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Real-time, on-line monitoring of organic chemical reactions using extractive electrospray ionization tandem mass spectrometry

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Extractive electrospray ionization mass spectrometry (EESI-MS) for real-time monitoring of organic chemical reactions was demonstrated for a well-established pharmaceutical process reaction and a widely used acetylation reaction in the presence of a nucleophilic catalyst, 4-dimethylaminopyridine (4-DMAP). EESI-MS provides real-time information that allows us to determine the optimum time for terminating the reaction based on the relative intensities of the precursors and products. In addition, tandem mass spectrometric (MS/MS) analysis via EESI-MS permits on-line validation of proposed reaction intermediates. The simplicity and rapid response of EESI-MS make it a valuable technique for on-line characterization and full control of chemical and pharmaceutical reactions, resulting in maximized product yield and minimized environmental costs. Copyright © 2008 John Wiley & Sons, Ltd.

Obtaining comprehensive information on chemical reactions is crucial for the characterization of reaction mechanisms as well as the maximization of production efficiency in the chemical and pharmaceutical industries. Usually, detection of process deviations and prompt modification of reaction conditions are key to achieving the best control of chemical reactions. However, this demands techniques that are suited for real-time, on-line monitoring of the chemical reaction processes. Among many other benefits, real-time, on-line characterization allows identification of theoretically proposed transients, which are usually short-lived species of low concentration, resulting in a better understanding of the reaction mechanisms. This improved understanding will allow the design of superior reaction schemes with higher efficiency and minimized cost. Suitable techniques for on-line monitoring of chemical reactions require high sensitivity, high specificity and fast response. Mass spectrometry-based methods are of particular interest for the on-line analysis of reactions,[1] due to their high sensitivity and high specificity. Tandem mass spectrometry (MS^n) is often used to acquire kinetic information on chemical reactions and to characterize the reaction intermediates in solution, providing advances in mechanistic studies in organic chemistry.[2,3] Although direct infusion electrospray ionization spectrometry (ESI-MS)[4–9] and membrane introduction mass spectrometry (MIMS)[10–12] are gaining popularity in this field, both techniques require a series of steps and specially designed equipment to complete the sample pre-treatments (e.g. extraction, separation, dilution, etc.), and this can cause a delay of several minutes in the analysis.[8–10] Moreover, ESI signal variations can occur due to changes in solution composition.[13] To address the delay problem, rapid mixing has been coupled to direct infusion ESI-MS to acquire pre-steady-state information of fast reactions, decreasing the delay to several tens of ms.[14] Even so, rapid mixing is not suitable for on-line monitoring of process scale reactions. MIMS is more amenable to compounds with appreciable vapor pressure and favorable permeability, which depends on the properties of the membrane used and the compounds being studied. Therefore, MIMS cannot be generally used for monitoring of organic chemical reactions. Recently, direct analysis in real time (DART) has been applied for reaction monitoring in drug discovery.[15] In the DART approach, the end of a tube was dipped into a solution to fetch analytes, and then put in front of a heated DART ion source. After volatilization of the solvent, the analytes on the glass surface were ionized, and then directed to the mass spectrometer for analysis.[15] However, the high temperature (up to 250°C) could cause degradation of sensitive compounds.[15]

Alternatively, neutral analytes in gaseous, liquid, aerosol form or liberated from a surface can be rapidly and directly detected by extractive electrospray ionization (EESI)-MS,[16–22] without any sample pre-treatment. In addition, EESI may be applicable to reaction suspensions and heterogeneous reaction mixtures which would otherwise be impossible to

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analyze by direct flow injection analysis. EESI has been successfully used to monitor complex mixtures (e.g. raw urine, milk, etc.),[16] showing its potential for on-line, real-time monitoring of trace amounts of chemicals.

We have extended the application of EESI to instantly follow organic chemical reactions in a straightforward manner, with a rather simple setup. Two important chemical reactions were monitored in real-time: a one-step Michael addition reaction of phenylethylamine (PEA) and acrylonitrile in ethanol, and a multiple-step acetylation reaction of benzyl alcohol with acetic anhydride catalyzed by 4-dimethylaminopyridine (4-DMAP) in dichloromethane. The ongoing reactions are not disturbed by the EESI-MS analysis, which is carried out on a quadrupole time-of-flight (Q-TOF) mass spectrometer. The relatively simple setup allows this method to be implemented on any type of MS instrument equipped with an ESI/APCI interface. The EESI technique provides an instant response and does not require sample pre-treatment, making it a powerful and convenient tool for the on-line characterization and full control of chemical and pharmaceutical reactions in real time.

EXPERIMENTAL

In the EESI source, the electrospray tip was placed 8 mm away from the cone inlet of the mass spectrometer at a 40° angle from the axis of the sampling cone (shown in Fig. 1). By introducing an intermittent, or if necessary continuous, N_2 gas flow (50 L/h) through one neck of a 100-mL three-necked flask with the middle neck capped, the compounds emerging from the bulk reaction solution were sampled at regular intervals, or continuously through the third neck, split in case of saturation, and then transported separately through a 30 cm long piece of Teflon tubing (6 mm, i.d.) heated to 80°C. The angle between the electrospray tip and the Teflon tubing was 60°, the ending of the tubing was 6 mm away from the cone inlet and 4 mm away from the sprayer orifice. A solvent mixture (methanol/water/acetic acid 40%/40%/20%) was electro-sprayed at a flow rate of 5 μL/min infused by a syringe pump (Harvard Apparatus, Holiston, MA, USA). The ESI voltage was +3 kV and the cone voltage was 40 V. The Q-TOF mass spectrometer (QTOF UltimaTM, Micromass/Waters, Manchester, UK) was running in positive ion detection mode, while other parameters were maintained at default values as suggested by the manufacturer. By taking into account the dead volume of the transporting line after adding all reactants and the flow rate of the N_2 gas, it can be deduced that the chemicals in the reaction mixture can be detected in less than 0.2 s. This time could easily be further reduced by taking a higher flow rate or a shorter transportation line, or both. The spectra were recorded for 40–60 s while the carrier gas was on, and followed by background subtraction over the *m/z* 50–800 range (MassLynx 4.0, Waters, Manchester, UK). Collision-induced dissociation (CID) was performed at a collision energy of 10–25 arbitrary units, as defined by the manufacturer.

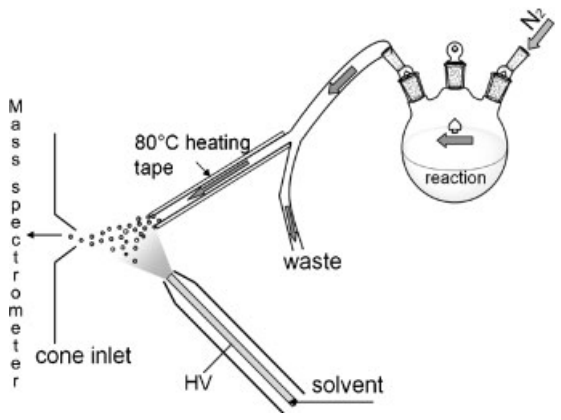

Figure 1. Schematic view of the EESI setup.

PEA (99%), benzyl alcohol (HPLC), acetic anhydride (HPLC), methanol (99% pure), UHP water, acetic acid (99%), and 4-DMAP (99%) were obtained from Fluka (Buchs, Switzerland), acrylonitrile (99%) from Acros (Geel, Belgium) and ethanol (HPLC) from Merck (Darmstadt, Germany). Dichloromethane was purchased from J.T. Baker (Deventer, The Netherlands).

RESULTS AND DISCUSSION

The Michael addition reaction of phenylethylamine (10.4 mL) and acrylonitrile (12.5 mL) stirred in ethanol (27 mL) occurs easily and can be run at room temperature. The reaction gives a good yield of phenylethylaminopropionitrile (PEAP, MW 174) after a short time, but also forms a side product, 3-[(2-cyanoethyl)phenylethylamino]propionitrile (CPEAP, MW 227) after a longer reaction time, by addition of a second molecule of acrylonitrile to PEAP.[10]

We monitored the reaction products continuously at the start of the reaction to determine the delay between the changes in solution and the corresponding signal. This was performed by putting all the Michael reaction components in the vessel except acrylonitrile. The PEAP signal was then monitored continuously while the acrylonitrile was added to the vessel. It took less than 1 s to observe the PEAP signal after the addition of acrylonitrile. As described above, the N_2 gas takes around 0.2 s to flow from the vessel to the ESI plume. Thus, the delay for this setup is estimated to be in the range from 0.2 to 1 s.

Representative mass spectra recorded at 20, 60 and 300 min individually after the addition of acrylonitrile (shown in Fig. 2) demonstrate the wealth of valuable information provided about ongoing chemical reactions by EESI-MS. At the beginning of the reaction, the protonated PEA (*m/z* 122) and the main product PEAP (*m/z* 175) were seen clearly in the spectra, with other ions originating presumably from impurities or side products. For example, the ions at *m/z* 105 and 158 are chemical noise. These ions were present and their behavior was the same when there was only pure ethanol in the flask, following the same experimental procedure. At around 40 min, the ion representing the side product (*m/z* 228) was observable and became quite intense after 60 min. In the final stage, the main product and the side product (*m/z* 228) were apparent in the spectra. An advantage of EESI for chemical reaction monitoring is the preferential detection of reactants and (side) products, since most solvents (such as alkanes) have low proton affinities

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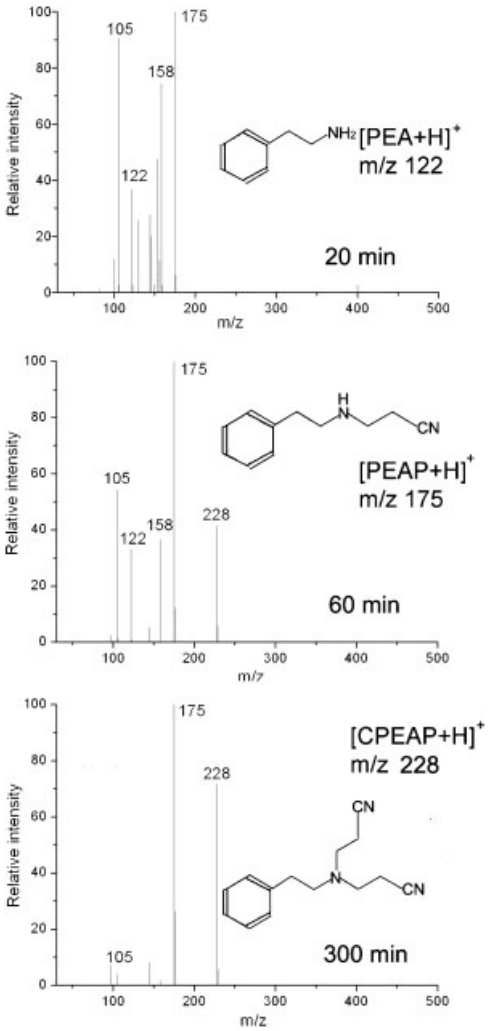

Figure 2. Mass spectra of the Michael addition reaction recorded at 20, 60 and 300 min, respectively. Inserts: molecular structures of PEA, PEAP and CPEAP.

(PAs) and remain undetectable, thereby simplifying the mass spectra. However, this is not a limitation, because analytes with low PA can be detected if desired by adding species which easily cationize low PA compounds, for example, by adding $AgNO_3$ to observe sulfur-containing compounds.[19]

The single ion responses for protonated PEA, PEAP and CPEAP during the course of the Michael addition reaction in Fig. 3 show that the intensity of the starting reactant, PEA, continues to decrease, while the products, both PEAP and CPEAP, increase over the same duration. It is seen that after 120 min the relative intensity of PEAP reached its maximum. This is in good agreement with previous studies performed using MIMS;[10] however, with a rather simple setup and fast response. The slight difference in the suggested endpoint of the reaction might originate from the differences in the laboratory environments. This validates the suitability of EESI-MS for the real-time, on-line monitoring of chemical reactions. EESI also offers instant response, a simple setup and no disturbance to the ongoing reactions. Although the absolute intensities of specific compounds are dependent on their vapor pressure and individual ESI response, the relative signal intensities suffice for most applications. The sensitivity of this technique can be improved by sampling more analytes, for example, through aerosolization. The facts mentioned above open up the possibility of EESI-MS being utilized for the real-time, on-line monitoring of chemical reactions in industry, providing instant data for the feedback loop to correct possible reaction deviations.

In addition to real-time monitoring, tandem mass spectrometry (MS^n) helps to identify unknown species, validate proposed intermediates and further understand the reaction mechanisms. To demonstrate this, an acetylation reaction of benzyl alcohol (10.8 mL) and acetic anhydride (10.1 mL) in the presence of 4-DMAP (0.11 g) as catalyst, stirred in dichloromethane (21 mL) at room temperature, was followed to track and fingerprint theoretically proposed intermediates with EESI. The acetylation reaction mechanism of 4-DMAP catalysis involves a nucleophilic attack of 4-DMAP on a carbonyl group of acetic anhydride, generating

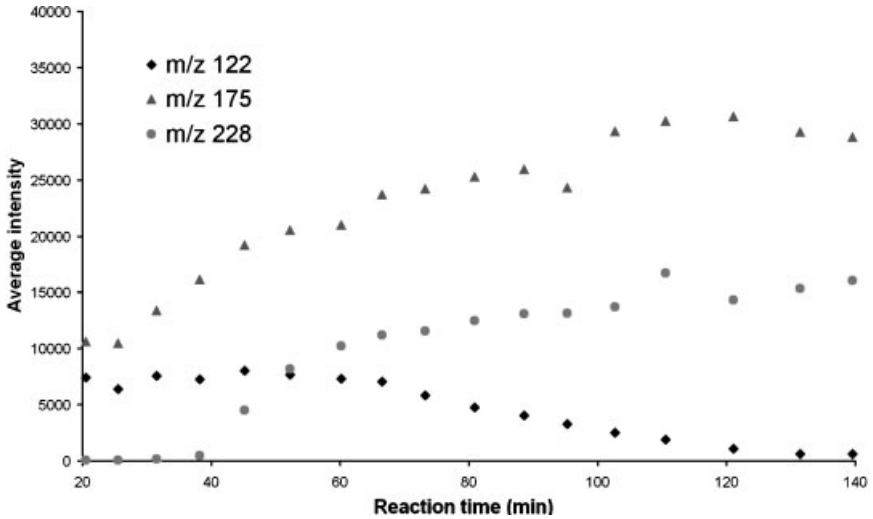

Figure 3. Traces of protonated PEA (*m/z* 122), protonated PEAP (*m/z* 175) and protonated CPEAP (*m/z* 228) by monitoring their individual averaged signal intensity.

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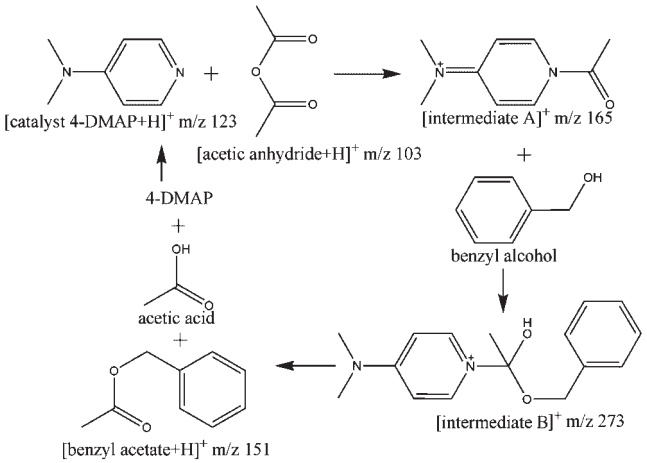

Figure 4. Proposed reaction mechanism of catalytic acetylation of acetic anhydride and benzyl alcohol in the presence of 4-DMAP.

a positively charged intermediate 'A', confirmed by nuclear magnetic resonance (NMR) spectroscopy.[23] The reaction of A with benzyl alcohol then leads to a second intermediate 'B',[24] which finally produces benzyl acetate, as the main product, and regenerates 4-DMAP (shown in Fig. 4). The single ion current (SIC) traces of some selected ions including protonated acetic anhydride (*m/z* 103), protonated benzyl acetate (*m/z* 151), a side product (*m/z* 301) and intermediate B (*m/z* 273) as a function of time are shown in Fig. 5. The spikes in these traces result from the intermittent sampling of the reaction mixture every 4–5 min. During one sampling cycle, the signal rose from 10% to 90% in less than 0.2 s, indicating a rapid response time. Note that there is a change of intensity during a sampling pulse (~40 s), as indicated, for example, by the arrows along the SIC trace of *m/z* 273 in Fig. 5. The more interesting thing is that the shape of individual pulses (indicated by the slope of the arrows) kept changing. For example, at the beginning of the acetylation reaction, the signal intensity of *m/z* 273 grew during one sampling event, but became less and less pronounced as the reaction proceeded, due to continuous consumption of benzyl alcohol in the solution. After reaching a steady state around 17 min, the *m/z* 273 signal continued to decrease until it disappeared. Another point to be noted is that, by looking into single sampling pulses carefully (zoomed view in Fig. 5), the changes of signal intensity of certain compounds can be observed in seconds. With a relatively high flow rate (50 L/h), virtually all of the original headspace will be flushed out of the flask within 3 s. The signal variation afterwards follows the changes in solution, as discussed above. The rising profiles of some single sampling pulses reveal that the changes of the compound concentrations in the solution phase are reflected very quickly (estimated to be in less than 1 s) by the analyzed headspace, making this EESI approach a real-time method for monitoring organic chemical reactions.

As shown in Fig. 5, the signal for protonated acetic anhydride (*m/z* 103) kept decreasing because it was consumed continuously for the generation of the intermediate. Due to the low PA of benzyl alcohol, its response in positive EESI is very low. In the case of the proposed intermediate A (*m/z* 165), background subtraction had to be performed. After careful comparisons of individual background-subtracted spectra, a signal at *m/z* 165 was observed after adding 4-DMAP, and it then decreased continuously until it disappeared (data not shown). Fragmentation of the *m/z* 165 ion yields *m/z* 123, which represents the protonated 4-DMAP, and *m/z* 107 through loss of one CH_4 molecule from *m/z* 123 (Fig. 6). The characteristic benzyl cation (*m/z* 91 by losing one acetic acid) is clearly seen in the MS/MS spectrum of *m/z* 151, confirming that the ion at *m/z* 151 represents protonated benzyl acetate. The benzyl acetate signal increased in the early stage of the reaction, but started to

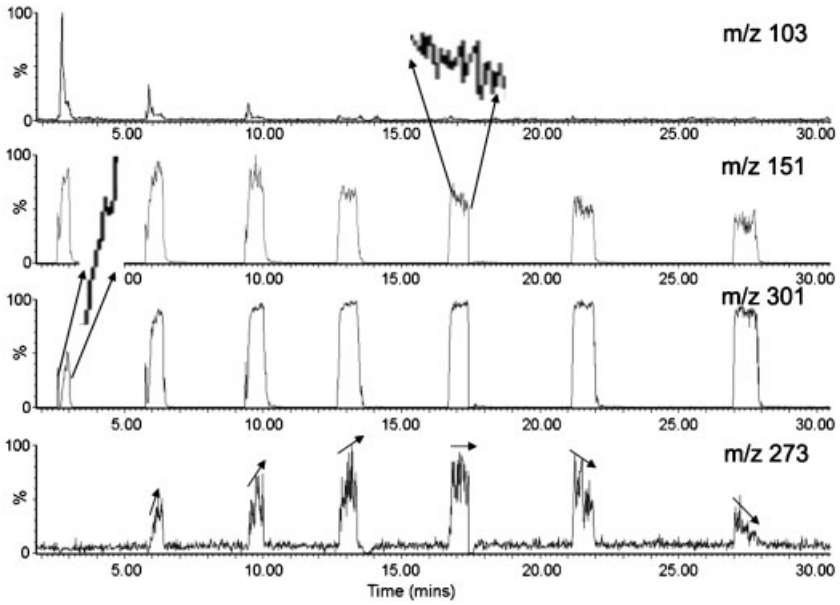

Figure 5. Selected ion traces of several compounds including *m/z* 103, 151, 301 and 273 as a function of time (min) in the 4-DMAP-catalyzed acetylation reaction.

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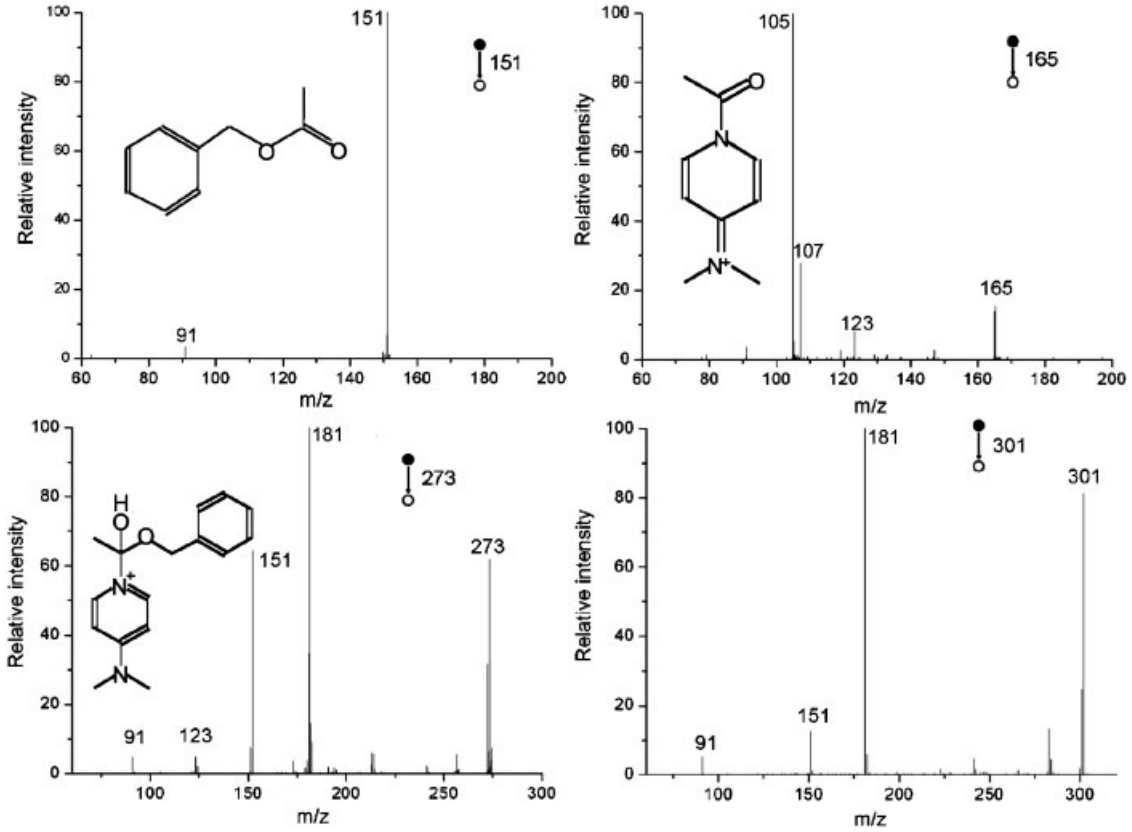

Figure 6. Collision-induced dissociation spectra of some $[M+H]^+$ ions of products and intermediates from the 4-DMAP-catalyzed acetylation reaction, including *m/z* 151, 165, 273 and 301.

diminish after 10 min, which may indicate that some side reactions that consume the main product were occurring. The ion at *m/z* 301 is the protonated dimer of benzyl acetate, which was produced by cluster formation due to the relatively high concentration of benzyl acetate in the resultant mixture. This assignment is supported by its MS/MS spectrum, which gives product ions at *m/z* 151 and 91, as shown in Fig. 6. Moreover, the formation of *m/z* 181 can be rationalized by two consecutive losses of acetic acid from *m/z* 301.[25] The intensity of *m/z* 301 reached a plateau at around 10 min, possibly due to the saturation of the detector of the mass spectrometer. The intermediate B was observed at *m/z* 273, and its main fragmentations were those yielding protonated benzyl acetate (*m/z* 151), protonated 4-DMAP (*m/z* 123), the benzyl cation from the main product (*m/z* 91), and *m/z* 181 as described above (Fig. 6). Note that the signal of the *m/z* 123 ion was absent after the reaction started. However, the ion at *m/z* 123 representing 4-DMAP can be clearly observed when there is only 4-DMAP dissolved in the solvent. The absence of the 4-DMAP signal during the reaction can be explained by the involvement of 4-DMAP in the catalytic cycle. Afterwards, the regenerated 4-DMAP reacts with the freshly produced acetic acid, yielding relatively stable ion pairing 'complexes', 4-DMAP · HOAc.[26] The protonated 4-DMAP ion was again seen immediately after adding an auxiliary base, triethylamine.

The application of EESI-MS is not limited to the detection of volatile compounds. Pick-up of highly water-soluble semi-volatile compounds by aerosol water droplets has been demonstrated.[17] Similarly, with the help of an aerosol formed from organic solvents usually present in reactions, the rapid detection and monitoring of both semi-volatile and non-volatile compounds by EESI can be carried out without changing the experimental setup.

CONCLUSIONS

EESI-MS is a useful technique for the on-line monitoring and characterization of chemical reactions in real-time by quickly sampling the chemicals emerging from a running reaction mixture. With a rather simple instrumental setup and convenient operation, EESI-MS can be easily implemented in either chemical industry or on common lab apparatus. As demonstrated in this study, together with an almost instantaneous response time and the ability to work with complex matrices, EESI-MS is able to track the chemical dynamics of simple reactions (e.g. elementary reactions) and complicated chemical reactions (e.g. heterogeneous chemical reaction, and reactions involving catalysts). Compared with other available techniques, EESI-MS allows a better control of chemical and pharmaceutical reactions due to its high sensitivity and rapid response, providing a practically useful tool which could allow the determination of the reaction endpoint for optimum yields and minimum cost (e.g. side products, waste, etc.). In addition, EESI-MS permits the confirmation of proposed transients, which leads to better understanding of chemical reaction mechanisms. This is particularly beneficial to organic chemistry, drug discovery and material sciences.

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