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Online monitoring of chemical reactions by contactless atmospheric pressure ionization mass spectrometry

Dear Sir,

Online monitoring of the appearance of the intermediates and products during chemical reactions is helpful to understand reaction mechanisms. Various analytical tools including nuclear magnetic resonance spectroscopy,[1,2] Raman spectroscopy[3,4] and mass spectrometry (MS)[5–29] have been employed for the study. MS is one of the most promising means among analytical tools that can provide information of mass-to-charge ratios for intermediates and products generated from chemical reactions. The main challenge for online monitoring of species generated from chemical reactions by MS is the interface design. The success of online monitoring of chemical reactions using MS relies on real-time recording of the generated species from reactions without sacrificing kinetic information during coupling. In addition, MS can only detect gas-phase ions. Nevertheless, most chemical reactions occur in liquid phase. Thus, generating gas-phase ions from liquid samples is vital for the success of this study. Electrospray ionization (ESI) is one of the most common atmospheric pressure ionization (API) methods that generate gas-phase ions from liquid-reacting species/products in the proximity of a mass spectrometer for online monitoring of chemical reactions.[7–18] In addition, some recently developed ambient ionization methods[19–29] such as desorption ESI,[19,20] extractive ESI,[21,22] electrospray desorption ionization,[23] fused droplet ESI,[24,25] low-temperature plasma probe[26] for ambient desorption ionization and ultrasonication-assisted spray ionization [27] also have shown their usefulness in monitoring reactions in real time. High-voltage electric power supply, gas supply, or laser is generally required for these approaches. High-voltage free approaches such as Venturi easy ambient sonic-spray ionization[28] with gas supply only have been used to online monitoring of organic reactions. A comprehensive description about the progress using MS to monitor chemical reactions can refer to the recently published book written by Santos.[29] Some of these techniques may be suitable for monitoring fast reactions that take place during desorption/ionization process.[19–26] However, our current study is focused on using a recently developed API technique for monitoring the changes of reacting species from an organic reaction requiring a long reaction time to reach the end of the reaction.

A continuous-flow API method was recently explored, called contactless API (C-API),[30] which uses a short tapered-capillary tip (~ 1 cm) as the spray emitter for the generation of gas-phase ions from a liquid solution. High-voltage electric power supply, gas supply and laser are not required for the C-API approach. The C-API approach only requires a tapered capillary for the generation of gas-phase ions in the proximity of MS (Fig. 1). The sample solution can flow continuously from the sample inlet to the tip outlet owing to capillary action. The putative ionization mechanism of the C-API approach may be based mainly on polarization processes induced by the high electric field in the mass spectrometer. When the silica capillary tip is placed close to a mass spectrometer equipped with a high voltage (−3 kV), polarization occurs on the tip because of the electric field provided by the mass spectrometer, leading to charge accumulation of the liquid on the meniscus. When electrostatic repulsion of the liquid droplet hanging on the meniscus overcomes surface tension, fine sample droplets are sprayed out of the tip. Figure 1 shows the photograph of the spray generated from a C-API capillary tip during C-API-MS analysis, in which the spray is directed at the orifice of the mass spectrometer. Gas-phase ions are readily formed for MS detection after solvent evaporation. The mechanism of ion formation in C-API is similar to what has been known in ESI, i.e. droplet charging via a high electrical field, although there is no direct high-electric contact on the C-API emitter. Thus, C-API can be alternatively called contactless ESI. When a very high electric voltage applied on the MS orifice, redox interferences resulting from the high electric voltage may be observed.

The capillary in the C-API setup functions as sampling tip and spray emitter. When using this setup for online monitoring of organic reactions, a capillary inserted into a reaction vial should be able to deliver the reacting species from the reaction solution based on capillary action to the capillary outlet. The tapered capillary outlet can be placed in front of the orifice of a MS for the generation gas-phase ions from the effluent. Therefore, reaction species, intermediates and products generated from reactions can be readily monitored by C-API-MS. Directing analytes from the reaction vial to the proximity of MS requires a capillary with sufficient length. We believed that a long capillary might work just as well as a short capillary (~ 1 cm) in the C-API-MS analysis. The possibility, by alternatively using a long capillary (~ 20 cm) with a tapered outlet as the sampling tube and spray emitter for the C-API-MS analysis of biomolecules, including bradykinin and cytochrome C, was examined initially. All the experiments in this work were preformed from an Esquire2000 ion trap mass spectrometer (Bruker Daltonics). Multiply charged ions derived from bradykinin (Fig. 2a) and cytochrome C (Fig. 2b) dominate the CAPI mass spectra. The results indicate that the C-API-MS analysis can be successfully conducted when a long capillary is used as the spray emitter. The C-API-MS approach can work relatively well in analyzing small and large molecules when a long capillary is used in the C-API setup.

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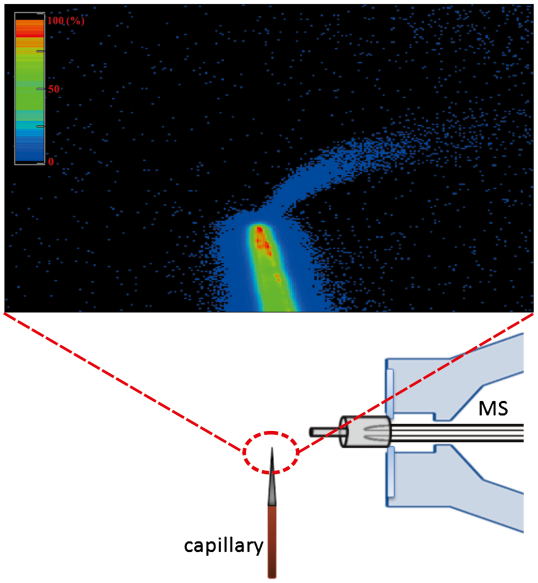


Figure 1. (a) Contactless-API setup. (b) Photograph of the CAPI spray taken by a Hamamatsu ORCA-Flash 2.8 CMOS.

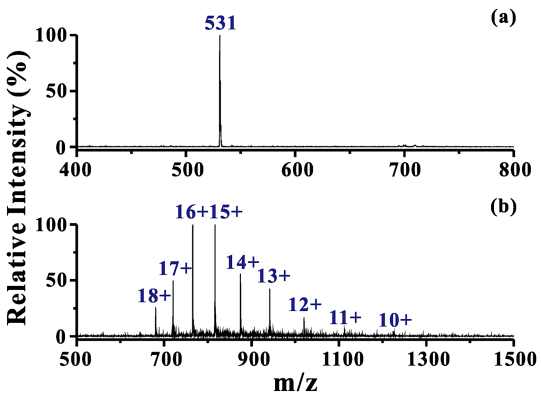


Figure 2. Contactless-API mass spectra of (a) bradykinin (10^{-6} M) and (b) cytochrome C (10^{-6} M in 1% acetic acid) prepared in water/acetonitrile (1:1, v/v). A tapered capillary (length: 20 cm) was used as the C-API sampling tube and spray emitter. The inner diameters of the C-API capillary obtained before and after tapping were 50 μm and ~10 μm, respectively.

In the current C-API approach, there is no electrode put in the sample inlet. For comparison, we put an electrode into the sample inlet and investigated the mass spectral results. Aspartic acid was used as the model sample, while negative ion mode was operated. The voltage applied on the orifice of the mass spectrometer was 3000 V. Figures 3a and 3b display the mass spectra of aspartic acid [$(M\text{-}H)^-$ = 132] obtained without and with introducing an electrode (0 V) in the sample vial, respectively. The signal-to-noise (S/N) ratio of the ion peak of aspartic acid at m/z 132 (S/N = 770) in Fig. 3a is higher than that (S/N = 369) in Fig. 3b. Furthermore, fewer background ions are observed in Fig. 3a. When applying −500 V to the sample inlet, the peak at m/z 132 still dominates the mass spectrum (Fig. 3c). However, the intensity of the background ions increases, and some unknown ions appear in the mass spectrum. The mass spectrum became worse when the voltage applied in the sample inlet was adjusted to −2000 V (Fig. 3 d). The results indicate that our C-API approach with uncomplicated setup can obtain better quality of mass spectra with few background ions.

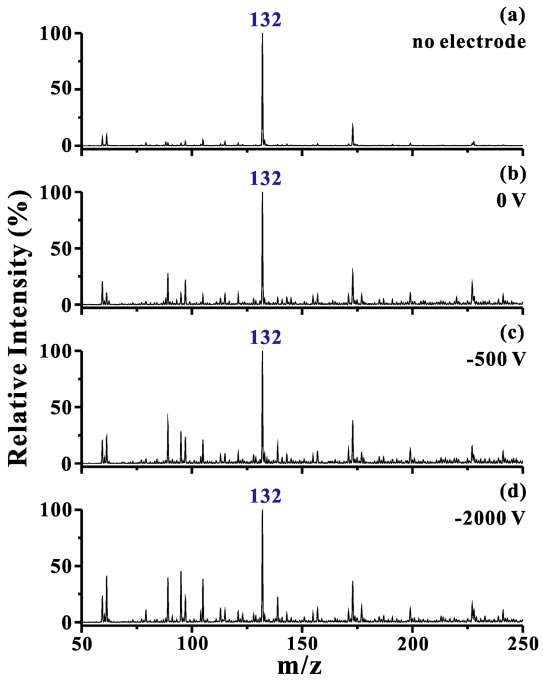
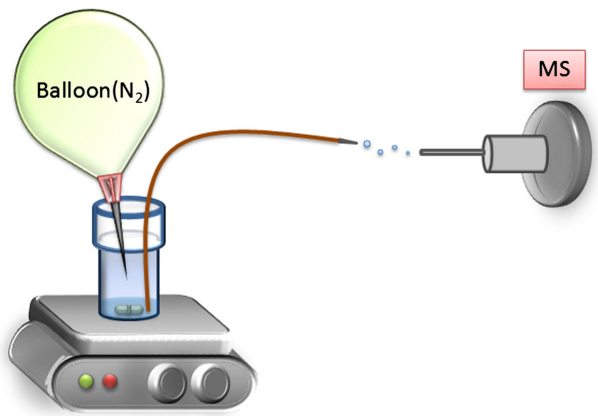


Figure 3. (a) Contactless-API mass spectrum of aspartic acid (10^{-6} M) prepared in water/acetonitrile (1:1, v/v) without introducing an electrode to the sample inlet. Mass spectra of aspartic acid (10^{-6} M) obtained by introducing an electrode with the voltages of (b) 0 V, (c) −500 V and (d) −2000 V to the sample inlet.

We attempted to employ the current approach for online monitoring of chemical reactions, so we considered that many chemical reactions are performed under oxygen-free condition. One of the approaches is using balloons filled with nitrogen (or argon) to connect the capped reaction vials to meet the requirement. Thus, it raises a question of whether the nitrogen balloon might cause any effect during CAPI-MS analysis. For the ease of comparison, bradykinin and cytochrome C were again used as samples. The sample vial was capped and charged with gas via a balloon (volume: ~4.2 L) filled with nitrogen (~0.6 psi) (Scheme 1). The doubly charged ions of bradykinin and multiply charged cytochrome C again dominated the CAPI mass spectra in Figs. 4a and 4b, respectively. The nitrogen balloon provides an additional



Scheme 1. Cartoon diagram of the online monitoring configuration of an organic reaction by contactless-API-MS.

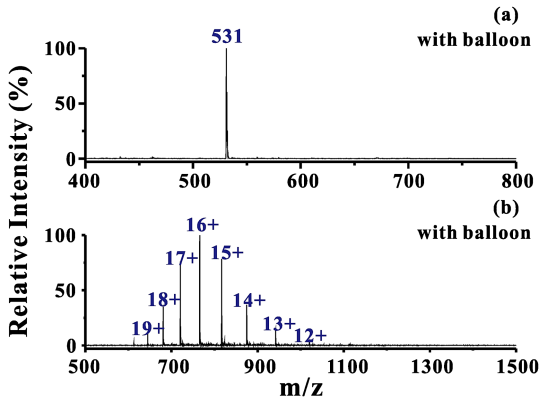
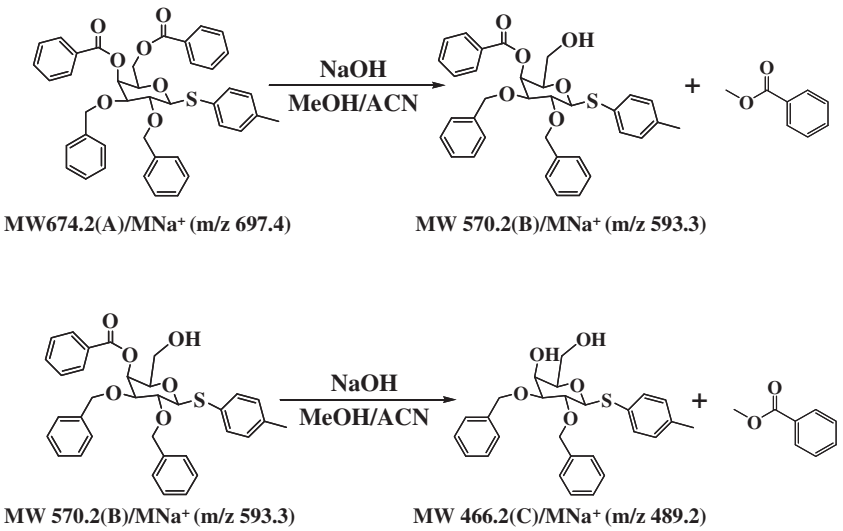


Figure 4. Contactless-API mass spectra of (a) bradykinin (10^{-6} M) and (b) cytochrome C (10^{-6} M in 1% acetic acid) prepared in water/acetonitrile (1:1, v/v). A balloon was attached to the sample vial as shown in Scheme 1. A tapered capillary (length: 20 cm) was used as the C-API sampling tube and spray emitter. The inner diameters of the C-API capillary before and after tapping were 50 μm and ~10 μm, respectively.

pressure to the sample vial, driving the flow of bradykinin to the capillary outlet in a shorter period of time. Furthermore, the ion intensity derived from the analytes in the presence of a nitrogen balloon is higher than that obtained in an opened reaction vial (cf. Fig. 2). The results indicate that a sample vial capped with a balloon is compatible with the C-API-MS setup. No apparent compromise in MS detection has been found. Furthermore, the spectral quality of the C-API mass spectra can further be improved when using a sample vial sealed with a nitrogen balloon in terms of S/N ratios. We believe that using other means to provide oxygen-free conditions such as charging the reaction vial with the gas from nitrogen/argon pipes can also be suited to this setup. The results shown above implied that the new C-API setup should be suitable for online monitoring of chemical reactions.

Zemplén reaction, which is generally used in deprotection of ester functions in carbohydrate chemistry,[31] was selected as the model reaction. Among different ester protecting groups, benzoyl ester is widely used in organic synthesis due to its chemical stability. Removal of the benzoate group is often performed under basic conditions.[31] The rate of deprotection for different benzoate functions depends on their position in sugar structure. C-API-MS was used to online monitor the Zemplén deprotection of a model substrate, i.e. *p*-tolyl-4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl- β-D-thio-galactopyranoside (A), under basic catalytic conditions. Scheme 2 shows the Zemplén reaction. The expected intermediate and the final product are labeled B and C, respectively. Reactant A (3×10^{-4} M, 1 mL) was dissolved in methanol:acetonitrile (1/1, v/v) and placed in a capped reaction vial, which was charged with nitrogen with a nitrogen-filled balloon (~0.6 psi). The reaction vial was placed on the top of a stirring plate (Scheme 1). A capillary filled with methanol:acetonitrile (1/1, v/v) was inserted into the capped vial containing reactant A prepared in methanol:acetonitrile (1/1, v/v), and its tapered outlet was placed close (~ 1 mm) to an ion trap mass spectrometer. The high voltage of the mass spectrometer was set at −3 kV, while no any electric connection on the C-API tip was made. After acquiring MS data for 1 min, a solvent containing sodium hydroxide (3 mM)/methanol (1 mL) as the base catalyst was injected into the reaction vial using a syringe while stirring. As can be seen in Scheme 1, the combination of C-API-MS with the Zemplén reaction is straightforward. If it is necessary, the reaction can be conducted at a high temperature by simply switching on the heating function from the plate. Although the reaction does not require an oxygen-free environment, a nitrogen balloon is still connected to the reaction vial, leading the species generated from the reaction vial to flow to the capillary outlet in a shorter period of time. The reactants, intermediates and products generated from the organic reaction were readily monitored through the mass spectrometer. Figure 5a shows the total ion current (TIC) chromatogram obtained during the reaction. During the first 5 min, the ion peak at m/z 697 derived from sodiated reactant A dominates the mass spectrum (Fig. 5b). A weak peak at m/z 593, corresponding to sodiated ion of the intermediate B with the loss of a benzoyl function group at the C-6 position, already appears in the same mass spectrum. Figure 5c presents the extracted ion current chromatogram at m/z 697. Apparently, the ion intensity at m/z 697 decreases as reaction time passes



Scheme 2. Zemplén deprotection of *p*-tolyl-4,6-di-*O*-benzoyl-2,3-di-*O*-benzyl- β-D-thio-galactopyranoside (A, 0.3 mM, 1 mL) prepared in a solvent of methanol/acetonitrile (1/1, v/v). The catalyst is sodium hydroxide (NaOH, 3 mM, 1 mL) dissolved in MeOH. ACN and MeOH stand for acetonitrile and methanol, respectively.

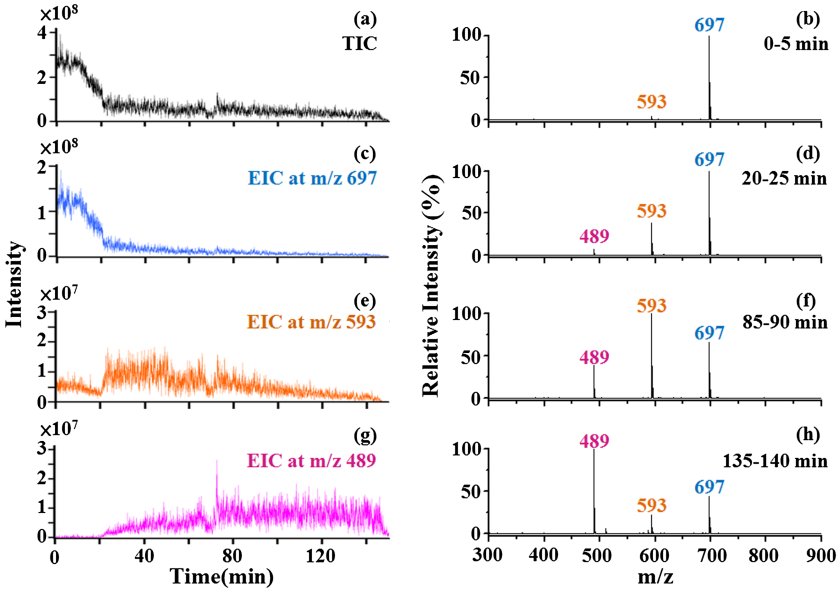


Figure 5. Zemplén deprotection of p-tolyl-4,6-di-O-benzoyl-2,3-di-O-benzyl-β-D- thio-galactopyranoside (A) prepared in the solvent of methanol/acetonitrile (1/1, v/v). The catalyst is sodium hydroxide (NaOH, 3 mM) dissolved in MeOH. ACN and MeOH stand for acetonitrile and methanol, respectively. (a) TIC chromatogram obtained during online monitoring of the Zemplén reaction using contactless-API-MS; (b) Contactless-API mass spectrum obtained from the average mass spectra acquired between the reaction time at 0 and 5 min in Panel a; (c) Extracted ion current (EIC) chromatogram at m/z 697; (d) Contactless-API mass spectrum obtained from the average mass spectra acquired between 20 and 25 min in Panel a; (e) EIC chromatogram at m/z 593; (f) Contactless-API mass spectrum obtained from the average mass spectra acquired between 85 and 90 min in Panel a; (g) EIC chromatogram at m/z 489; (h) Contactless-API mass spectrum obtained from the average mass spectra acquired between 135 and 140 min in Panel a.

by because the reactant is gradually consumed as the reaction continues. After the reaction proceeds for 20 min, the intensity of the sodiated ion peak at m/z 593 grows continually although the peak at m/z 697 (A) is still the base peak in the mass spectrum (Fig. 5 d). In addition, the ion peak at m/z 489 corresponding to the final product with the loss of two benzoyl functional groups of reactant A is observed in the same mass spectrum. The ion intensity at m/z 593 increases as the reaction continues and decreases gradually after the reaction proceeds for 90 min (Fig. 5e). The peak at m/z 593 dominates the mass spectrum (Fig. 5f) obtained from the average mass spectra between 85 and 90 min in Fig. 5a, whereas the peak of reactant A at m/z 697 becomes weak. However, the ion intensity at m/z 489 (C) grows continually (Fig. 5 g). The peak at m/z 489 is the base peak of the mass spectrum obtained from the average mass spectra between 135 and 140 min (Fig. 5 h). The results demonstrate that the approach can be used readily in online monitoring of the reaction without using any complicated interfacing design. We also estimated the flow rate of this setup by using the reagent A as the sample and methanol/acetonitrile (1:1, v/v) as the running solvent. Without attaching a balloon to the sample vial, the flow rate was estimated ~97 nL/min. When a balloon (~0.6 psi) was attached to the sample vial as shown in Scheme 1, the flow rate was increased to ~190 nL/min. The presence of extra pressure provided by the balloon helps for increasing the flow rate, resulting in a better S/N ratio as we can compare the results shown in Figs. 2 and 4. Nevertheless, the balloon shrank gradually after for a period of time, which resulted in the decrease of the flow rate in the capillary and the decline of the ion intensity. This observation also can explain the apparent decline of the TIC in Fig. 5a. A better pressure control using a gas supply with a fixed pressure should be able to avoid the problem of the decline of the TIC during a long time monitoring.

In summary, a long capillary can be used as the sampling tool and the spray emitter in the C-API-MS analysis for small and large molecules. A straightforward approach for online monitoring of a chemical reaction using the C-API-MS as the detection method is also demonstrated. There is no compromise in combining the monitoring of the reaction and C-API-MS detection. It only requires a capillary with a tapered outlet to connect the reaction vial to C-API-MS. Thus, this approach can be potentially used in various types of chemical reactions with different experimental conditions at a high temperature, in a water/oil bath, or under oxygen-free conditions. Nevertheless, a better pressure control should be used to improve the C-API setup. The reaction vial can be subjected to required experimental conditions without affecting C-API-MS detection. We have demonstrated that our current approach is quite promising for online monitoring of a model Zemplén reaction. This approach should benefit the study of the kinetics for chemical reactions and the mechanism interpretation of chemical reactions of interest.

Yours,

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