Introduction

Acoustic cavitation induced by ultrasound forms vapor-filled bubbles in liquids, followed by expansion, which leads to the generation of small and energetic bubbles.\(^\text{[1,2]}\) These small bubbles can be considered as micro reactors that facilitate the efficiency of chemical reactions.\(^\text{[3]}\) Consequently, ultrasonication has been widely used to accelerate chemical reactions.\(^\text{[4–7]}\) For example, ultrasonication remarkably accelerates enzymatic digestion.\(^\text{[7,8]}\) The reaction time can be shortened from hours to minutes. The main advantage of ultrasonication-assisted reactions is the improved efficiency of chemical reactions in terms of reaction time and yield.

Ultrasonication has been used to assist ionization in massmetric analysis.\(^\text{[9–18]}\) For example, a low-frequency ultrasonicorator (\(\sim 40\) kHz) has been used to facilitate ionization in ultrasonication-assisted spray ionization mass spectrometry (UASI–MS).\(^\text{[12–14]}\) Capillary tubes are used as sampling tubes for delivering samples to facilitate sample delivery and to generate the ultrasonic spray at the outlet of the capillary. The ultrasonic spray containing analyte ions can then be directly detected using UASI–MS although no electric contact is made with the UASI capillary. Furthermore, UASI–MS with a low-frequency ultrasonicorator (\(\sim 40\) kHz) has been readily used for online monitoring of chemical reactions.\(^\text{[14]}\) However, the acceleration of the chemical reactions was not observed by the low-frequency ultrasonic transducer-based UASI setup.

Reactive MS was lately popularized by desorption/ionization spray ionization (DESI)\(^\text{[19]}\) that can facilitate chemical reactions \(\textit{in situ}\) during desorption/ionization process. In DESI, a stream of charge droplets generated from electrospray is directed hit onto the sample deposited on a solid substrate at ambient conditions.\(^\text{[19–21]}\) As a result, the secondary ions are generated from the interaction between the charged droplets with analyte on the DESI substrate for MS analysis. When the charge droplets are composed of reactive reagents, the droplets can selectively react with their target analytes from the sample deposited on the sample substrate through ion/molecule reactions during desorption/ionization process. The reaction products derived from target analytes are simultaneously detected by MS. Thus, reactive MS can be defined as a technique in which target analytes are selectively reacted with reactive additives during ionization process and allow the generated product ions to be detected by MS instantaneously. High-frequency ultrasonicorators are known to prominently accelerate chemical reactions.\(^\text{[22–26]}\)

We propose herein using MHz-based UASI–MS as the reactive ion source. Namely, when liquid solution containing reactants and samples is deposited directly on a MHz-based-ultrasonic transducer, it is expected that chemical reactions and ionization occur simultaneously. The generated product ions can be

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* Correspondence to: Yu-Chieh Chen, Department of Applied Chemistry, National Chiao Tung University, Taiwan. E-mail: yuchie@mail.nctu.edu.tw

Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan

\(^*\) Correspondence to: Yu-Chie Chen, Department of Applied Chemistry, National Chiao Tung University, Taiwan. E-mail: yuchie@mail.nctu.edu.tw

Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan
simultaneously detected by MS. In this work, we proposed that the UASI–MS equipped with a high-frequency ultrasonic transducer can be used to detect small/large analytes and to accelerate and monitor organic reactions from the time resolved MS$^{(26)}$ in real time.

Experimental section

Reagents and materials

All the chemicals including acethydrazide trimethylammonium chloride (Girard T reagent), formic acid, bradykinin, melittin, insulin, cytochrome c, myoglobin, hydroxylamine, cortisone and androsterone were purchased from Sigma (St. Louis, MO, USA). Ethanol and methanol were obtained from C-Echo (MoLi, Taiwan). Capillaries (50 μm inner diameter (i. d.), 366 μm outer diameter (o. d.)) were obtained from Polymicro Technologies (Phoenix, AZ, USA).

Setup of UASI–MS

The main components of the reactive UASI were an ultrasonic transducer (frequency: ~1.7 MHz, diameter: 25 mm, voltage: 24 V, current: 450 mA) (Centenary Materials, Taiwan) and a MicrOTOF-Q II mass spectrometer (Bruker Daltonics, Bremen, Germany). The voltage set at the orifice of the mass spectrometer was ~1500 V, while the temperature of the capillary for ion transfer was set at either 220 °C or 300 °C. The samples were generally prepared in a mixture of 0.25% formic acid prepared in the solvent of methanol and deionized water (1:1, v/v) unless otherwise specified. By simply depositing the sample droplets on the surface of the ultrasonic transducer placed right underneath (~3 cm) of the orifice of the mass spectrometer as shown in Scheme 1, the ultrasonic spray derived from the sample solution can be readily monitored by the mass spectrometer.

Reactive UASI–MS

When conducting the reaction of hydroxylamine with cortisone (or androsterone), the mixture of cortisone (or androsterone) (5 mg/ml) and hydroxylamine (5 mg/ml, 0.9 ml) prepared in the solvent (0.1 ml) of methanol/water (3:1, v/v) was prepared prior to MS analysis. Urine samples were prepared by removing precipitate after centrifugation at 7000 rpm for 30 min. Supernatant was diluted 50-fold by deionized water. A given amount of cortisone (5 mg/ml, 10 μl) prepared in methanol was spiked into the diluted urine (990 μl). The other urine sample with the addition of hydroxylamine was prepared by mixing the diluted urine (900 μl), cortisone (5 mg/ml, 10 μl) prepared in methanol and hydroxylamine (5 mg/ml, 90 μl) prepared in deionized water right prior to MS analysis.

Results and discussion

Scheme 1 shows the components of the UASI including a miniature ultrasonicator (~1.7 MHz) and a syringe (or a pipette). Simply positioning the miniature ultrasonic transducer in front of the orifice of a mass spectrometer, analyte ion signals can be readily observed by the mass spectrometer upon depositing sample solution on the ultrasonic transducer. Figures 1A–1F show the resultant mass spectra of acethydrazide trimethylammonium (Girard T reagent), bradykinin, melittin, insulin, cytochrome c and myoglobin, respectively. A high-intensity analyte ion peak at m/z 132 was observed in the mass spectrum (Fig. 1A). Multiply charged ions derived from peptides (Figs. 1B and 1C) and proteins (Figs. 1D, 1E and 1F) were observed in the mass spectra. The results indicated that the current approach is suitable for analyzing small and large molecules. Furthermore, the mass spectral profiles of small and large molecules obtained from the UASI–MS are similar to those obtained from ESI–MS (Fig. S1).

After demonstrating that the current approach can be used for analytes with a wide mass range, we further demonstrated the feasibility of using the UASI approach to simultaneously accelerate and monitor the reaction of Girard T reagent with a ketosteroid. Scheme 2A shows the reaction of androsterone (MW = 290) with Girard T reagent (MW = 132). We expected the product ion peak to appear at m/z 404. We initially used conventional ESI to determine whether the product ions can be observed in the mass spectra. The reactant solutions were mixed prior to ESI injection (flow rate: 3 μl/min). Figure 2A shows the extracted ion chromatograms (EICs) of the reactant ions at m/z 132 (black) and at m/z 291 ([M + H]$^+$, red) and the expected product ion at m/z 404 (blue). The ion intensity of these monitored ions remained constant during the monitoring period. Figure 2B shows the corresponding mass spectrum. Only the peaks at m/z 132 and at m/z 291 appeared in the mass spectrum; no peak was observed at m/z 404. The results indicate that the reaction barely progressed in this setup during the hour. The UASI equipped with a miniature ultrasonic transducer (frequency: ~1.7 MHz) was employed to monitor the same reaction. Figure 2C shows the EICs at m/z 132 (black), at m/z 291 (red), and at m/z 404 (blue), and Fig. 2D shows the corresponding mass spectrum. Evidently, the ion intensity at m/z 404 derived from the product ion slightly increased. The product ion was observed right after the droplets of the reactants were deposited on the miniature transducer. On the basis of the acquisition time (duty cycle: 40 ms/cycle) required to collect the ion signal through the micro-Q-TOF II mass spectrometer, the product ion was readily generated and monitored by the MS within 1 s. Furthermore, we employed the Q-TOF mass spectrometer to accurately identify the product ion. Table S1 lists the experimental and theoretical m/z of the reactant and product ions. The experimental m/z was relatively close to the theoretical value (<2 ppm). Thus, the peak at m/z 404 represents the product.
Figure 1. UASI mass spectra of (A) Girard T reagent (5 × 10^{-5} M), (B) bradykinin (5 × 10^{-5} M), (C) melittin (10^{-6} M), (D) insulin (5 × 10^{-5} M), (E) cytochrome c (10^{-4} M) and (F) myoglobin (10^{-4} M). The temperature of the ion-transfer capillary was set to 220 °C.

Figure 2. Monitoring of the reaction of Girard T reagent (m/z 132, 5 μg/ml) and androsterone (m/z 291, 50 μg/ml) prepared in the solvent of methanol/water (3:1, v/v) with a volume ratio of 1 to 9. (A) EICs at m/z 132, m/z 291 and m/z 404 obtained using ESI–MS and (B) its corresponding mass spectrum acquired from 2118 s to 2123 s. (C) EICs at m/z 132, m/z 291 and m/z 404 obtained using the UASI–MS with ~1.7 MHz ultrasonic transducer and (D) its corresponding mass spectrum acquired from 15 s to 20 s. The arrow indicated in panel C indicated the time of the sample solution was dropped on the ultrasonicator. The total sample volume deposited on the ultrasonicator during the UASI–MS analysis was 1 ml. This figure is available in colour online at wileyonlinelibrary.com/journal/jms.
ion. Additionally, we further confirmed the results by collecting the reaction droplets containing androsterone with Girard T reagent from the miniature ultrasonicator for off-line MS analysis. Figure S2A shows the EICs at m/z 132 (black), m/z 291 (red) and m/z 404 (blue), whereas Fig. S2B shows its corresponding mass spectrum. Apparently, the product ion at m/z 404 appeared in the mass spectrum, indicating the collected droplets containing the product species. The signal-to-noise ratio (S/N) at m/z 404 was not good. The results confirmed again that the reaction really happened on the surface of the miniature ultrasonicator in the UASI process. The results indicated that the reaction can be accelerated by the high-frequency ultrasonicator using the proposed UASI–MS setup. The UASI–MS setup can be used to monitor the product ions in real time. Furthermore, the reaction was speeded up from hours to less than 1 s. Additionally, the results also suggested that better S/N can be obtained from on-line monitoring of the chemical reaction by UASI–MS analysis in real time.

Additionally, the reaction of hydroxylamine with cortisone was further used to demonstrate the UASI–MS setup for simultaneously accelerating and monitoring chemical reactions. Furthermore, only 10 μl of sample solution was deposited on the ultrasonicator to examine if a small volume of sample was sufficient for conducting the monitoring of the reaction by the current approach. Cortisone can be used as a drug to relieve inflammation and pain and to cure disease.[27] Scheme 2B shows the reaction of hydroxylamine with cortisone. Protonated cortisone ion signal was expected to appear at m/z 361, the intermediate ion at m/z 394, and the product ion at m/z 376. We initially used cortisone as the sample to observe whether any interference ions might be overlapped with the m/z values of the intermediate and product ions. Figure 3A shows the EICs of cortisone at m/z 361 ([(M + H)+] (black), 376 (red) and 394 (blue) obtained from the UASI–MS. The ion intensity at m/z 376 and 394 remained zero in the EICs. Figure 3B shows the corresponding mass spectrum. In addition to the peak at m/z 361, there are two main peaks derived from alkali metal adduct ions of cortisone, i.e. 383 ([M + Na]+) and 399 ([M + K]+), and some background ions appearing in the same mass spectrum. The result indicated that there were no background ions overlapped with the m/z values of the intermediate and the product ions. The reaction of hydroxylamine and cortisone was then examined by the UASI–MS. Figure 3C shows the resultant EICs at m/z 361 (black), at m/z 376 (red) and at m/z 394 (blue). The product ion at m/z 376 and the intermediate ion at m/z 394 were observed in the EICs. The intermediate ion and product ion immediately appeared upon the deposition of the reactant solution. Figure 3D shows the corresponding mass spectrum acquired from the period of 7 s to 12 s. Apparently, the peaks derived from protonated cortisone (m/z 361), the intermediate ion (m/z 394) and the product ion (m/z 376) dominated the mass spectrum. Background ions were also greatly suppressed (cf. Fig. 3B). Presumably, the presence of hydroxylamine suppressed the alkali metal adduct ions because NH3 can compete with alkali metal ions to bind with analyte species. The results demonstrated again that the current UASI–MS is suitable for simultaneously accelerating and monitoring of the reaction of hydroxylamine with cortisone. Furthermore, intermediate ions can be observed in the UASI mass spectra, and only 10 μl of the reaction solution was sufficient to obtain the result.

We also examined if similar results to Fig. 3 could be obtained by ESI–MS. When using ESI–MS to analyze cortisone (Figs. S3A and S3B), the results were similar to what we obtained in Figs. 3A and 3B. That is, there were no interference ions appearing at m/z 376 and m/z 394 (Figs. S3A and S3B). The EIC plot at m/z 361 (black) remained certain intensity, while the intensity at m/z 376 (blue) and m/z 394 (red) remained zero (Fig. S3A). Protonated

Figure 3. Monitoring of the reaction of hydroxylamine with cortisone. (A) EICs at m/z 361, m/z 376 and m/z 394 from the cortisone (5 μg/ml) obtained using UAISI–MS with 1.7 MHz ultrasonic transducer and (B) its corresponding mass spectrum. (C) EICs at m/z 361, m/z 376 and m/z 394 from the cortisone sample (5 μg/ml) containing hydroxylamine (45 μg/ml) obtained using UAISI–MS with 1.7 MHz ultrasonic transducer and (D) its corresponding reactive UAISI mass spectrum acquired from 7 s to 12 s. The arrows marked on panels A and C were the time point that the sample solution was dropped on the ultrasonicator. The total sample volume deposited on the ultrasonicator during the UASI–MS analysis was 10 μl. This figure is available in colour online at wileyonlinelibrary.com/journal/jms
cortisone ion at m/z 361 dominated the mass spectrum (Fig. S3B), while the sodium and potassium adduct ions of cortisone at m/z 383 and 399, respectively, were also observed in the same mass spectrum. Hydroxylamine and cortisone were mixed prior to the analysis of ESI–MS. Figure S3C shows the resultant EICs at m/z 361 (black), at m/z 376 (blue) and at m/z 394 (red), while Fig. S3D shows the corresponding mass spectrum. The product ion at m/z 376 was observed in the EIC, but the intermediate ion at m/z 394 was missing. The relative intensity of the peak m/z 376 was much lower (Fig. S3D) than that obtained from UASI–MS (cf. Fig. 3D). Although ESI–MS can be used to observe the product ion at m/z 376, it is unable to detect the intermediate ion at m/z 394. These results suggest that UASI–MS can be potentially considered as an alternative method to monitor chemical reactions for the short-lifetime intermediate ions. As the model reactions demonstrated in this work, the product ions from the UASI–MS setup can be observed within 1 s upon depositing the reactants. The results suggest that the current technique can efficiently accelerate the chemical reactions while monitoring the generation of the product ions simultaneously. However, the reactants and products have a short retention time on the ultrasonic transducer during UASI–MS analysis. Thus, the intensity of the generated product was generally low. Nevertheless, upon depositing the sample solution on the ultrasonic transducer, the UASI spray and reactions of the sample solution were readily generated and immediately detected by the mass spectrometer.

One of the potential applications of the reactive MS is to selectively detect target species from complex samples through the generated product ions. For example, traces of steroids are present in urine samples, but detecting target steroids directly from urine samples using MS may be difficult. Interference from complex background ions may suppress the ion signals derived from target steroids. Reactive-MS such as the current UASI–MS provides an alternative method for detecting trace steroids from urine samples with minimal sample pretreatment. We prepared a diluted urine sample spiked with a steroid, i.e. cortisone, which can be used to relieve pain and reduce the swelling from inflammation. The daily dose for adults is usually 25–300 μg.[27] Considering urine volume (~1.5 l) generated by adults per day,[29] the concentration in biological fluids was estimated to be 4.6 × 10⁻⁵–4.6 × 10⁻⁴ M. The concentration (~1.4 × 10⁻⁴ M) used in the experiment was within this range although the metabolism needs to be further considered in real cases. Herein, we only demonstrate the possibility to detect the reactive product ion directly from a simulated urine sample using our approach. First, the urine sample spiked with cortisone without addition of hydroxylamine was examined by the UASI–MS. Figure 4A shows the EICs at m/z 361 (black), m/z 376 (blue) and m/z 394 (red), while Fig. 4B shows the corresponding UASI mass spectrum of the diluted urine spiked with cortisone. The EICs in Fig. 4A were not very stable presumably owing to the presence of complex matrix such as salts in urine. The intensity at the expected intermediate ion (m/z 394) and the product ion (m/z 376) remained nearly zero, which indicated no interference ions overlapped with the m/z values of the expected ions. The ion peak at m/z 399 derived from the potassium adduct ions of cortisone dominated the mass spectrum (Fig. 4B) owing to the presence of a high concentration of potassium ions in the urine sample. The peak at m/z 361 represents the protonated cortisone and the peak at m/z 383 represents the sodium adduct ions. Presumably, the peak at m/z 114 represents protonated creatinine ions and that at m/z 227 represents the dimer ions of creatinine. Next, we added hydroxylamine to the urine sample prior to the UASI–MS analysis.

**Figure 4.** Selective detection of trace cortisone from a urine sample. (A) EICs at m/z 361, m/z 376 and m/z 394 from the 50-fold diluted urine (990 μl) spiked with cortisone (5 mg/ml, 10 μl) obtained using UASI–MS with ~1.7 MHz ultrasonic transducer and (B) its corresponding UASI mass spectrum acquired from 24 s and 29 s. (C) EICs at m/z 361 and m/z 376 from the 50-fold diluted urine sample (900 μl) cortisone (5 mg/ml, 10 μl) with the addition of hydroxylamine (5 mg/ml, 90 μl) obtained using UASI–MS with ~1.7 MHz ultrasonic transducer and (D) its corresponding reactive UASI mass spectrum acquired from 12 s to 17 s. The arrows marked on panels A and C were the time point that the sample solution was dropped on the ultrasonicator. The signal-to-noise ratio at m/z 376 was ~315. This figure is available in colour online at wileyonlinelibrary.com/journal/jms
Figure 4C shows the resultant EICs of the protonated cortisol at m/z 361 (black) and the product ion at m/z 376 (blue). These two ions dominated the corresponding mass spectrum as shown in Fig. 4D. However, the intermediate ion at m/z 394 was not observed, presumably owing to its low ion intensity. The peaks derived from the alkali metal adduct ions of cortisol at m/z 383 ([M+Na]⁺) and at m/z 399 ([M+K]⁺) with low-intensity were also observed, whereas background ions such as at m/z 114 and at m/z 227 that were presumably derived from creatinine were barely observed in the same mass spectrum. The results indicated that the steroid traces can be simply confirmed by examining the appearance of the product ions using this reactive UASI–MS approach. Furthermore, the interference from background ions was dramatically suppressed using the current UASI approach. Additionally, the S/N ratios of target analytes were dramatically improved using the current reactive-MS approach.

Conclusions

We have demonstrated that the UASI–MS system with an ~1.7 MHz ultrasonic transducer can be used to analyze analytes with a wide mass range. Furthermore, this approach can be used to efficiently accelerate chemical reactions and to instantaneously monitor the reacting species including reactants, intermediates and product ions. The proposed method is potentially useful for investigating chemical reactions within a short period of time. The results also suggest the possibility of using the proposed setup for reactive-MS analysis that can be conducted by reacting reactants during mass spectrometric analysis. The target species and their product ions can be easily observed in the reactive UASI mass spectra within 1 s. The results also suggest that this reactive-UASI MS approach can be readily used to confirm the identity of target steroids from complex urine samples by selectively reacting and detecting of target species/product ions from complex samples. The background ions were suppressed and the S/N ratios were greatly improved during the reactive UASI–MS analysis. The applications of this approach in the selective detection of trace target species from complex samples look quite promising. The main advantages of this approach include ease of operation, elimination of tedious sample pretreatment, improving S/N ratios and shortening the analysis time. Additionally, the setup of this approach is quite simple, and its essential part, i.e., the miniature ultrasonic transducer, is inexpensive (~$30 USD). Furthermore, simply wiping the sample residues by proper solvents from the surface of the ultrasonic transducer after analysis, the transducer can be readily used for the analysis of new samples. Thus, we believe that this approach should potentially benefit in understanding mechanisms of specific chemical reactions and determining trace target species from complex samples with minimum sample pretreatment.

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References


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