Neutral Desorption Using a Sealed Enclosure to Sample Explosives on Human Skin for Rapid Detection by EESI-MS

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A novel air-tight neutral desorption enclosure has been fabricated to noninvasively sample low picograms of explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX), triacetone triperoxide (TATP), and nitroglycerin (NG) from human skin using a neutral nitrogen gas beam. Without further sample pretreatment, the explosive mixtures collected from the skin surface were directly transported by a nitrogen carrier gas over a 4-m distance for sensitive detection and rapid identification by extractive electrospray ionization tandem mass spectrometry. (J Am Soc Mass Spectrom 2009, 20, 719–722) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

The detection of explosives using ambient mass spectrometric techniques is of increasing interest because these techniques provide high sensitivity and high specificity with minimal sample pretreatment [1–7]. Ambient ionization techniques such as DESI [1–4], DART [8, 9], and DAPCI [5, 10, 11] generate ions of analytes by impacting sample surfaces with charged particles or metastable atoms. Alternatively, by using a neutral desorption (ND) device [12, 13], analytes on virtually any type of surfaces can be softly sampled for extractive electrospray ionization (EESI). ND-EESI tolerates highly complex matrices due to the separation of the sampling and ionization processes in both space and time. Both volatile and nonvolatile analytes can be sampled and transferred, using a gentle air beam, over a long distance space with high efficiency. ND-EESI allows the biological samples to retain maximally their native conditions during the sample analysis. Thus, ND-EESI is particularly suitable for rapid characterization of biological surfaces [12, 13]. In this study, trace amounts of non-volatile explosives, which accumulate easily on skin surfaces, were directly sampled by using a novel air-tight ND enclosure for rapid tandem EESI mass spectrometric analysis.

Experimental

Unlike previously reported open-air ND experiments [12–15], a C-shaped glass cell (i.d. 20 mm) was fabricated in this study to air-tightly cover the sampling area (ca. 10^2 mm) and the ND device (as shown in Figure 1). A pure nitrogen gas beam (room temperature, velocity 300 m/s, flux 2.7 mL/s) ejected from an aperture (i.d. 100 μm) impacted the skin surface for ND sampling. The distance between the ND gas emitter and the skin was 2.5 mm. This distance was determined by the thickness of an inert rubber spacer installed at the bottom of the glass shell. The analytes desorbed were introduced as an aerosol flow (velocity 3.4 m/s, flux 2.7 mL/s) into the ESI source through the sample transfer line (STL) (Teflon tube, i.d. 3 mm). A home-made EESI source was coupled to a ThermoFinnigan LTQ-XL mass spectrometer (San Jose, CA). The sample outlet formed an angle of 60° with the electrospray beam. The angle between the sample outlet and the heated capillary of the LTQ instrument was 150°, which was equivalent to the one formed by the electrospray beam and the heated capillary of the LTQ instrument. The EESI source assembly was coaxially mounted to the heated capillary of the LTQ instrument. The distance between the inlet of the LTQ instrument and the EESI source was 10 mm.

Healthy volunteers gave their consent for their skin to be noninvasively sampled. The explosives deposited on the skin were properly washed out immediately after sampling. Chemicals such as RDX, TATP, HMX were bought from Accu Standard, Inc. (New Haven, CT, USA); NG was imported from Dr. Ehrenstorfer Gmbh (Augsburg, Germany), and TNT was purchased from Chem Service, Inc. (West Chester, PA, USA). Water used was deionized.

Explosives standards (1~10 ng/mL), prepared by dilution using methanol/water (1:1) solution, were

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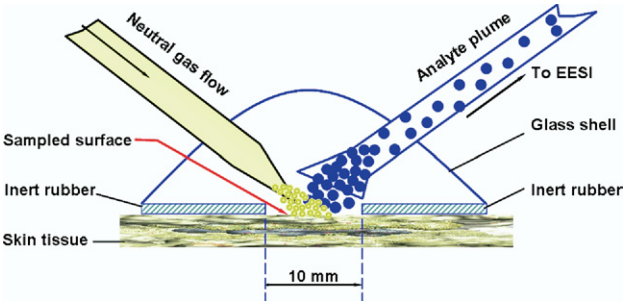

Figure 1. Schematic of the air-tight ND enclosure.

placed on the unwashed skin using a micropipette, ensuring the final amount of explosive was a few picograms. The sample spots were ~10 mm^2, which was fully covered by the ND sampling probe. The skin surface was sampled immediately after liquid evaporation.

For EESI, the reagent solvent (i.e., ammonium acetate aqueous solution 0.1 mM) or acetic acid methanol (10%, vol:vol) solution)] was electrosprayed at 5 μL/min with a high voltage (±4 kV, for positive/negative ion detection). The temperature of the heated capillary was optimized to be 100° C so that thermal dissociation of explosives was avoided. The default values of voltages for the heated capillary, ion optics and the detectors were used without further optimization. Collision-induced dissociation (CID) experiments were done by applying 18% to 35% collision energy to the precursor ions isolated with a window width of 1 mass/charge unit. Blank spectra were collected from clean skin patches where no explosive standards had been deposited.

Results and Discussion

Figure 2 presents the mass spectrum of TNT (10 pg) and RDX (20 pg) mixtures deposited on human skin, showing the radical anions of TNT (*m/z* 227) and a complex of $(RDX + CH_3COO)^-$ (*m/z* 281). In the CID spectra (the insets of Figure 2), the radical anions of TNT (*m/z* 227) generated fragments of *m/z* 212, 210, 197, and 183 by the loss of CH_3, OH, NO, and probably $NOCH_2$, respectively. The TNT fragmentation data were the same as those obtained when pure TNT was only used as an analyte, and were in agreement with previous studies [4]. The precursor ions of *m/z* 281 produced major fragments of *m/z* 263, 237, and a small peak at *m/z* 221 by the loss of water, CO_2 and acetic acid, respectively. The product ions of *m/z* 263 further fragmented to yield ions of *m/z* 221 and *m/z* 204 in the MS/MS/MS spectrum, by the loss of CH_2CO and OH group, successively. Similar to RDX, NG and HMX formed $(M + CH_3COO)^-$ complexes under the EESI conditions for negative ion detection. Characteristic fragments rather than the molecular ions of the analyte were preferably observed in the CID experiments of the $(M + CH_3COO)^-$complexes (seen in Table 1), indicating that the $(M + CH_3COO)^-$complexes were strongly bound. The fragmentation patterns of these analyte complexes differ from those of non-covalent complexes (e.g., $(RDX + Cl)^-$ complexes [2, 4], providing complementary information for specific detection of explosives (e.g., RDX) present in highly complicated matrices.

Extra experiments were done in 2, 4, 6, 12 h after 1 ng TNT and RDX were deposited on the forearm skin. The data showed no serious sensitivity loss after 2~12 h because the explosives do not vaporize readily. No correlation between RDX signal levels and the alteration of the skin temperature was detected (seen in the supporting information, which can be found in the electronic version of this article). This confirmed that the ND sampling method worked based on a desorption-based sampling mechanism rather than volatilization or evaporation. Other studies also demonstrated that explosives on skin can be desorbed for post-ionization [16]. However, for volatile or water-soluble compounds on skin, signal loss is expected after exposure to ambient environment for long time, due to the materials loss caused by analyte migration/degradation.

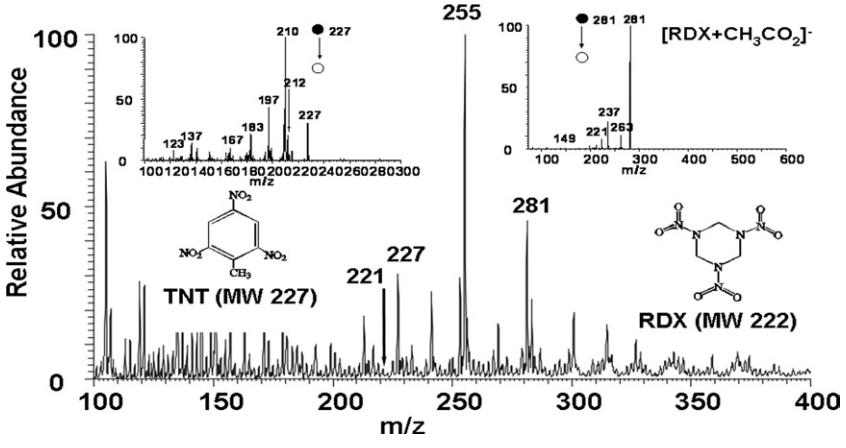

Figure 2. Simultaneous detection of TNT and RDX mixtures on human skin by ND-EESI-MS^n. The insets show the CID spectra of ions of interests. The mass spectra were recorded with an average time of 1 min.

Table 1. Explosives detected on skin surface

Explosives (MW)	Ions observed: Ionic species	Ions observed: *m/z*	Major fragments (MS/MS, *m/z*)
TNT (227)	TNT^-	227	212, 210, 197, 183
RDX (222)	$(RDX\text{-}Ac)^-$[a]	281	263, 237, 221
NG (227)	$(NG\text{-}Ac)^-$[a]	286	268, 242, 226
HMX (295)	$(HMX\text{-}Ac)^-$[a]	355	309, 295
	$(TATP\text{-}NH_4)^+$[b]	240	225, 223, 222
TATP (222)	$(TATP\text{-}N_a)^+$[b]	245	215
	$(TATP\text{-}H)^+$[b]	223	208, 207

[a]Ac = CH_3COO.
[b]Ions detected using ammonium acetate aqueous solution (1 mM) as the spray solvent.

Similarly, other explosives were detected in either negative or positive ion detection mode. Table 1 summarizes the analytical results, where the characteristic fragments observed confirmed the detection of the explosives. Interestingly, the TATP formed protonated molecule (*m/z* 223), proton bound ammonia adducts (*m/z* 240), and sodium cation bound adducts (*m/z* 245), respectively. These data were identical to those observed in previous studies [17]. The $(TATP + Na)^+$ complex probably forms in the EESI step. The observation of the sodium adducts, along with the protonated TATP, shows the gentle character of the ND-EESI method for the ionization of this extremely fragile molecule [17].

For most explosives tested, ND-EESI-MS provided a limit of detection (LOD) of 0.5~10 pg ($S/N \geq 3$) on skin surfaces. Compared with previous studies [17], a lower detection limit was achieved in this experiment because the ND enclosure prevented material loss and the EESI step provided high ionization efficiency. Studies showed that ambient ions generated by DESI can be sampled with improved efficiency by using the same concept [18]. All the signals detected based on the characteristic fragments were well correlated with the explosives. Typically, the signal level rose up to 90% in two scans, and after the measurement, the signal dropped down to the noise level in about one~two scans when the sample was taken away. This validated that the signals detected were sample-related. The dynamic response range was found to be about four orders of magnitude for all the explosives. For example, a linear response ($y = 3.7x$ (pg) + 2.6, $R^2 = 0.96$) was found for RDX in the range of 0.01~100 ng, using the fragment (*m/z* 237) generated in the CID experiments.

For the analysis of explosives in a real world, remote analysis is highly desirable, especially in cases under hazardous environments (e.g., extremely low-temperature, radioactive environment, etc.). Previously, ions of explosives (total amount of 0.5–20 ng) created in atmospheric pressure were sampled in open air for mass detection [1]. On the other hand, neutral analytes can be easily transported using a long STL. For example, the signal intensities of either RDX (20 pg) or TNT (20 pg) were maintained at almost the same levels (shown in Supplemental Figure S1) when the length of the STLs varied from 2 to 400 cm, generating signals of 200 cps for RDX (*m/z* 237) and 400 cps for TNT (*m/z* 210) in the MS/MS spectra. The total amounts of explosives were remarkably lower than those detected by transferring ions in open air [1] because the air-tight ND-STL system transported the analytes to the EESI source without notable material loss. So far, no serious sample carryover effect was found when a Teflon tube (400 cm length; 3 mm i.d.) was used.

Transferring samples over a long distance interval may delay the signal response in ND-EESI-MS. The response time is mainly dependent on the ND gas flow rate and the length of the STL. In this study, the signal responded about 1s when a 4 m length tube was used. Furthermore, the EESI source and the ND device required no optimization when the sample was reloaded for the ND process. This further facilitates the high throughput analysis of explosives in complex matrices.

Conclusion

Our data show that ND is capable of sampling explosives at low picograms levels directly from biological surfaces such as human skin, for rapid sensitive detection by EESI-MS^n. Selective ion/molecule reactions can be easily implemented in the EESI process, resulting in enhanced specificity for the detection of trace amounts of explosives present in complex matrices such as biological surfaces. The neutral sample plume created by a ND technique can be transported over a long distance path along a sample transfer line for remote analysis, providing an easy way for suitably fast screening of explosives.

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