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Coupling of single droplet micro-extraction with desorption electrospray ionization-mass spectrometry

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Dedicated to Prof. Michael L. Gross in the occasion of his 70th birthday.

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ABSTRACT

Single droplet micro-extraction (SDME) is a powerful preconcentration and purification technique for trace chemical analysis, but the detection of the resulting single droplet extract is often time-consuming with traditional detection methods such as GC/MS or LC/MS. In this study, desorption electrospray ionization-mass spectrometry (DESI-MS) was coupled with SDME to serve as a fast detection method for the first time, demonstrated by the trace analysis of methamphetamine (MA) in aqueous solution and the detection of an organic reaction product from an ionic liquid (IL). In the former application, three-phase liquid SDME was conducted to enrich MA in aqueous solution to an organic solvent and then to back-extract the analyte to a single aqueous droplet. Subsequent DESI-MS analysis can be performed either to a single droplet or multiple droplets in a row. The average enrichment factor obtained for MA was 390-fold. In the latter case, two-phase liquid SDME was conducted to directly extract the product of an organic reaction performed in a room temperature ionic liquid (i.e., the nucleophilic addition of aniline to phenyl isothiocyanate to form N,N′-diphenylthiourea in 1-butyl-3-methyl-imidazolium tetrafluoroborate). The ionization of the resulting droplet extract by DESI allows one to directly examine the reaction product without interference from the ionic liquid. Such an analysis uses a few microliters of an organic solvent, achieving green chemistry. This combined SDME/DESI-MS method is characterized with high preconcentration efficiency, high throughput capability and minimizing organic solvent waste.

1. Introduction

Desorption electrospray ionization-mass spectrometry (DESI-MS), first introduced by Cooks and co-workers [1,2], is a powerful detection method for a variety of analytes encompassing illicit drugs, chemical warfare agents, pharmaceuticals, biological samples, etc. [3–22]. DESI-MS is characterized by no or little need of sample pretreatment, satisfactory performance under ambient conditions, short analysis time (as short as a few seconds), and equivalent specificity to other mass spectrometric methods. Recently, in our and other laboratories, DESI-MS has been extended to the direct ionization of liquid samples [23–28] such as biological samples and drugs. It has been demonstrated that liquid DESI-MS has the advantages of direct analysis of liquid samples as opposed to drying the sample in conventional DESI-MS or adding additives of organic solvents/acids to samples prior to ionization as required by ESI-MS. Liquid DESI-MS was advantageous to a protein conformation study [29] and coupled to an electrochemical cell for the reduction of protein disulfide bonds [25]. Notably, liquid DESI-MS is suitable for direct analysis of droplets or thin liquid films [23,28].

Single droplet micro-extraction (SDME) is a preconcentration/purification protocol used to preconcentrate or separate analytes from complex sample matrices. As an efficient enrichment and separation method, micro-extraction has been developed for various applications ranging from environmental monitoring [30,31], anti-doping screening [32], to forensic detection [33,34]. Among all the micro-extraction techniques, single droplet micro-extraction (SDME) [33–36] has displayed advantages of low cost, simplicity and effective preconcentration capability. Because of the microliter volume of extraction solvent used, only a small extraction stock solution is needed. Moreover, a small volume of organic solvent is consumed in the process, producing less amount of waste than traditional extraction methods and enabling easy clean-up [37]. A microliter sized droplet used in SDME offers a larger phase ratio compared with solid phase micro-extraction, leading to a highly efficient extraction of the analyte into the desired phase. In the traditional two-phase liquid SDME experiments, a single droplet is immersed into another liquid layer for analyte extrac-

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tion. In a variant form of SDME such as three-phase liquid SDME, the protocol involves a two-step extraction so that low concentration analytes can be preconcentrated from a bulky solution to another liquid phase and then into a single droplet of a suitable solvent. In both cases, the resulting single droplet extract is subsequently subjected to analysis. For this purpose, SDME was typically coupled with various analytical methods such as capillary electrophoresis (CE) [38–40], high-performance liquid chromatography (HPLC) [30,31,33,37], GC/MS [41] and fluorescence (FL) spectroscopy [38]. However, the detection of the resulting single droplet extract by these methods involving separation is often either time-consuming or limited to volatile/chromophoric compounds. It is necessary to develop a rapid and general analysis platform for SDME.

Methamphetamine (MA) [42] as a highly addictive and easy-to-make drug has been commonly abused among a vast population with the promotion of increasing production by illicit drug laboratories worldwide [43]. MA abuse can cause impairments to neurotransmitter systems distributed throughout the brain and further damage cognitive ability [44]. Because of the reasons mentioned above, fast, convenient and economical detection methods of MA are needed. For this reason, MA was selected as one of the model compounds in this work to couple SDME with DESI-MS.

Green chemistry has become important with recent concerns of climate change and overpopulation. Ionic liquids (ILs) have been applied frequently as green solvents in organic chemistry because of their special properties such as negligible vapor pressures and simplicity in recycling and reuse [45–49]. It would be informative to monitor the progress of a reaction by instrumental methods such as MS during the reaction process with high chemical specificity and sensitivity. Although matrix-assisted laser desorption/ionization (MALDI) mass spectrometry can be used to detect peptides or proteins from ILs directly [50], the direct detection of reaction products in ILs using electrospray ionization (ESI) based mass spectrometric methods is challenging because ILs are not amenable to ESI-MS. As a result of the low volatility and the ionic nature of ILs, they may either contaminate the MS instrument or suppress the ion signals of product compounds. Therefore, new approaches for simplifying the MS detection of the reaction products in ionic liquids are needed.

Because of the capability of direct analysis of nonvolatile compounds in small volume of liquid films (sub-microliter) by DESI-MS (even in a high throughput manner [23]) and the power of SDME in preconcentration, it would be reasonable to combine these two techniques for enhanced performance in analysis of liquid samples. As demonstrated in this work, for the first time SDME was coupled with DESI-MS to detect a trace amount of MA in aqueous solution. In addition, a reaction product of N,N′-diphenylthiourea (DPTU), generated from aniline addition onto phenyl isothiocyanate in a room temperature ionic liquid (RTIL) of 1-butyl-3-methyl-imidazolium tetrafluoroborate [Bmim][BF_4], was successfully extracted into a small organic droplet and subsequently detected by DESI-MS. Such an analysis provides higher chemical specificity in comparison with traditional reaction monitoring techniques such as thin-layer chromatography (TLC). This combination benefits both techniques because DESI-MS allows fast chemical analysis of SDME extracts and SDME can be an ideal preconcentration and separation protocol for DESI-MS. The coupled SDME/DESI-MS will find useful applications in trace chemical analysis as well as in the green chemistry field.

2. Experimental

2.1. Chemicals and reagents

Methamphetamine hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO) and used as received. The deionized water used for sample preparation was obtained using a water purification system (Nanopure Diamond Barnstead, Thermo Scientific). High-performance liquid chromatography grade methanol was purchased from GFS Chemicals (Columbus, OH) and glacial acetic acid was purchased from Fisher Chemicals (Fair Lawn, NJ). ACS grade hexane was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Reagent grade tetramethylammonium chloride was supplied by Sigma Aldrich. Sodium hydroxide was obtained from Spectrum Chemical MFG Corp. (Gardena, CA, USA). Aniline (ACS reagent, purity >99.5%), phenyl isothiocyanate (ACS reagent, purity of 99%), tetrachloromethane (anhydrous, ACS reagent, purity of 99.9%) and acetonitrile (HPLC grade) were supplied by Sigma-Aldrich. The room temperature ionic liquid [Bmim][BF_4] (HPLC grade) was from Fluka.

2.2. Three-phase SDME for trace analysis of MA

2.2.1. Sample preparation for MA extraction

All the MA samples were prepared from a 1.0 μg/mL stock MA·HCl solution in water. The calibration series included 50., 100., 200. and 800. ng/mL MA·HCl solutions in 5% acetic acid. The suspended droplet used for SDME was 4 μL of a 5% acetic acid aqueous solution.

2.2.2. Extraction procedure for MA

An extraction procedure from the literature [33] was followed with some modifications. The extraction set-up is displayed in Fig. 1(A). First 4.00 mL of 0.50 M NaOH solution was added to a 10 mL test tube followed by the addition of 4.0 μL of 1.0 μg/mL MA·HCl aqueous solution. After mixing, the test tube was sealed with aluminum foil. This solution served as a mimetic of 1. ng/mL of MA in aqueous solution and the added base NaOH was for neutralizing the MA molecules. For the 1st step extraction, 400.0 μL of hexane was added and the test tube was shaken manually for 5 min. The test tube with a stirring bar (length: 10 mm; diameter: 3 mm) then was mounted on a magnetic stirrer (Corning Inc., Model PC-220) and it was stirred for 15 min at the highest speed (1100 rpm) for accelerating the hexane extraction of MA from the bulk aqueous phase. After the stirring was stopped a 4 μL droplet of 5% acetic acid in water was suspended in the organic layer of hexane by using a 50 μL flat-top HPLC syringe (Hamilton) with the needle replaced with 7 cm of a deactivated fused silica capillary (i.d.: 0.25 mm; o.d.: 0.36 mm; Alltech Associates Inc., Deerfield, IL, USA) after being inserted through the aluminum foil seal of the test tube and fastened with a ring stand clamp. About 10 μL of air above the droplet was kept in the syringe for the convenience of handling the droplet. The solution then was stirred at the highest speed (1100 rpm) for 40 min to convey MA to the suspended droplet while the test tube was sealed with aluminum foil. The whole extraction procedure took about an hour. After extraction, the hanging droplet was withdrawn back into the syringe and then subjected to DESI-MS analysis as described below.

2.2.3. Calibration of DESI-MS detection of MA

A series of calibration solutions were used to calibrate the DESI-MS detection of MA in aqueous solution. The concentrations of the calibration solutions were 50., 100., 200. and 800. ng/mL of MA·HCl in 5% acetic acid and the extractions and measurements were conducted in triplicate. The experiment procedures contained three parts. First, methanol/water/acetic acid (50:50:1 by volume) was used as the DESI spray solvent and a blank solution of 5% acetic acid in water was sampled for obtaining the background spectrum for signal correction; secondly, 4 μL of the prepared MA calibration solutions was sampled with the syringe used for microextraction from which they were exposed to DESI-MS detection. An internal standard of 1.0 μM tetramethylammonium chloride was contained in the DESI spray solvent for correction of signal

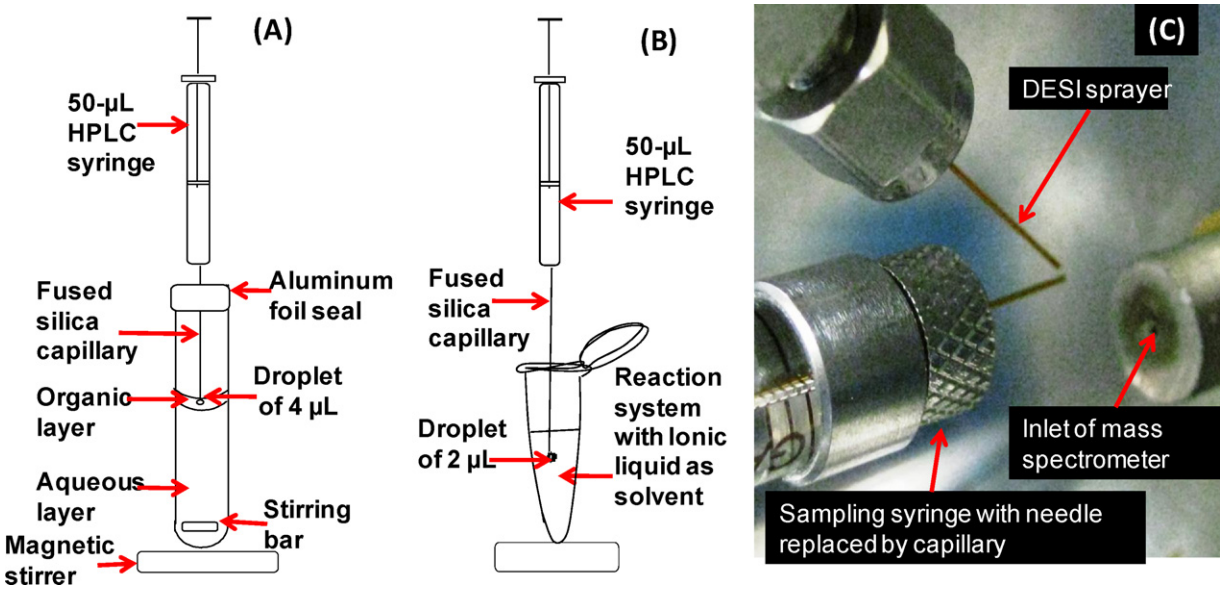


Fig. 1. Schematics showing (A) the three-phase SDME set-up for MA analysis and (B) the two-phase SDME set-up for DPTU detection from an ionic liquid; (C) image showing the direct desorption and ionization of the obtained single droplet extract contained in the syringe with a needle of the deactivated fused silica capillary.

fluctuation. The DESI-MS analyses of MA extracts were conducted following the same procedure under the same conditions as the calibration experiments. The calibration results were modeled by linear regression analysis [51]. The 95% confidence intervals for the calibration line, the slope, and the intercept were calculated from the averages of the replicates for each standard concentration. The average concentration for the single droplet sample containing the extracted MA was calculated from three extraction replicates and reported with a 95% confidence interval.

2.3. Detection of the products of organic reactions performed in ionic liquids

The reaction procedure in Ref. [52] was followed with some modifications. Briefly, 200 μL of [Bmim][BF4] was added to a 0.5 mL plastic vial. Under stirring with two mini stir bars (diameter: 2.0 mm; length: 2.2 mm) at the highest speed (1100 rpm), 0.1 mmol of each reactant (aniline and phenyl isothiocyanate) was added to the ionic liquid solvent and the reaction was allowed to proceed for 20 min. As shown in Fig. 1(B), the same type of 50 μL HPLC syringe (the metallic needle was also replaced with a 4 cm fused silica capillary) as used in MA extraction was used to suspend a CCl_4 droplet of 3 μL in the layer of ionic liquid without stirring. Extraction times of 1, 2 and 10 min were evaluated for the best signal intensity. To prevent accidental withdrawing of the ionic liquid into the syringe, only about 2 μL of the 3 μL CCl_4 droplet was withdrawn into the syringe. Subsequently, fused silica capillary was cut to a length of ca. 0.5 cm after withdrawing the droplet into the syringe and prior to DESI-MS analysis. This step was necessary to avoid the vibration of the capillary during DESI ionization of the sample.

2.4. Instrumentation

An ion trap mass spectrometer (Thermo Finnigan LCQ DECA, San Jose, CA) was used in this work. The DESI source set-up is described in detail elsewhere [23] with a slight modification as illustrated in Fig. 1(C) for which no surface was used as in the conventional DESI-MS. Briefly, for all the experiments a heated capillary temperature of 150 °C, a spray voltage of +5.0 kV, and a nebulizing gas (N_2) pressure of 180 psi were maintained. For the MA detection a typical DESI spray solvent consisted of methanol:water:acetic acid (50:50:1 by volume) with an injection flow rate of 10.0 μL/min. For quantification of MA extracts, the spray solvent was doped with 1 μM tetramethylammonium chloride as an internal standard. The ionization of the single droplet obtained from SDME occurred by directing the charged microdroplet beam from DESI spray onto the silica capillary of the syringe that was used in the micro-extraction while the sample was expelled from the capillary. The resulting ions were collected and detected by the mass spectrometer. As shown in Fig. 1(C) the angle of the DESI sprayer with respect to the sampling capillary needle was about 45° and the tips of these two capillaries were approximately 1 mm apart.

For MA detection a 4 μL droplet was expelled from the capillary at 40 μL/min by a Chemyx Model F100 syringe pump (Huston, TX), which took 4–6 s. Full MS scans were acquired in positive ion mode. For the detection of the DPTU extract, similar DESI-MS parameters were used with the differences listed below. First, acetonitrile with 0.5% acetic acid was used as the DESI spray solvent. The spray speed was adjusted to 5 μL/min and the DPTU extract was expelled from the syringe at a rate of 20 μL/min. A 2 μL droplet of the blank solvent of CCl_4 was detected under the same conditions to characterize the background signal. Collision induced dissociation (CID) mass spectra were also collected for the product identification.

3. Results and discussion

3.1. SDME/DESI-MS for trace analysis of MA

3.1.1. Optimization of three-phase SDME for MA extraction

In our experiment for MA extraction, three-phase liquid extraction procedure was selected for preconcentration because with two-phase micro-extraction of MA from an aqueous solution, the acceptor phase should be an organic solvent, which gave a relatively weaker mass spectrometric signal of MA than from an aqueous solution. One approach to improve the extraction efficiency of an extraction is to adjust the pH of aqueous phase by adding acid or base. For MA as a model compound for controlled phenethylamine drugs, this approach was adopted. In the process of preparing the stock solution for extraction of MA, NaOH was added to reach a final concentration of 0.50 M of NaOH. By adding NaOH, MA·HCl in water was converted to the free-base form of MA, hence improving its solubility in the hexane layer. According to the literature, 0.50 M NaOH is basic enough to convert all of the MA to its free-base form [33]. Subsequently, 5% acetic acid was used in the suspended droplet

to convert the MA to its protonated form; therefore the equilibria would direct MA into the aqueous suspended droplet phase. Acetic acid was used to acidify the acceptor droplet because it was also used with the DESI spray. A concentration of 5% acetic acid was sufficient to convert MA to its protonated form. The size of droplet was selected to be 4 μL because a droplet of this size allowed a balance between better extraction efficiency with larger sized droplets [33] and the better preconcentration of MA with smaller sized droplets.

The smaller the volume of hexane, the better the extraction efficiency; however, if the volume of hexane is too small, the organic layer is not thick enough to separate the aqueous droplet from the aqueous bottom layer. Therefore, 400 μL was chosen as the smallest volume of hexane possible to give an enough thickness of the organic layer to maintain the droplet.

3.1.2. Optimization of the DESI apparatus for MA detection

Two improvements were made to the DESI apparatus. In traditional DESI-MS, a surface is involved, serving as a sample desorption platform. In this study no surface was used in the DESI set-up. As seen in Fig. 1(C), the syringe was mounted on a syringe pump and the tip of the capillary was placed in between the tip of DESI sprayer and the MS inlet, similar to the apparatus that was used to couple with an electrochemical cell in one of our previous studies [25]. Another improvement was based on the observation that conductive surface material such as metal or graphite may cause neutralization of the charged droplets from the DESI sprayer, reducing the ionization efficiency [53]. Although a surface was not used in this experiment, the metallic syringe needle would act as a metal surface that might cause partial neutralization of the charged droplets, leading to the weakening of the MS signal. The replacement of the syringe needle with the deactivated fused silica capillary avoided this problem. For the DESI-MS detection of extracted MA in the acceptor droplet, because the needle of syringe was already replaced with a capillary (7 cm for MA extraction to match with the dimension of the test tube used for MA extraction) before the extraction, there was no need to transfer the droplet from the syringe to a surface, a capillary or a vial. After the suspended droplet was withdrawn into the syringe, the extract could be directly detected after the fused silica capillary was cut to a length of 0.5 cm to avoid vibration of the capillary.

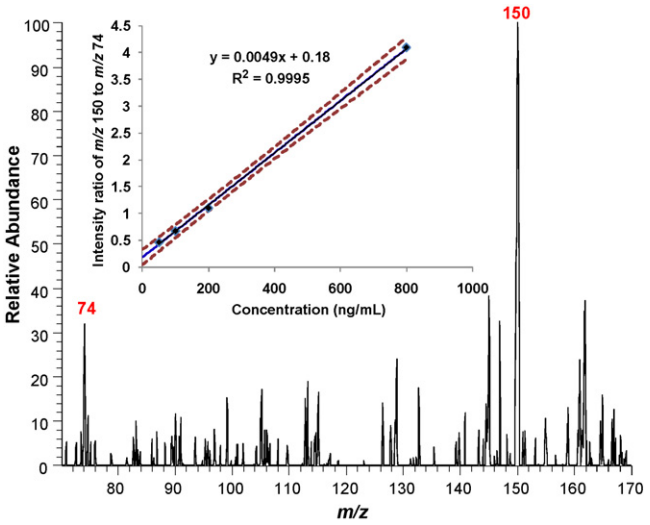


Fig. 2. Representative DESI-MS spectrum of an 800. ng/mL MA standard solution for calibration. Inset: standard calibration line for the DESI-MS detection of single droplet extract samples. The ratios of peak heights of *m*/*z* 150 to those of *m*/*z* 74 were plotted versus the concentrations of standard MA solutions. (-) represents the upper and lower boundaries of the 95% confidence intervals; (—) represents the calibration line obtained from the average data points of three replicates of standard solutions.

3.1.3. Calibration of single droplet DESI-MS analysis for MA

All the calibration samples were in 5% acetic acid to match up with the pH condition of the MA extract. Fig. 2 is the DESI-MS spectrum of the standard solution containing 800. ng/mL of MA. The observed peaks at *m*/*z* 74 and 150 correspond to the tetram-

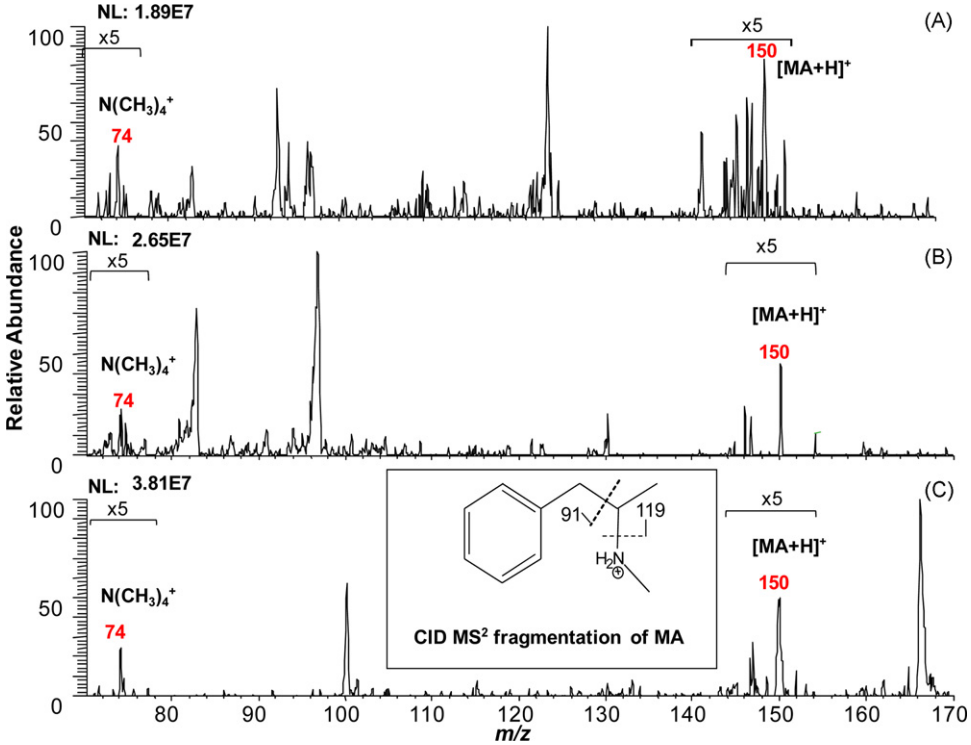


Fig. 3. DESI-MS spectra of three extraction replicates (a single scan is displayed for each spectrum of three replicates A–C). Inset shows the CID MS/MS spectrum of the protonated MA ion $[MA+H]^+$ (*m*/*z* 150).

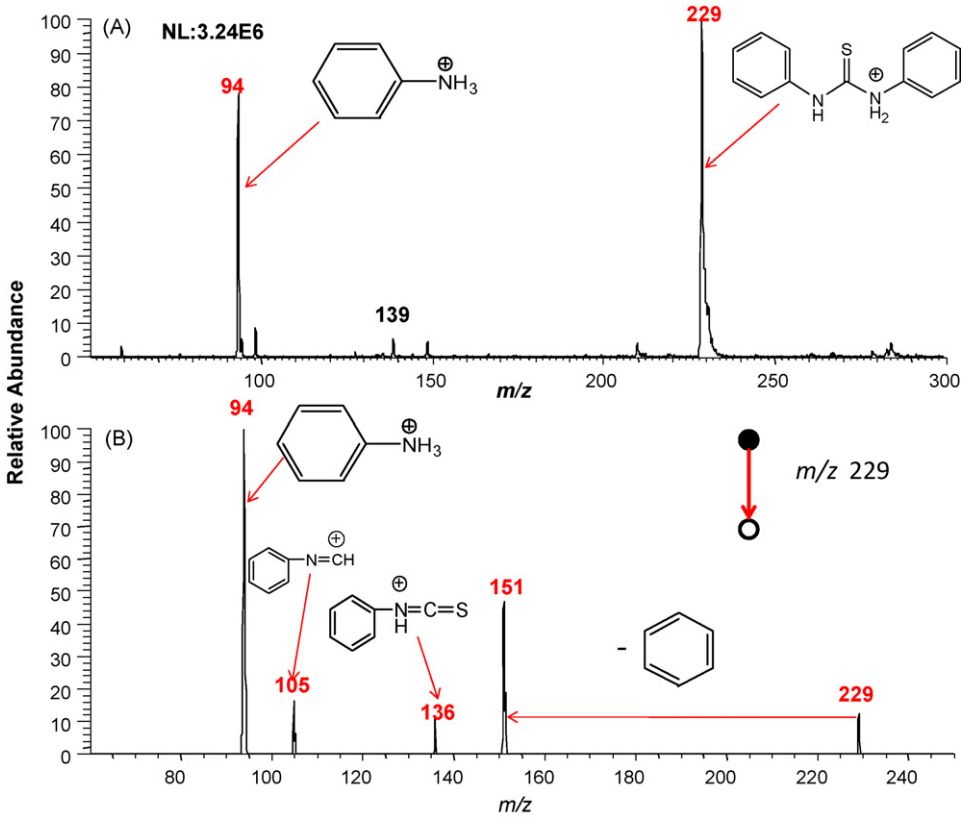


Fig. 4. (A) DESI-MS spectrum of the extracted DPTU from [Bmim][BF_4] by using CCl_4 as the extractant; (B) CID MS/MS mass spectrum of the protonated reaction product $[DPTU+H]^+$ (*m*/*z* 229).

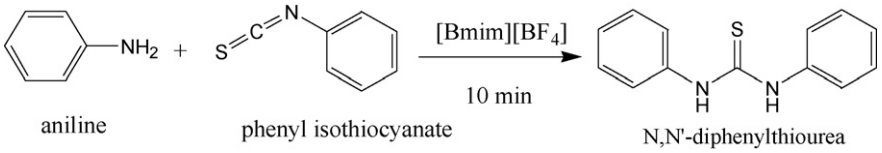
ethylammonium cation $N(CH_3)_4^+$ from the internal standard and the protonated MA, respectively. The ratios of peak heights of *m*/*z* 150 to those of *m*/*z* 74 with respect to MA standard concentrations gave a linear calibration (inset of Fig. 2). The calibration line is displayed with the upper and lower confidence intervals (discrete lines) at the 95% confidence level. The slope and intercept are 0.0049 ± 0.0003 mL/ng and 0.2 ± 0.1, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of the MA in the droplet were 51 ± 4 and 127 ± 9 ng/mL, respectively, with full MS scan by direct ionization of DESI-MS.

3.1.4. Extraction result and enrichment factor for MA

The mass spectra of three extraction replicates are displayed in Fig. 3. The MS/MS collision induced dissociation (CID) fragmentation of *m*/*z* 150 gave characteristic fragment ions *m*/*z* 91 and *m*/*z* 119 via consecutive losses of CH_3NH_2 and C_2H_4 (illustrated in the inset of Fig. 3). This fragmentation pattern confirms the presence of MA in the single droplet extracts. The average calculated concentration of the three replicates with 95% confidence interval was 390 ± 39 ng/mL. The original solution prepared for the extraction was 1. ng/mL. Therefore, the average enrichment factor was 390-fold for the three replicates. This result demonstrates the effective preconcentration and subsequent successful DESI-MS detection of MA. By contrast, without extraction direct DESI-MS full scan could not detect the MA signal of the 1. ng/mL MA original solution for extraction because the concentration was below the LOD. Previously it has been reported that solid phase micro-extraction (SPME) was coupled with DESI-MS [54]. This work is an addition to the coupling of preconcentration techniques with DESI-MS but with the focus on liquid micro-extraction. The selection of DESI-MS as the detector for SDME is advantageous over other methods such as GC/MS as it allows detection of nonvolatile analytes without derivatization steps and shortens the analysis time due to direct sampling and ionization capability.

3.2. SDMS/DESI-MS detection of products in organic reactions with ILs as solvents

As another application, SDME can be used to detect products of reactions performed in ionic liquids. Different from previous work regarding micro-extraction involving the use of ionic liquids in which ILs have been mainly used as the extractants in a micro-extraction [55,56], here an IL was used as the supporting solvent. The organic reaction product was the analyte. The nucleophilic addition of aniline to phenyl isothiocyanate to form N,N′-diphenylthiourea (DPTU, given in the equation below) in 1-butyl-3-methyl-imidazolium tetrafluoroborate (a room temperature ionic liquid) was chosen as a model reaction. Note that in this case, the ionic liquid used also functioned as a catalyst to expedite the reaction [52]:



To efficiently extract the product DPTU by SDME from the reaction system, an extractant must have good solvation strength for DPTU while not being miscible with [Bmim][BF_4]. The extrac-

tant also must have a density similar to or greater than that of [Bmim][BF_4] (ρ of 1.20 g/mL at 20 °C) for the suspended droplet to be held stably in the [Bmim][BF_4] layer. Organic solvents such as diethyl ether, dichloromethane and chloroform can solvate DPTU, but they are either miscible with [Bmim][BF_4] or have a density less than that of [Bmim][BF_4]. After multiple attempts to find a suitable extractant, tetrachloromethane was found to meet all the requirements.

To balance extraction efficiency with time, a few extraction times were investigated. Comparisons were made among 1, 2 and 10 min of extraction times. While all of these extraction times gave evident signals of DPTU under full MS scan mode, the 10 min extraction gave the highest signal intensity for the protonated product DPTU at m/z 229. As seen in Fig. 4(A), the peak at m/z 229 is the base peak in the spectrum. Another major peak at m/z 94 corresponds to the protonated aniline, probably resulting from the remaining reactant. Therefore, 10 min extraction time was sufficient for SDME of DPTU followed by the direct detection by DESI-MS. The MS/MS CID spectrum of DPTU is given in Fig. 4(B), in which fragment ions of m/z 151, 136, 105, and 94 are seen. These fragments arose from the losses of PhH, $PhNH_2$, C_6H_6NS and PhN=C=S, respectively, from the precursor ion of m/z 229, confirming the assignment of the product ion. In this experiment, 3 μL of CCl_4 was used for the extraction. The negligible volume of organic solvent CCl_4 used in the whole process was in accordance with the concept of green chemistry and the use of ILs as green reaction solvents. In our case, with micro-extraction by CCl_4, the majority of the ionic liquid was excluded from the single droplet extract, thus preventing possible contamination of the MS instrument and the ion suppression effect by the ionic liquid solvent. The peak at m/z 139 corresponding to the [Bmim] cation in Fig. 4(A) is small, demonstrating that the measures were successful in minimizing [Bmim][BF_4] introduced into the MS instrument.

In most cases of traditional SDME, ILs were simply used as the extractants for SDME of organic analytes or metal ions from an aqueous system and detected by GC/MS, LC/MS, GC/ion mobility spectrometry, etc. [52,56–60]. In some other cases, hollow fibers were used for which ILs were the intermedia [39]. Compared with these methods, our approach allows the fast detection of the reaction product with high chemical specificity. As increased attention is being given to the IL-based reactions, this reported method would provide a novel method for real-time reaction monitoring and reaction kinetic studies [14].

3.3. High throughput analysis

The analysis of the single droplet samples by DESI-MS can be conducted in such a manner that when the collection of data was started, only syringes with different samples needed to be changed. As in our MA extraction experiments, the interval between calibration samples was 1–2 min for manually switching samples. Because of the high speed of sample injection and microliter sample size, the sample signal was a sharp spike in the extracted ion chromatogram of m/z 150 and the scan time of each sample was about 4–6 s in our experiments. A short analysis time per sample and the manner of continuous collection of data resulted in high throughput analysis.

4. Conclusions

In summary, desorption electrospray ionization-mass spectrometry was coupled with single droplet micro-extraction for the first time. This coupling technique is promising in convenience, high throughput capability, low cost, versatility and high detection speed (a few seconds per extract sample). The DESI-MS analysis after the extraction of reaction products from an ionic liquid with a single droplet of organic solvent offers a fast and green approach for monitoring and optimizing organic reactions. Compared with other detection methods such as GC/MS used for micro-extraction experiments, DESI-MS allows the direct and fast analysis of non-volatile compounds preconcentrated or separated from matrices in the single droplet extract resulting from SDME. A large enrichment factor of 390 was observed in the three-phase SDME experiment for the model compound MA. The coupling of these two powerful techniques holds the potential for biological sample analysis from complex matrices. This method has extended the scope of SDME applications and also is an improvement for the DESI-MS method. Future work will focus on automation of the DESI-MS detection for high throughput analysis [27] of extraction droplets with positioning control for better reproducibility and further exploration of SDME with selective extractions other than simple pH control.

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