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RESEARCH ARTICLE

A Combined Desorption Ionization by Charge Exchange (DICE) and Desorption Electrospray Ionization (DESI) Source for Mass Spectrometry

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Abstract

A source that couples the desorption ionization by charge exchange (DICE) and desorption electrospray ionization (DESI) techniques together was demonstrated to broaden the range of compounds that can be analyzed in a single mass spectrometric experiment under ambient conditions. A tee union was used to mix the spray reagents into a partially immiscible blend before this mixture was passed through a conventional electrospray (ES) probe capillary. Using this technique, compounds that are ionized more efficiently by the DICE method and those that are ionized better with the DESI procedure could be analyzed simultaneously. For example, hydroquinone, which is not detected when subjected to DESI-MS in the positive-ion generation mode, or the sodium adduct of guaifenesin, which is not detected when examined by DICE-MS, could both be detected in one experiment when the two techniques were combined. The combined technique was able to generate the molecular ion, proton and metal adduct from the same compound. When coupled to a tandem mass spectrometer, the combined source enabled the generation of product ion spectra from the molecular ion and the $[M + H]^+$ or $[M + metal]^+$ ions of the same compound without the need to physically change the source from DICE to DESI. The ability to record CID spectra of both the molecular ion and adduct ions in a single mass spectrometric experiment adds a new dimension to the array of mass spectrometric methods available for structural studies.

Key words: Desorption ionization, Ambient pressure, Charge exchange, DICE, DESI

Introduction

The recently introduced desorption ionization by charge exchange (DICE) technique has been demonstrated to generate molecular ions from many classes of polar and nonpolar compounds under ambient conditions [1]. Upon further research work, we observed that the DICE technique does not produce gaseous metal cation adducts, even though they are commonly generated by other desorption methods. This phenomenon can be viewed as a unique advantage of the DICE technique because the spectra generated are less complicated. Nevertheless, some mass spectrometrists might prefer the flexibility of observing spectral peaks for the metal adducts as well. Metal-adduct ions fragment differently upon collision-induced dissociation (CID) compared to the fragmentation pathways followed by their protonated counterparts, and thereby generate additional structural information [2, 3]. The desorption electrospray ionization (DESI) technique is a widely used method for forming both the protonated and sodiated species simultaneously [4, 5]

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from a wide range of compounds such as proteins [6, 7], metabolites, [8, 9], carbohydrates [4], and pharmaceuticals [10]. However, the conventional DESI procedure is not a universal desorption ionization technique. For example, it does not efficiently ionize compounds of low polarity [11]. However, the detection of compounds of low proton affinity and low acidity, such as cholesterol, has been achieved by reactive DESI procedures [12, 13]. Clearly, combining the DESI and DICE methods should broaden the array of compounds that can be analyzed by ambient-ionization mass spectrometry in a single experiment. In this report, we present data that demonstrate how DESI and DICE techniques can be coupled and used to screen for a wider variety of compounds in a single experiment. The procedure also provides the flexibility of producing different types of precursor ions at the discretion of the analyst.

Experimental

Materials

Tylenol® tablets (for a severe cold) containing 325 mg acetaminophen, 10 mg dextromethorphan HBr, 200 mg guaifenesin and 5 mg phenylephrine HCl (McNeil Consumer Healthcare, Fort Washington, PA, USA), Equate tablets containing 250 mg acetaminophen, 65 mg caffeine and 250 mg aspirin (Wal-Mart, Westbury, NY, USA), Claritin® tablets containing10 mg loratadine (Schering-Plough, Memphis, TN, USA) and Advil® tablets containing 200 mg ibuprofen (Wyeth, Madison, NJ, USA) per tablet were purchased from an over-the-counter pharmacy. Vitamin K was purchased from Alfa Aesar (Wardhill, MA, USA). 2-Naphthol was purchased from TCI America (Portland, OR, USA). 1,4-Hydroquinone was purchased from Sigma–Aldrich (St. Louis, MO, USA). Formic acid and toluene (99.8%) were purchased from EMD Chemical (Gibbstown, NJ, USA). Methanol (100%) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Water purified by a Milli-Q purification system (Millipore, Billerica, MA, USA) was used. A piece of braided stainless steel wire (O.D. ~1/8″), manufactured by OOK (Miami, FL, USA), was used as the probe to which the samples were applied. The probe surface was cleaned with methanol before use. The polycarbonate sheet, 8.0″×10.0″×0.1″, was purchased from SABIC-IP (Pittsfield, MA, USA). The PEEK tee union (0.02″ bore) was obtained from Upchurch Scientific Inc. (Oak Harbor, WA, USA).

Mass Spectrometer

All experiments were conducted using a Waters Quattro Micro triple quadrupole mass spectrometer (Milford, MA, USA). The front glass panel that isolates the ion-source region from the outside ambient conditions was replaced with a piece of polycarbonate sheet cut to similar dimensions of ~3.75″×3.75″. A slit of dimensions ~0.25″×0.15″ was milled into the polycarbonate panel in order to introduce the sample probe onto which analytes had been deposited.

Reagent Delivery Pumps

A Waters Separation Module 2695 was used as an auxiliary pump to deliver the DICE reagent spray. The built-in syringe pump of the Quattro Micro mass spectrometer was used for the metered delivery of the DESI reagent spray.

Instrumental Parameters

The DICE reagent (toluene) was infused at a flow rate of between 50 to 100 μL/min using the auxiliary pump. The DESI reagent was infused as a solution of 0.1% formic acid in 70% water/30% methanol at a flow rate of 10–15 μL/min. For combined DICE/DESI experiments, the two reagents were mixed in a tee union prior to passing the mixture through the ESI metal capillary (100 μm), which was held at 5.0 kV. The ratio of the DICE/DESI reagent ranged from 75/25 to 90/10 v/v. When performing individual DICE or DESI experiments, only one reagent solvent was allowed to pass through the tee union; this was achieved by switching the other pump off. The nebulizing gas (nitrogen) flow rate and temperature were set at 750 L/hr and 350 °C, respectively. The cone voltage was set at 25 V, and the cone gas was applied at 25 L/hr. The source temperature was kept at 125 °C. (The small amount of solvent vapor emanating from the source represents a safety issue; the use of a movable exhaust apron is recommended to minimize exposure to solvent vapors.)

Sample Preparation

A mixture of 1,4-hydroquinone, 2-naphthol and vitamin K was prepared in methanol (0.6 mg/mL for each compound). To deposit ~10 μg of each compound onto the target surface, ~15 μL of the mixture solution was pipetted onto a target surface (braided stainless steel wire) area of about 40 mm^2 (the surface area was calculated by assuming that the braided wire is similar in form, and thus surface, to a solid rod). After the deposition, the surface was air-dried for 0.5–3 min before subjecting the deposits to desorption ionization experiments. The over-the-counter tablets were cut into two halves, and one half was used for the desorption experiments.

Sample Introduction

The target probe was inserted through the slit that was cut into the polycarbonate panel. The probe tip was held about 1–2 cm away from the ESI capillary orifice and about 2–3 cm away from the sampling cone. The incident (α) and collection (β) angles had to be set at $\alpha \sim \beta \sim 80°$ because of the constraints of the instrumentation. A clean wire was used as the control to obtain background spectra. The over-the-counter tablets were subjected to desorption ionization

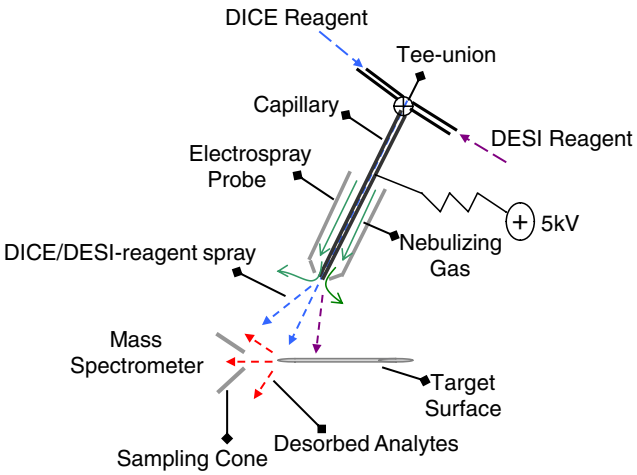

Figure 1. Schematic diagram of the combined DICE–DESI experimental setup

directly by introducing the tablets with a pair of tweezers after removing the polycarbonate panel that isolates the ion-source region from the outside atmosphere.

Results and Discussion

DESI, a technique that is widely used to generate gaseous ions from a vast range of analytes [14], is known to be less efficient for relatively nonpolar compounds [11, 15]. The recently introduced DICE technique, on the other hand, has been demonstrated to generate gaseous molecular ions under ambient conditions from many polar and nonpolar compounds that possess ionization energies lower than that of the selected spray reagent [1]. We envisaged that a combination of the DICE and DESI techniques would enable the ionization of a broader range of analytes within a single desorption ionization experiment. Although the solvents used for the two techniques are immiscible in each other, the flows from two separate pumps could be blended via a tee union and then passed through the metal capillary held at a high electrical potential, nebulized with nitrogen, and then sprayed onto the target surface bearing analytes. In other words, it was thought that directing the combined spray reagents as a partially miscible mixture [16] would be a more versatile approach than employing two separate DICE and DESI sprayers to desorb analytes from a target surface (Figure 1). This approach is possible because the reagents used for the DICE technique are usually nonpolar (toluene for example), and those employed for the DESI technique are relatively polar (e.g., methanol/water) [16].

A mass spectrum generated from the gaseous ions produced by spraying nebulized toluene (DICE) on an analyte mixture of 1,4-hydroquinone, 2-naphthol and vitamin K deposited on a metal surface is shown in Figure 2a. Three prominent peaks were observed at *m/z* 451, 144, and 110 for protonated vitamin K, the positively charged molecular ion of 2-naphthol, and that of 1,4-hydroquinone, respectively. In contrast, the spectrum generated by the DESI procedure did not show a peak for the hydroquinone in positive-ion mode (Figure 2b). Evidently, spraying a nebulized mixture of toluene (DICE) and water, methanol and formic acid (DESI) in the combined procedure increases the versatility of the method, because it enabled the hydroquinone that was not observed by DESI-MS in positive-ion

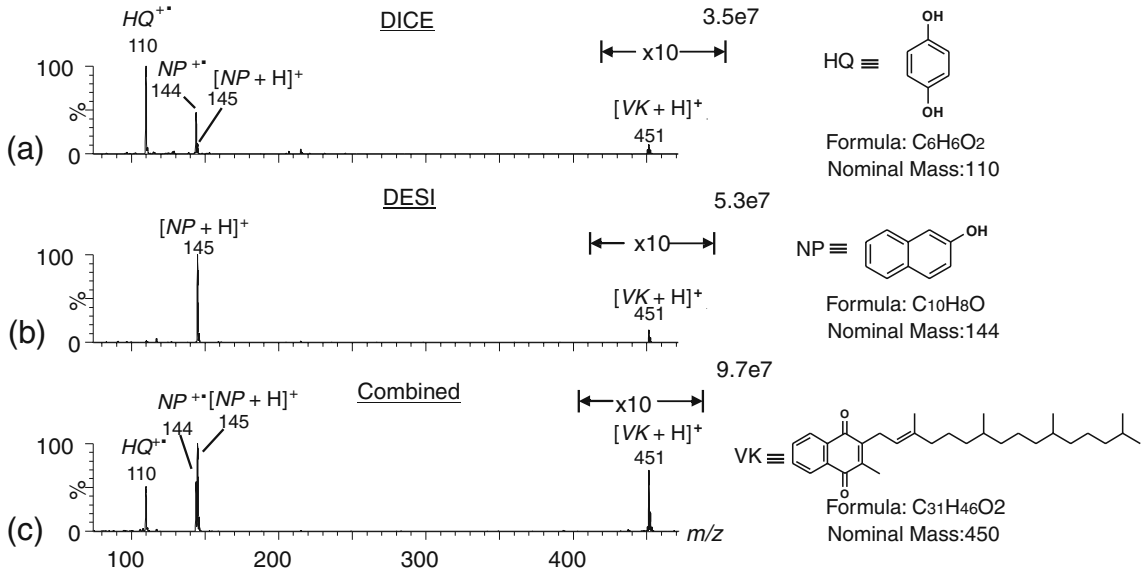

Figure 2. Background-subtracted mass spectra recorded on a Quattro Micro triple quadrupole mass spectrometer by the DICE technique (**a**), the DESI technique (**b**), and the combined method (**c**) from a mixture of 1,4-hydroquinone (*HQ*), 2-naphthol (*NP*) and vitamin K (*VK*) deposited onto a metal surface at about 250 ng/mm^2

mode to be detected in a single experiment (Figure 2c). Moreover, peaks for positively charged molecular ions (*m/z* 110 for 1,4-hydroquinone and *m/z* 144 for 2-naphthol) that were not observed when using the DESI technique could still be observed when the two procedures were combined. In fact, enhanced total ion intensities of the respective analytes were also noted when the two methods were combined. We hypothesized that when the DICE–DESI spray mixture is used, the analytes are more readily desorbed from the target surface.

In order to further demonstrate the versatility of the combined technique, it was applied to a mixture of analytes in a complex sample matrix. For example, when a tablet of an over-the-counter drug for the common cold (Tylenol®) was subjected to DICE-MS in the positive-ion generation mode, peaks were detected for three of the active ingredients in the tablet (acetaminophen, guaifenesin and dextromethorphan) (Figure 3a). The DESI spectrum, on the other hand, showed a peak at *m/z* 221 for the sodium adduct of guaifenesin that was not detected with DICE-MS, but failed to exhibit a peak for dextromethorphan (Figure 3b). The combined technique, in contrast, not only generated the sodium adduct of guaifenesin, which corresponds to the peak at *m/z* 221, but also showed a peak at *m/z* 272 for $[M + H]^+$ of dextromethorphan (Figure 3c). Thus, mass spectrometric peaks for the ions that were not generated by each method when used alone were observed when the combined procedure was applied.

In addition to peaks for the precursor ions of the active ingredients in Tylenol®, peaks for certain product ions formed in the source region were also observed in the spectra, as illustrated in Figures 3a and b. The peaks observed at *m/z* 125, 151, and 163 in Figure 3 are in fact product ions derived from protonated guaifenesin. This was confirmed by a product ion spectrum recorded from *m/z* 199 ion from guaifenesin [Electronic Supplementary Material (ESM) Figure S-1a]. The peak at *m/z* 151 in Figure 3b, on the other hand, could represent either the molecular ion of acetaminophen or a product ion from guaifenesin. The product ion spectrum of the ion with *m/z* 199 showed a peak at *m/z* 151, which confirmed that the *m/z* 151 peak in Figure 3a does in fact represent a product ion derived from the *m/z* 199 ion of guaifenesin (ESM Figure S-1a) rather than the molecular ion of acetaminophen. Similarly, the peak at *m/z* 124 in Figure 3a represents a product ion derived from the molecular ion of guaifenesin, and not a fragment ion from its $[M + H]^+$ ion (see ESM Figure S-1b).

Similar results were obtained with another pharmaceutical tablet, Claritin®, which has loratadine as its principal active ingredient. The spectrum generated by the DICE method showed peaks at *m/z* 382, and 383 for the molecular ion and the proton adduct, respectively (ESM Figure S-2a). A peak for the sodium adduct, which was not observed in the DICE spectrum, was seen at *m/z* 405 in the DESI spectrum (ESM Figure S-2b). On the other hand, the spectrum generated by the combined technique showed peaks for all three species (ESM Figure S-2c).

Although we have demonstrated that the DICE and DESI techniques can be combined to ionize a broader range of compounds within a single mass spectrometric experiment, it is difficult to estimate the relative contribution from each

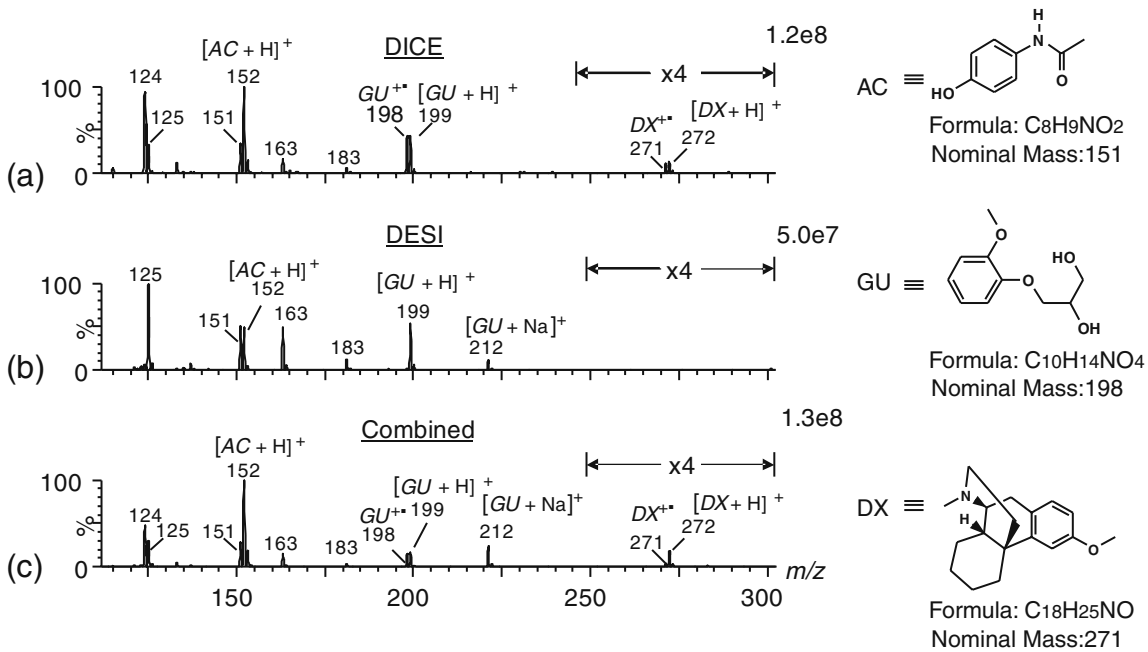

Figure 3. Mass spectra of ions desorbed from a Tylenol® tablet containing acetaminophen (AC), guaifenesin (GU), and dextromethorphan (DX), as recorded by DICE (**a**), DESI (**b**) and the combined (**c**) mass spectrometric techniques on a Quattro Micro triple quadrupole mass spectrometer

method and comprehend the underlying mechanisms. Presumably, the desorption process involved in the combined technique is a composite of the mechanisms of droplet "pick up" [15, 17], thermal desorption [15], and momentum transfer from the heated nebulizer gas stream [15]. We hypothesized previously that the molecular ions of toluene for the DICE technique could be formed in both the solution phase (by an electrochemical oxidation process) and the gas phase (by an electric discharge process) when the nebulized spray reagent is directed onto a surface [1]. Most likely, the ionization of analytes occurs in both the DICE and the DESI methods in the microdroplets after the analytes have been "picked up" [15, 17] by the spray reagent. In contrast, neutral analytes, liberated from the surface by thermal and momentum transfer via the stream of nebulizing gas, are ionized by an APCI-related mechanism in the gas phase. Song et al. have proposed a transient microenvironment mechanism (TMEM) that describes this APCI-related ionization that occurs in the gaseous phase [18]. Although the spray reagents of DICE and DESI are partially immiscible, the net ionization efficiency of the combined technique is expected to be different from those of the two techniques conducted separately. A more in-depth study to determine the dominant desorption and ionization processes that take place when two or more solvents are infused simultaneously will be conducted in the future.

It is well known that molecular ions and protonated products derived from the same molecule upon collision-induced dissociation (CID) produce spectra that are different from each other [19]. Table 1 summarizes a list of product ions that were detected by subjecting DICE-derived molecular ions of caffeine (one of the active ingredients in Equate tablets) to CID. In contrast, subjecting the ions of the protonated species $[M + H]^+$ generated by the DESI procedure to CID resulted in very few product ion peaks under identical collision energy settings (ESM Figure S-3). Similar observations were noted with the molecular ion and the $[M + H]^+$ ion of ibuprofen (Table 1, ESM Figure S-4). The ability to record CID spectra of both the molecular ion and the $[M + H]^+$ ion in a single mass spectrometric run adds a new dimension to the array of analytical methods available for structural studies.

The initial molecular ions produced by the charge exchange technique (DICE) are not as vibrationally excited as those produced by the conventional electron ionization (EI) method using 70 eV electrons. Nevertheless, collision-induced dissociation product-ion spectra recorded from DICE-derived molecular ions show similarities to those recorded under 70 eV conditions, enabling meaningful comparisons to be made with spectra found in commercial EI libraries. In fact, the CID product ion spectrum of caffeine and that of ibuprofen produced in this way from molecular ions show many similarities to the corresponding 70 eV EI spectra reported in the National Institute of Standards mass spectral database (ESM Figure S-5) [20].

The product ion spectra from the molecular ions do not, however, always provide additional structural information in comparison to that obtained when the protonated species are recorded under similar collision energies. The molecular ion of acetaminophen (Equate tablet) produces fewer product ions than those from the protonated species when subjected to low-energy CID fragmentation (Table 1, ESM Figure S-6). Although fewer product ion peaks were recorded from the molecular ion of acetaminophen, the spectrum obtained in this way nevertheless shared some similarities with its 70 eV EI spectrum [20]. On the other hand, a simpler CID spectrum is sometimes more useful, because the fragmentation of a precursor ion into a single product ion provides the higher signal intensities necessary for better quantification.

Conclusion

The results presented in this paper demonstrate that combining the DESI and DICE techniques through a simple instrumental modification broadens the range of compounds that can be screened under ambient conditions in a single mass spectrometric experiment. In this way, analysts can choose to select either DICE, DESI, or the combined method as their desired ionization method according to the chemistry of the analytes. Moreover, the product ion spectra generated from the DICE-derived molecular ions, as demonstrated in this work, share some similarities with the spectra found in EI libraries, which facilitates the characterization of unknown samples.

Table 1. Product ions obtained from caffeine, ibuprofen, and acetaminophen under different ionization and experimental conditions

Analyte	Experimental conditions	Activation energy	Detected ions (*m/z*)
Caffeine	CID product ion spectrum of $[M + H]^+$ ion generated by DESI	15 eV	195, 138, 110
	CID product ion spectrum of $M^{+\cdot}$ ion generated by DICE	15 eV	194, 193, 165, 155, 152, 137,124, 110, 109, 82, 55
	Full-scan spectrum* of $M^{+\cdot}$ ion generated by EI	70 eV	194, 193, 165, 137,138, 110, 109, 82, 67, 55
Ibuprofen	CID product ion spectrum of $[M + H]^+$ ion generated by DESI	10 eV	207, 161
	CID product ion spectrum of $M^{+\cdot}$ ion generated by DICE	10 eV	206, 188, 164, 163, 161, 159, 150, 145, 119
	Full-scan spectrum* of $M^{+\cdot}$ ion generated by EI	70 eV	206, 164, 163, 161, 121, 120, 119, 107
Acetaminophen	CID product ion spectrum of $[M + H]^+$ ion generated by DESI	20 eV	152, 110, 109, 93, 92, 82, 65
	CID product ion spectrum of $M^{+\cdot}$ ion generated by DICE	20 eV	151, 109
	Full-scan spectrum* $M^{+\cdot}$ ion generated by EI	70 eV	151, 109, 81, 80

* Data from the NIST Chemical Database [20]

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