Ultrasonication-assisted spray ionization mass spectrometry for on-line monitoring of organic reactions†

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Received 17th July 2010, Accepted 28th September 2010
DOI: 10.1039/c0cc02629h

A straightforward on-line monitoring of organic reactions by ultrasonication-assisted spray ionization mass spectrometry (UASI MS) is demonstrated in this work.

Ultrasonication has been employed to accelerate organic syntheses[1–8] and enzymatic reactions.[9–11] For example, high biodiesel yields can be obtained through the transesterification of vegetable oils in the presence of alcohol and base catalyst under ultrasonication within a much shorter time than those obtained through conventional approaches.[6,7] Under ultrasonic mixing, smaller droplet sizes of alcohol/oil dispersions accelerate the reaction, leading to high transesterification yield.[8] The hydrolytic activity of lipase[10] and tryptic digestion of proteins[11] can be enhanced dramatically under ultrasound irradiation. The main advantages of ultrasonication-assisted reactions include short reaction time, high yields, and ease of use.

Recently, we explored a new atmospheric pressure ionization mass spectrometry, so-called ultrasonication-assisted spray ionization mass spectrometry (UASI MS),[12] which has been demonstrated to be suitable for the analysis of small and large biomolecules. The background in the UASI mass spectra is rather low, allowing for a good signal to noise (S/N) ratio[12] compared with that obtained using the conventional electrospray ionization mass spectrometry (ESI MS). The good S/N ratio is due to the fact that a high electric field is not required for the generation of sample spray in the UASI MS approach. Therefore, the background ions derived from electrochemical reaction are eliminated. In the UASI MS, a capillary is placed into an Eppendorf tube containing the sample solution subjected to an ultrasonicator used to provide a sufficient driving force to direct the liquid sample solution from the inlet to the tapered outlet of the capillary, leading to the generation of the ultrasonic spray. The analyte ions derived from the ultrasonic spray are directly detected using MS. As ultrasonication has been used in assisting organic reactions, there are no compromises in coupling the UASI MS with ultrasonication-assisted reactions (Fig. 1). Reactants dissolved in a suitable solvent are placed in an Eppendorf tube, which has been subjected to an ultrasonicator. Once the ultrasonicator is switched on, ultrasonication-assisted organic reaction is carried out. The reaction intermediates and products continually run through the capillary, spraying out from the capillary outlet with the assistance of ultrasonication and simultaneously monitored by the MS. Thus, monitoring reactions through this approach is very straightforward. In this study, we employed the UASI MS to monitor ultrasonication-assisted reactions in real time.

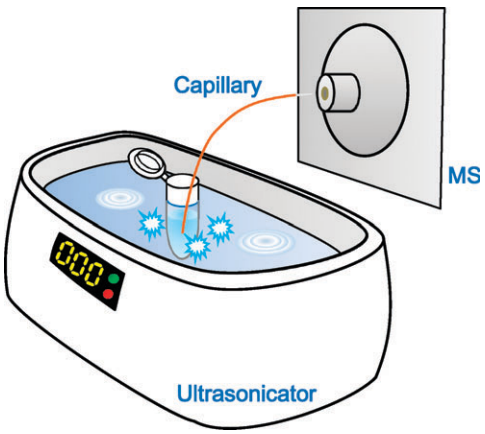

Fig. 1 Cartoon representation of the configuration of the on-line monitoring of ultrasonication-assisted reactions by UASI MS.

A transesterification reaction called the Zemplen reaction is generally used in deblocking reactions in carbohydrate chemistry.[13] Ultrasound has been successfully employed in promoting the reaction.[14] We selected a Zemplen reaction as the model reaction for on-line monitoring using the UASI MS. Esters are versatile groups in hydroxyl functions during oxidation, peptide coupling reactions, or glycosylations and are typically deprotected by base-catalyzed solvolysis. Among the large number of ester protecting groups, benzoyl ester is less susceptible to the undesirable acyl migration; consequently, it is widely used in organic synthesis. The removal of the benzoate group is usually accomplished using Zemplen conditions, where a base catalyst is employed to catalyze a transesterification process. When multiple benzoyl functions are present in the same molecule, an analytical method that can assess the removal rate of each individual function is imperative. In doing so, a regioselective deprotection may be realized. Thus, the real-time monitoring UASI MS is applied to monitor the Zemplen deprotection of a model substrate, namely, 4-*O*-benzyl-2,3,6-tri-*O*-benzoyl thioglucopyranoside (**A**). Sodium methoxide (MeONa) is used as the catalyst for the Zemplen reaction. The synthesis of reactant **A** was described in ESI.† Scheme 1 illustrates the Zemplen reaction. The expected intermediates and the final product are labeled **B**, **C**, and **D**, respectively.

Prior to performing the on-line monitoring of the Zemplen reaction, the reaction products obtained from off line were collected at different reaction time points and subjected to ESI MS for analysis. For comparison, the reaction was performed

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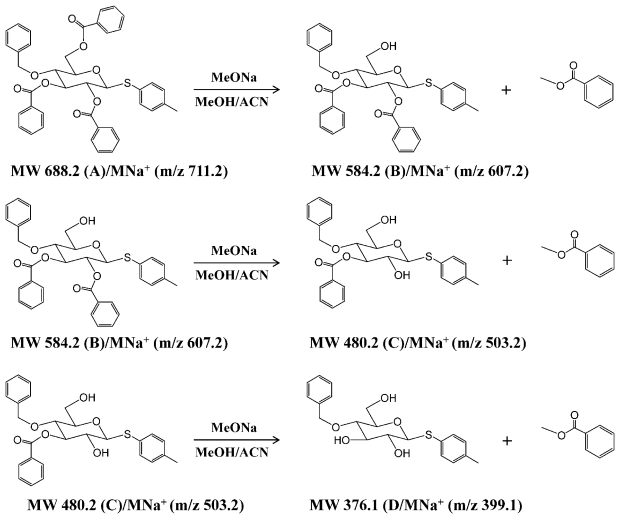
† Electronic supplementary information (ESI) available: Experimental details and supplementary results are provided. See DOI: 10.1039/c0cc02629h



Scheme 1 Zemplen deprotection of 4-*O*-benzyl-2,3,6-tri-*O*-benzoyl thioglucopyranoside (**A**) in the solvent of methanol/acetonitrile (1/1, v/v). The catalyst was MeONa. ACN and MeOH stand for acetonitrile and methanol, respectively.

under different conditions, including vortex-mixing and Fig. 2a–f display the ESI mass spectra of the reaction products collected at the reaction time points of 0, 12, 27, 35, 42, and 65 min, respectively. The peaks at *m*/*z* 437, 620, and 647 were presumably derived from the solvent background and observed in each mass spectrum. The peak at *m*/*z* 711 derived from sodiated reactant **A** dominated the mass spectrum in Fig. 2a prior to reaction. A weak peak at *m*/*z* 607 representing the sodiated intermediate resulting from the loss of a benzoyl function group of reactant **A** began to appear in the mass spectrum after reacting for 12 min (Fig. 2b). After the reaction had proceeded for 27 min, the peak at *m*/*z* 607 was slightly raised in the mass spectrum as seen in Fig. 2c. A very weak peak at *m*/*z* 503, a sodiated peak, representing the loss of two benzoyl functional groups from the reactant began to appear in the same mass spectrum. As the reaction time reached its 35th min, the peak at *m*/*z* 607 became enhanced (Fig. 2d). The peak at *m*/*z* 503 was revealed in the same mass spectrum. After the reaction went on for 42 min, the ESI mass spectrum of the reaction sample resembled that of Fig. 2d (Fig. 2e). The product ion derived from the sodiated product through the loss of three benzoyl functional groups was not observed in the mass spectrum after reacting for 65 min (Fig. 2f). The intensity of the peak at *m*/*z* 607 continued to elevate in the mass spectrum (Fig. 2f), whereas the peak at *m*/*z* 711 dominated the mass spectra after the reaction had stood for 65 min (Fig. 2f).

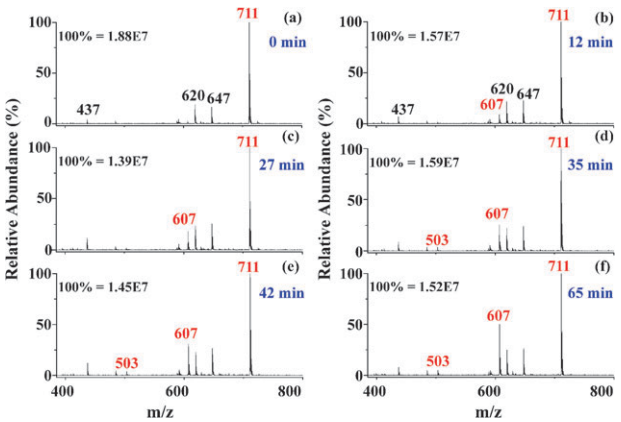


Fig. 2 ESI mass spectra of the samples obtained from the Zemplen reaction using 4-*O*-benzyl-2,3,6-tri-*O*-benzoyl thioglucopyranoside (**A**) prepared in the solvent of methanol/acetonitrile (1/1, v/v) as the product; MeONa was used as catalyst. The reaction was conducted under vortex-mixing. The samples were collected for ESI MS analysis at the following reaction time points: (a) 0, (b) 12 min, (c) 27 min, (d) 35 min, (e) 42 min, and (f) 65 min.

As mentioned earlier, ultrasonication has been demonstrated to be effective in accelerating organic reactions; thus, we also performed the Zemplen reaction under ultrasonication. The reaction products were collected at specific time points. Fig. 3a–f display the ESI mass spectra of the reaction products obtained at the reaction time of 0, 12, 27, 35, 42, and 65 min, respectively. At the beginning, the peak at *m*/*z* 711 corresponding to sodiated reactant **A** dominated the mass spectrum (Fig. 3a). The peaks at *m*/*z* 437, 620, and 647 derived from the solvent and background were also observed in the same mass spectrum. After the reaction had proceeded for 12 min, the peak at *m*/*z* 711 still dominated the mass spectrum, whereas a weak peak at *m*/*z* 607 derived from the loss of a benzoyl function group was revealed in the mass spectrum (Fig. 3b). In addition, a peak at *m*/*z* 503 resulting from the loss of two benzoyl functional groups also appeared in the same mass spectrum. The peaks at *m*/*z* 503 and 607 were slightly raised after the reaction continually reacted for 27 and 35 min (Fig. 3c and d). As the reaction time reached 42 min, the peak at *m*/*z* 607 dominated the mass spectrum. An extra peak at *m*/*z* 399 with a low intensity was also revealed in the same mass spectrum (Fig. 3e). The peak at *m*/*z* 607 remained as the base peak after the reaction went on for 65 min (Fig. 3f). However, the peak at *m*/*z* 711 corresponding to sodiated reactant **A** reduced substantially; while the intensities of the peaks at *m*/*z* 399 and 503 increased to a slight extent. The results presented in Fig. 3 indicate that the reaction was effectively accelerated under ultrasonication compared with those obtained by the conventional procedures as shown in Fig. 2.

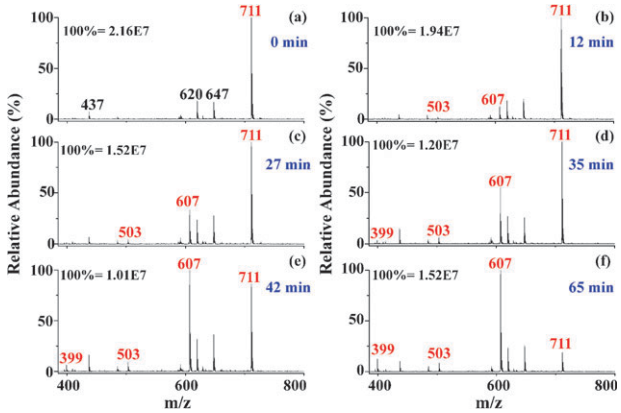


Fig. 3 ESI mass spectra of the samples obtained from the Zemplen reaction using 4-*O*-benzyl-2,3,6-tri-*O*-benzoyl thioglucopyranoside (**A**) prepared in the solvent of methanol/acetonitrile (1/1, v/v) as the product; MeONa was used as catalyst. The reaction was conducted under ultrasonication. The samples were collected for ESI MS analysis at the following reaction time points: (a) 0, (b) 12 min, (c) 27 min, (d) 35 min, (e) 42 min, and (f) 65 min.

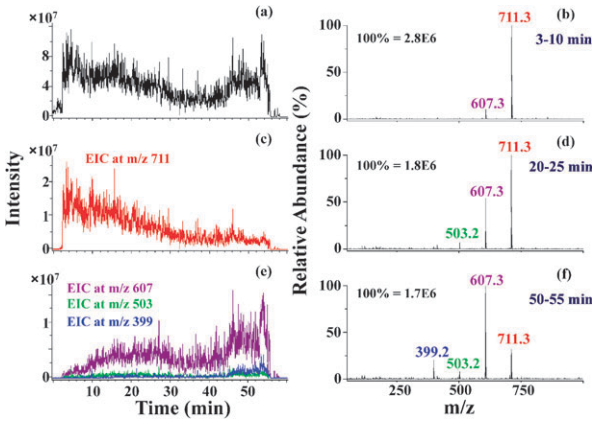


Fig. 4 Zemplen deprotection of 4-*O*-benzyl-2,3,6-tri-*O*-benzoyl thio-gluco-pyranoside (**A**) prepared in the solvent of methanol/acetonitrile (1/1, v/v). The catalyst was MeONa. (a) TIC chromatogram obtained during the on-line monitoring of the Zemplen reaction using the UASI MS; (b) UASI mass spectrum obtained from the averaged mass spectra acquired between the reaction time at 3 and 10 min in Panel a; (c) EIC at *m*/*z* 711; (d) UASI mass spectrum obtained from the averaged mass spectra acquired between 20 and 25 min in Panel a; (e) EIC at *m*/*z* 607, 503, and 399 plotted in purple, green, and blue, respectively; and (f) UASI mass spectrum obtained from the averaged mass spectra acquired between 50 and 55 min in Panel a.

After demonstrating that the Zemplen reaction accelerated effectively under ultrasonication, we monitored the reaction with the UASI MS in real time. The setup of the UASI MS and the fabrication of the capillary tip are briefly stated in ESI†, which are mainly based on the approach described in the previous study.[12] The combination of UASI MS with the ultrasonic-assisted reaction was straightforward; a capillary filled with solvent was placed into the reaction vial subjected to an ultrasonicator, and the tapered outlet of the capillary was placed close to the inlet of a mass spectrometer. The products generated from the ultrasonic-assisted reaction were monitored readily through the mass spectrometer upon switching on the ultrasonicator.

We initially filled out a capillary with acetonitrile/methanol (1/1, v/v) solvent and placed it into the reaction vial containing reactant **A** prepared in acetonitrile/methanol (1/1, v/v). The reaction vial was subjected to ultrasonication, whereas the tapered outlet of the capillary was placed close to the inlet of an ion trap mass spectrometer (Fig. 1). The catalyst, MeONa, was added into the reaction vial when the ultrasonicator was switched on. The ions generated from the capillary outlet *via* ultrasonic spray were monitored using the mass spectrometer in real time. Prior to powering the ultrasonicator on, the mass spectrometer was employed to acquire ions during the first 2 min (Fig. 4a). The mass spectrometer detects low intensities of ions derived from a volatile solvent at the beginning. Once the ultrasonicator was switched on, the total ion current (TIC) increased dramatically as seen in Fig. 4a. After monitoring the ions for 56 min, the ultrasonicator was switched off, and the intensity of the ions suddenly decreased. During the first 3–10 min, the sodiated reactant **A** at *m*/*z* 711 marked in red dominated the average mass spectrum (Fig. 4b). Fig. 4c presents the extracted ion chromatogram (EIC) at *m*/*z* 711 during the on-line monitoring of the Zemplen reaction by the UASI MS. The intensity of the EIC at *m*/*z* 711 decreased as the monitoring time increased. Such results are attributed to continuous consumption of the reactant **A** in the reaction course. The peak at *m*/*z* 607 (**B**), which is a sodiated ion, attributed to the loss of the benzoyl function group at the C-6 primary position, was also observed in the same mass spectrum. Fig. 4d displays the mass spectrum obtained from the averaged mass spectra between 20 and 25 min in Fig. 4a. Aside from the peaks at *m*/*z* 711 and 607 observed in Fig. 4b, there was an extra peak that appeared in the mass spectrum at *m*/*z* 503, which is a sodiated peak corresponding to the loss of two benzoyl functional groups of reactant **A**. As the reaction time increased, the peak at *m*/*z* 399, which represents the loss of three benzoyl functional groups, began to appear in the mass spectra. Fig. 4e displays the EIC at *m*/*z* 607, 503, and 399 plotted in purple, green, and blue, respectively. The ion current for *m*/*z* 607 increased continually, whereas the ion current for *m*/*z* 503 remained the same. The ion current for *m*/*z* 399, a sodiated ion, became noticeable after reaction for ~44 min. Fig. 4f presents the mass spectrum obtained from the averaged mass spectra acquired between 50 and 55 min in Fig. 4a. As expected, the peak at *m*/*z* 399 was observed in the mass spectrum except for the peaks at *m*/*z* 503, 607, and 711. The peak at *m*/*z* 607 dominated the mass spectrum. This result indicates that the regioselective removal of C-6 benzoate ester in substrate **A** is made possible by the real-time monitoring of the reaction with the use of UASI MS method. Additionally, the background ions at *m*/*z* 437, 620, and 647 appearing in conventional ESI mass spectra in Fig. 2 and 3 are not observed in Fig. 4. The results demonstrate that background in UASI mass spectra is rather low.

In conclusion, we have demonstrated a very straightforward approach for on-line monitoring of organic reactions using the UASI MS as detection method. There are no compromises in combining ultrasonication-assisted organic reactions and the UASI MS analysis. This approach can be further used for various types of organic reactions assisted by ultrasonication such as enzymatic reactions and polymer degradations.

We thank the National Science Council (NSC) of Taiwan for the financial support of this work.

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