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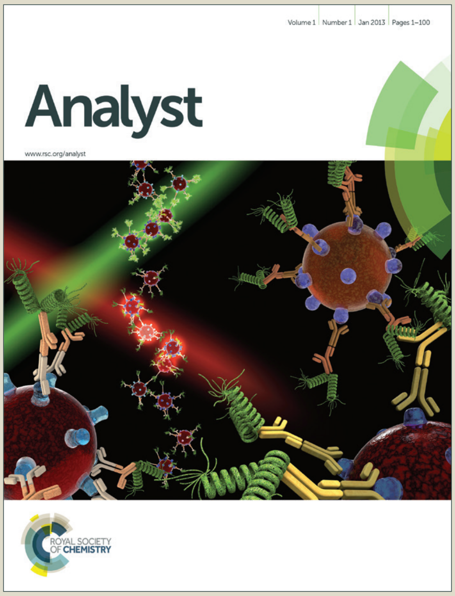

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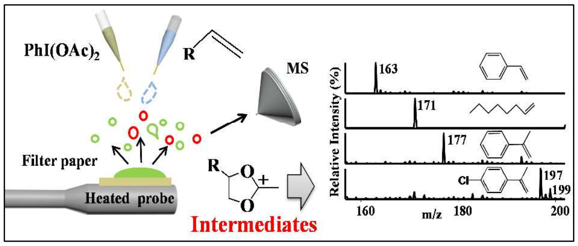
Graphical abstract



Interception of short-life reactive intermediates could be achieved with paper assisted thermal ionization mass spectrometry in real time.

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Reactive intermediate detection in real time via paper assisted thermal ionization mass spectrometry

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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To capture reactive intermediates from bulk solution in real time, mass spectrometry with paper assisted thermal ionization has been developed. Two representative organic reactions, Eschweiler–Clarke methylation at high temperature and acid-catalyzed olefin dioxygenation at room temperature, were chosen as models to evaluate the present method.

Among the elucidation of organic reaction mechanisms, reactive intermediates play a significant role. Generally, those intermediates are studied by optical or magnetism-related techniques.[1-3] However, these methods commonly suffer from inaccurate identification of intermediates, especially for those with short-life and low concentration. With the advent of electrospray ionization (ESI), the number of mass spectrometry (MS)-based approaches has grown dramatically for reaction monitoring[4-7] by virtue of its high specificity, sensitivity and speed. Nevertheless, dilution prior to analysis is obligated to avoid possible contamination from the highly concentrated reaction solution for traditional ESI MS monitoring of organic reactions. The delay between chemical reaction and detection of either on-line or off-line ESI MS would cause missing of some "fleeting" intermediates, thus more straightforward strategies for MS monitoring of reactive intermediates are to be developed.

Since the invention of desorption electrospray ionization (DESI),[8] ambient ionization MS methods have been rapidly explored due to the principle of no sample pretreatment. That principle fits perfectly with chemical reaction monitoring. Recently, DESI[9] and inductive ESI[10] have been developed to monitor reaction intermediates. The utilization of other relative techniques has also been reported such as low temperature plasma (LTP),[11] extractive electrospray ionization (EESI),[12,13] electrospray-assisted laser desorption/ionization (ELDI)[14] and probe electrospray ionization (PESI).[15] However, these methods are inconvenient to monitor some harsh reactions in real time, such as those requiring high temperature, or additional heating device was obligatory to initiate the reaction off-line before MS analysis. Thus we aimed at developing a more facile and easy-to-operate ambient ionization method for reaction intermediate study.

Based on the fact that heating the reaction solution would cause thermal desorption[16-19] as well as thermal ionization,[20-23] we proposed an ambient ionization via adding bulk reactant solution on a heating device as shown in Fig. 1a. When bulk reactant solution was loaded onto a heated probe (electric soldering iron used here), solvent would bump intensively due to the rapid heating, and chemicals were found to be ionized during that process. For monitoring of chemical reaction, the heated probe not only plays the role of ionizing reagents but also has the function of reaction vessel. Immediately the reaction was initiated on the heated metal probe, the reactants, intermediates and products were ionized for MS analysis, by which the goal of real-time monitoring was achieved. Besides, it was also found that with the assistance of a piece of filter paper the chemical reaction efficiency was greatly improved. The filter paper used here is in triangular form with the sharp angle pointing at the MS inlet but without spray high voltage applied as described in paper spray ionization.[24] This ionization method was named and abbreviated as "PATI" (paper assisted thermal ionization) in the following. In this study, reactive intermediate detection via PATI was evaluated by two model reactions: 1) Eschweiler–Clarke methylation (E-C reaction),[25] a reaction at high temperature and 2) alkenes dioxygenation,[26] a reaction at room temperature.

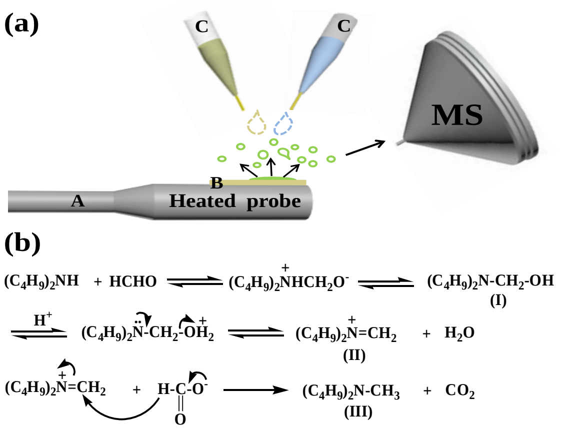


Fig. 1 (a) Schematic diagram of PATI setup. A: heated metal probe, B: filter paper, C: home-made pipette tip. (b) Proposed mechanism of E-C reaction.

E-C reaction is a nucleophilic additive-elimination reaction at high temperature.[25] With excess formaldehyde (HCHO) and formic acid (FA), primary or secondary amines would be methylated as tertiary amine through the proposed mechanism described in Fig. 1b. The lifetime of aliphatic iminium ions in aqueous solution has been reported to be quite short due to possible hydrolysis reaction,[27] which is challenging the MS interception via conventional ionization methods. We firstly began our investigation of E-C reaction monitoring with conventional ESI MS. The reaction was initiated in a heating reflux unit (76 °C). After 60 mins' reaction, an aliquot of the bulk solution was taken out and diluted (dilution ratio 1:100) with CH_3OH/H_2O (v/v, 1:1) for ESI MS analysis. No product ion (*m/z* 144) was observed with reactant concentrations as 0.6 mM (dibutylamine, *n*-DBA), 36 mM (HCHO) and 54 mM (FA) (Fig. 2a, black line). While all reactant concentrations were increased 10 times ($C_{n\text{-DBA}}$ = 6 mM, C_{HCHO} = 360 mM and C_{FA} = 540 mM), the product ion (*m/z* 144) was observed. However, intermediate ions for neither I (*m/z* 160) nor II (*m/z* 142) were observed (Fig. 2a, red line). For E-C monitoring with PATI, the metal probe was heated to 125 °C first, then 5 μL of the bulk reaction solution was loaded onto the heated metal probe with a home-made silica capillary-pipette tip. After evaporation of the reaction solvents the probe cooled slightly by the observation of less violent bumping. Nevertheless, the probe temperature could be recovered to the setting value in seconds for the next measurement. Since E-C reaction requires high temperature, the bulk solution hardly reacts at room temperature. Immediately the bulk solution contacted the heated probe, high temperature initiated the reaction accompanying the ionization of reactants, intermediates and products for MS analysis. While PATI was used to monitor E-C reaction, ions of intermediates I, II and product could be detected even with low reactant concentrations mentioned above ($C_{n\text{-DBA}}$ = 0.6 mM, C_{HCHO} = 36 mM and C_{FA} = 54 mM) (Fig. 2b). Moreover, the reaction process could be monitored step-by-step via PATI. When the mixture solution of *n*-DBA and HCHO was loaded onto the thermal probe, protonated intermediates I and II were observed (Fig. 2b, black line). After FA was added into the mixture solution to initiate the second step of E-C reaction, the signal intensity of intermediate II decreased dramatically and protonated ion of product was detected (Fig. 2b, red line). The relative signal intensity of each species upon addition of FA may also be impacted by their different ionization efficiencies with this additive. The corresponding identifications of reactant, intermediates I, II and product ions were confirmed according to their MS^2 mass spectra as shown in Fig. 2c-f.

It should be noted that much higher signal intensity of product ion (III *m/z* 144) was observed with the assistance of filter paper (Fig. 2b, green line). The improved ion intensity implies that higher reaction yield might be achieved as well as higher ionization efficiency. The improved reaction yield might be attributed to increased surface area for reactants contact due to the large amount of micro channels in filter papers or longer contact time between reactants and filter paper with slower desorption rate. The higher ionization efficiency could be inferred by the recent studies[24,28-30] using porous material to improve ionization efficiency via decreased size of initial droplet.[31]

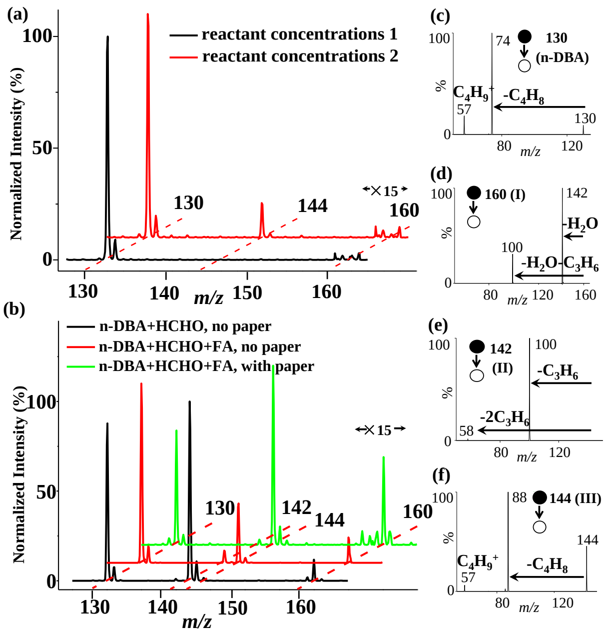


Fig. 2 (a) Monitoring of E-C reaction with conventional ESI MS. Reaction conditions: reactant concentrations 1, $C_{n\text{-DBA}}$ = 0.6 mM, C_{HCHO} = 36 mM, C_{FA} = 54 mM; reactant concentrations 2, $C_{n\text{-DBA}}$ = 6 mM, C_{HCHO} = 360 mM, C_{FA} = 540 mM; solvent: CH_3OH/H_2O (v/v, 1:1); reaction temperature: 76 °C. Before MS analysis, the bulk solutions with reactant concentrations 1 and 2 are diluted 100 and 1000 times with CH_3OH/H_2O (v/v, 1:1) respectively (b) Step-by-step study of E-C reaction using PATI with and without filter paper (conditions: $C_{n\text{-DBA}}$ = 0.6 mM, C_{HCHO} = 36 mM, C_{FA} = 54 mM; reaction solvent, CH_3OH/H_2O (v/v, 1:1); T_{probe} = 125 °C). Black line: the first step of E-C reaction without filter paper (*n*-DBA+HCHO). Red line: the second step of E-C reaction after addition of FA to the mixture of *n*-DBA and HCHO without filter paper (*n*-DBA+HCHO+FA). Green line: The second step of E-C reaction with the assistance of filter paper. MS^2 mass spectra of the reactant (c), intermediates (d, e) and product (f). Collision energy = 30%. Note: the mass ranges (*m/z* 158-161) for intermediate I (*m/z* 160) in all spectra of (2a) and (2b) were zoomed in 15 times.

To verify PATI's feasibility in monitoring reactions at room temperature, alkenes dioxygenation was tested with the present method. Although dioxygenation of alkenes with $PhI(OAc)_2$ has been well investigated using various catalysts, its reaction mechanism is not fully discovered yet. In previous study,[26] acid was speculated to serve as a possible alternative catalyst to metal ions.[32,33] The possible mechanism is shown in Scheme S1. In this study, alkenes dioxygenation with four substrates (styrene, octene, methylstyrene and 4-chlorostyrene) was tested. For conventional ESI experiment, all reaction reagents (substrate, $PhI(OAc)_2$, acetic acid (HOAc) and trifluoromethanesulfonic acid (TfOH)) were mixed in CH_2Cl_2 solvent in a vial first. After 30 mins' reaction, 5 μL of the bulk solution was taken out and diluted 10000 times with CH_3CN/H_2O (v/v, 1:1) for MS analysis. However, only part of intermediates B were intercepted while intermediates E could not be observed either in previous study[26] or with present ESI MS except for substrate 1 (Fig. S1, ESI†). For PATI MS, the experiment was performed by mixing two solutions on the heated paper with simultaneous MS analysis. One solution was the mixture of $PhI(OAc)_2$, substrate and HOAc in CH_2Cl_2, while the other one was TfOH (served as catalyst) in CH_2Cl_2. Once reactants contacted the catalyst on the heated paper, the reaction was initiated and the reactive intermediates were detected by mass spectrometer as shown in Fig. 3. Intermediates B were detected as B-HOAc for all of the four substrates (1, 2, 3 and 4) at *m/z* 307, 315, 321 (Fig. 3a) and 341/343 (Fig. 3b). Besides, intermediates E could also be identified for all four substrates at *m/z* 163, 171, 177 (Fig. 3a) and 197/199 (Fig. 3b). Moreover, the isotope distribution of the observed intermediate ions for substrate 4 (197/199, 341/342) was well in accordance with the calculated values ((M+2)/M = 32%) (Insets of Fig. 3b), which further confirmed the interception of the proposed intermediates. Similar to ESI MS, ions at *m/z* 345, 361, 483 and 585 were observed in the mass spectra as well. They corresponded to the monomer or dimer cations of reagent $PhI(OAc)_2$, being $[PhI(OAc)_2 + Na]^+$, $[PhI(OAc)_2 + K]^+$, $[(PhIO)_2Ac]^+$ and $[(PhIOAc)_2OAc]^+$ respectively (Detailed structures are shown in Scheme S2, ESI†). It should also be noted that filter paper used in alkenes dioxygenation reaction is not only to improve reaction and ionization efficiency but also to adsorb extra reagents. The highly concentrated $PhI(OAc)_2$ reagent dissolved in CH_2Cl_2 solvent easily formed crystals after solvent evaporation, which generally led to contamination and clogging of the MS inlet capillary. With the assistance of filter paper, most of the reagents were retained on the paper, and therefore contamination to the mass spectrometer was reduced and clogging of the inlet capillary could be avoided (ESI†, Fig. S2).

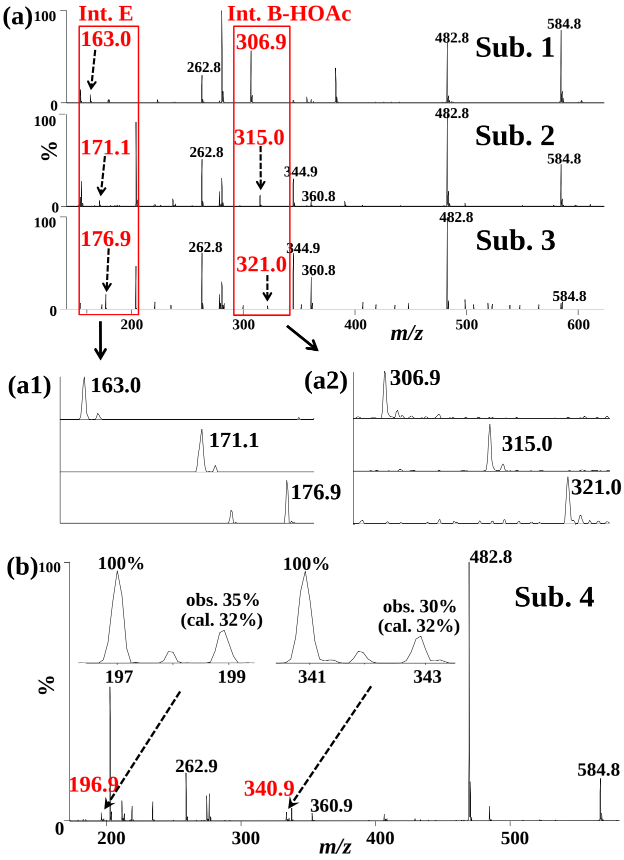
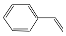
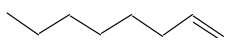
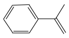
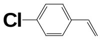


Fig. 3 Mass spectra of alkenes dioxygenation monitored by PATI with substrates 1-4 being (a) styrene, octene, 4-methylstyrene and (b) 4-chlorostyrene respectively. Reaction conditions: solution A, 0.38 M $PhI(OAc)_2$, 0.25 M substrate and 20% (v/v) HOAc in CH_2Cl_2; solution B, 10 mM TfOH in CH_2Cl_2. 5 μL of solution A and 5 μL of solution B were loaded onto the heated filter paper simultaneously. $T_{probe} = 90$ °C. (a1, a2) Amplification of the spectra corresponding to the mass ranges of intermediates E and B-HOAc respectively.

The comparison of intermediate monitoring with ESI and PATI MS is summarized in Table 1. It can be concluded that PATI could capture more intermediates than conventional ESI MS. It might be partly attributed to its real-time monitoring capability, which enables direct MS analysis of short-life intermediates without dilution or other treatments.

Table 1. Comparison of alkenes dioxygenation monitored with ESI and PATI MS.

Substrates	*m/z* (ESI)		*m/z* (PATI)	
Intermediates	B[a]	E	B	E
Sub. 1 	307	163	307	163
Sub. 2 	315	n.d.[b]	315	171
Sub. 3 	n.d.	n.d.	321	177
Sub. 4 	341/343	n.d.	341/343	197/199

[a] Intermediate B was detected as B-HOAc
[b] n.d.: not detected.

Conclusions

In summary, PATI-MS could be utilized to intercept reaction intermediates. Due to the direct and rapid ionization process, reaction intermediates could be detected in real time. Besides, PATI could detect intermediates of chemical reaction both at high temperature and room temperature. For reaction requiring high temperature, PATI source could serve as the heating device for reaction initiation as well as ionization tool. The availability of this method to monitor high temperature reaction was illustrated with E-C reaction. For room temperature reactions such as acid-catalyzed olefin dioxygenation, PATI was found to be able to confirm some of the reactive intermediates. With the development of portable MS instruments,[34-36] this facile ambient ionization method would be potentially useful in reaction mechanism investigations without rushes between fume hood and MS instrument.

Acknowledgements

This work was supported by National Natural Science Foundation of China (21205111) and Recruitment Program of Global Experts (Thousand Young Talent Program).

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and methods, supplementary figures and schemes (Fig. S1, S2, Scheme S1, S2). See DOI: 10.1039/b000000x/

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