate synthesis in dry acetonitrile with triethylamine (TEA) catalyst.

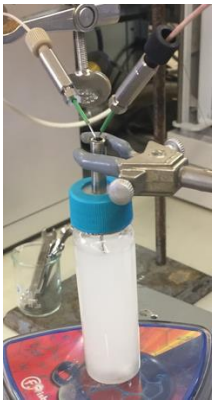

Figure 5. Photograph of an alkylation reaction mixture with CP-MIMS-LEI probe in place illustrating crystallized product formation.

Conclusions

The coupling of CP-MIMS and LEI exploits advantages of both methodologies, resulting in a robust system that allows the direct measurements of neutral organic analytes in complex, heterogeneous and corrosive reaction mixtures. CP-MIMS and LEI are ideally paired to suit the online measurement of a variety of synthetic reactants and products. Further, CP-MIMS-LEI quantitatively measures molecules that are not amenable to other online reaction monitoring strategies, such as direct sampling ESI approaches, therefore addressing a significant gap in current synthetic reaction monitoring methodology using mass spectrometry. The quality of online quantitative data is ensured by both membrane selectivity and LEI's capacity to operate in the presence of co-permeating reagents, with concentrations varying in time. Future work includes the use of online CP-MIMS monitoring simultaneously employing both LEI-MS and ESI-MS to enable the simultaneous online measurement of both charged and neutral species in synthetic reactions.

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