Chapter 2

EASILY FABRICATED ION SOURCE FOR CHARACTERIZING MIXTURES OF ORGANIC COMPOUNDS BY DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY

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2.1 Abstract

The increasing use of atmospheric pressure mass spectrometry has led to the development of many ambient ionization sources, for which sampling versatility and low cost are desired features. One such recent ambient ionization method is direct analysis in real time mass spectrometry (DART-MS), which has proven to be well suited to the analysis of native samples of both simple and complex natures. We describe a home-built DART source (EZ-DART) with versatile sampling capabilities, low power requirements, and low assembly cost which can be easily interfaced to mass spectrometers equipped with an atmospheric pressure inlet. The operating temperature range (22-250°C) enables the acquisition of both temperature programmed desorption-based DART mass spectra and the collection of multistep collision-induced dissociation (CID) mass spectra. We present here the validation of the EZ-DART source and a demonstration of its performance in a number

of relevant applications. Additionally, we introduce the new DART application of reagent assisted desorption ionization (RADI) for the targeting of specific chemical functionality in complex organic mixtures through a host-guest chemical system.

2.2 Introduction

Ambient mass spectrometry comprises a range of experimental methodologies by which analyte ions can be produced at atmospheric pressure and then transferred to the vacuum region of a mass spectrometer for analysis. Ambient analysis allows for rapid examination of a variety of samples with minimal to no sample pretreatment, compared to traditional methods requiring more complex procedures to access the analytical information. Recent reviews by Bodzon-Kulakowska and Suder¹ and Ding and Duan,² among others,^{3, 4} have discussed the multitude of different ambient ionization techniques, likely mechanisms by which they operate, and possible applications. Among the many different techniques for ambient ionization mass spectrometry, a solvent spray or plasma discharge are two of the most common.⁵

Applications for ambient mass spectrometry include, but are not limited to, food science,⁶⁻⁹ pharmaceutical characterization,¹⁰⁻¹⁵ forensics,¹⁶⁻²⁴ detection of volatile and semi-volatile organic compounds,²⁵⁻²⁹ biological samples,³⁰⁻³⁴ and the analysis of complex organics such as aerosols³⁵⁻³⁹ and hydrocarbons.⁴⁰⁻⁴² To be effective, an ambient ionization method must both desorb the target analyte into the gas phase and ionize it, or directly desorb ions from the sample. For the analysis of samples in a native state, the use of spray based methods such as desorption electrospray ionization (DESI),^{43, 44} or plasma methods such as

direct analysis in real time (DART),^{45, 46} have moved to the forefront. DESI uses a solvent spray to desorb and ionize a sample; DART utilizes a plasma discharge to produce metastable gas molecules that are optionally heated to desorb and ionize the sample through a complex mechanism.^{45, 47} The mechanism of ionization for DART is proposed to occur either by direct Penning ionization by the helium metastable or proton transfer from protonated water clusters resulting from ion molecule reactions of trace atmospheric species initially ionized by Penning ionization.⁴⁵ The formation of various positively and negatively charged gas phase adducts from atmospheric molecules such as ammonia are also commonly observed and assist in ionization processes.^{45, 48}

DART analysis has been applied successfully in the majority of applications where other ambient mass spectrometry methods have been utilized.^{11,21,4749} The selection of DESI or DART as the ionization source for a particular analytical application requires consideration of the target analyte's solubility in potential DESI spray solvents, and vapor pressure as well as potential reactivity in known DART ionization mechanisms. Prior work has shown that DART analysis can be readily applied to applications with highly complex sample compositions.⁴²

For difficult to detect analytes, either due to low ionization efficiencies or low detection limits, ambient mass spectrometry methods such as DART and DESI often employ chemical modification. This process involves the introduction of a chemical reagent that changes the detected analyte ion in a variety of possible ways. When used with DART, prior studies have utilized gas phase reagents such as chloroform, dichloromethane, ammonia, or trifluoroacetic acid to form gas phase adducts.^{48, 45} Similar chemical modifications using DESI have also been reported, using the designation "reactive DESI". That method involves

host-guest supramolecular chemistry with either the host or guest in the spray to complex with a target functionality present on the analyte in the sample, or derivatization chemistry involving attachment of a fixed charge to the target functionality within the molecule, often with the reagent molecule being delivered in the spray.⁵⁰ Many different host-guest supramolecular interactions⁵¹⁻⁵³ and chemical derivatization methods^{50, 54} have been used successfully with reactive DESI. We report in this study a similar method with DART ionization, referred to as reagent assisted desorption ionization (RADI), with which the introduction of host-guest supramolecular chemistry allows for enhanced detection of target species in a complex mixture and simplification of resultant spectra.

In this work, we describe an affordable, versatile, and easily fabricated DART source (which we refer to in this paper by the designation EZ-DART) that has proven effective for a wide variety of samples with varying degrees of complexity. Capabilities of this source are demonstrated in applications including commercial pharmaceutical identification, forensics, and analysis of organic mixtures. By reducing the temperature the sample ion signal persists over a timescale of several seconds. This facilitates the acquisition of multistep CID mass spectra to provide information relating to analyte structure. Systematic variation of the operating temperature over the range of 25-250°C enables the acquisition of temperature programmed desorption-based DART mass spectra. The minimal expense associated with fabrication of the EZ-DART source, which can be used with any mass spectrometric instrument having an atmospheric pressure inlet, offers the potential to expand the applications and user group of this ionization methodology.

2.3 Experimental

2.3.1 EZ-DART Source

The EZ-DART source was fabricated by the Caltech glass shop using borosilicate glass tubing and 1/16" flat ended tungsten wire electrodes. 1/2" glass tubing was used for the body with 1/8" glass tubing for the gas input line. The EZ-DART source design enables the use of helium (99.995% purity used in this study) at a flow a rate of 1.5 L/min. The glass construction enables visual inspection of the electrodes and discharge, the color of which changes if there is an air leak in the helium supply line. Source temperature and helium flow rate both impact sample depletion rates, but only temperature was modified in this study; consistent gas flow and discharge conditions were maintained for all experiments. The operating conditions used for the needle electrode are 1.5-2.5 kV DC with a current of 0.1-0.2 mA limited by a 10 M Ω resistor. The filter electrode is held at ground for most experiments compared to the 110-250 V DC filter voltage commonly used.^{11, 21, 45, 49} Filter voltages in this range were applied for the pharmaceutical samples reported below and showed no difference other than decreased overall intensity of the signal. The voltage for both electrodes is controlled by two digital high voltage DC power supplies (Stanford Research Systems, INC. Model PS350, Sunnyvale, CA).



Figure 2.1. a) Scale drawing of EZ-DART source with relevant dimensions. The discharge gap is approximately 4mm b) The actual EZ-DART source in operating configuration without heating tape

In typical operation, the outlet of the EZ-DART is oriented at approximately 45° from normal, with the sample no more than 5 mm from the EZ-DART outlet and ion transfer tube inlet for the angled configuration (shown in figure 2.1b)^{55, 56}. The reagent assisted desorption ionization (RADI) experiments were performed with the EZ-DART aligned in a linear configuration with the DART outlet approximately 1 cm from the mass spectrometer inlet. The resultant ions are analyzed by a linear ion trap mass spectrometer (LTQ-XL, Thermo Scientific, San Jose, CA). The inlet capillary for the mass spectrometer was held at 50°C for the angled configuration experiments and 250°C for the linear configuration experiments.

A lower temperature was used in the angled configuration to minimize ambient heating of the sample due to conductive heating of the ion transfer tube. This was not a concern in the linear configuration. The capillary (and surrounding font cone) voltage was tuned to allow for highest signal intensity of the target analyte during acquisition. This was a bias of 1.4V for negative ions and 0.2V for positive ions. Sampling occurs by aspiration of ions into the capillary from a nearly field free region. To heat the gas stream for temperature programmed desorption (TPD) analysis, a heating cord (Omega Engineering, Stamford, CT) was wrapped around the EZ-DART between the ion filter and the gas outlet, and then insulated with glass wool and aluminium foil or cloth fiberglass tape. The temperature is controlled with a variable transformer (Variac Co., Cleveland, OH) and measured with a K-type thermocouple placed between the heating tape and the glass (Omega Engineering, Stamford, CT). Reported temperatures are accurate within +/- 3°C. For collision induced dissociation (CID) spectra, a normalized CID energy of 20-25 (arbitrary units) was found to yield nearly 100% dissociation of the selected ion. These spectra are reported in the Supplemental Information, where relevant, with the targeted m/z values for each spectra indicated on the side.

2.3.2 Sample Preparation and Reagents

To show the efficacy and versatility of the EZ-DART source for both positive and negative ion analysis, a variety of representative analytes were chosen in the validation tests. Liquid standard samples were applied with borosilicate glass melting point capillaries (Kimble Chase, 1.5-1.8 x 90 mm) to glass microscope slides (Gold Seal microslides, 25 x 75

mm, 0.97-1.07 mm thickness) and then placed in the sample area for analysis. Maleic acid (Sigma Aldrich, 99%) was dissolved in methanol (Fisher Scientific, HPLC Grade), and then applied to the glass slide and allowed to evaporate into a film. Poly(propylene glycol) (Aldrich, average mass 2,700 amu; containing 120-190 ppm proprietary antioxidant) was applied directly to the glass slide. A reference solution of RDX (Cerilliant, 1 mg/ml in acetonitrile, certified reference material) was diluted to concentrations between 0.25 ng/ μ l and 1.75 ng/ μ l in acetonitrile (Omnisolv, HPLC Grade) for application and analyzed immediately.

Pharmaceutical samples were directly analyzed by the EZ-DART source. The coating was removed from ibuprofen, (Walgreens, 200 mg tablet with wax coating) pseudoephedrine (Walgreens, 30 mg tablet with wax coating), and the naproxen sodium tablet (Amneal Pharmaceuticals, 500 mg) before placement on the sampling stage to ensure an exposed surface. A viscous liquid hydrocarbon sample was obtained from the La Brea Tar Pits (the George C. Page Museum, Los Angeles, CA) by collecting a small amount of the surface-exposed tar in a vial. The collected tar was introduced to the EZ-DART source as a film applied directly to a glass slide without solvent. Neurotransmitters dopamine hydrochloride (Sigma Aldrich), norepinephrine hydrochloride (Sigma Aldrich >97%), gamma-aminobutyric acid (Sigma Aldrich >99%), and serotonin hydrogenoxalate (Sigma Aldrich) were tested as neat solids in precision glass capillaries (0.4 mm I.D., Drummond Scientific Co., Broomall, PA) in the linear configuration. The complexation agent 18-crown-6 ether (Sigma Aldrich 99%) was placed in a separate precision glass capillary and held in line either between the EZ-DART outlet and capillary holding a neurotransmitter, or in a reversed configuration between the same capillary and the mass spectrometer inlet.

2.4 Results and Discussion

2.4.1 Pharmaceutical Samples

The examination of pharmaceutical tablets with DART was one of the initial applications proposed by Cody et al., upon which further analyses have been built.^{11, 45, 57, 58} While there are other methods by which pharmaceuticals can be characterized, the majority of these methods typically require some amount of sample preparation, which increases the amount of time necessary for analysis.^{59, 60} The desire to minimize sample preparation and analysis time makes pharmaceutical identification an ideal application for EZ-DART.

A commercial ibuprofen tablet was chosen as a pharmaceutical sample for positive mode ionization due to its prior analysis by DART and its ease of ionization.¹¹ The wax-based pill coating was removed and the tablet placed in front of the gas stream in the angled configuration while the EZ-DART was operated at ambient temperature. Figure 2.2a shows the spectrum obtained with this procedure. The protonated species, ammonia adduct, and their associated dimers are detected.¹¹ The carboxylic acid group in ibuprofen allowed for detection in negative mode as the [M-H]⁻ species. The same procedure as above produces the spectrum shown in 2.2b when negative ions are detected.

Pseudoephedrine was examined in tablet form for its forensic relevance to the illicit manufacture of methamphetamine. The spectrum, shown in figure 2.2c, exhibits not only protonated pseudoephedrine, but also the ammonium adduct of triacetin and a dimer of triacetin and pseudoephedrine. Triacetin is a known matrix molecule in pharmaceuticals. This observation indicates that some additives included in the pill matrix may be observed during routine DART analysis. The ability of the DART to detect some matrix molecules may be of assistance to the analyst in providing additional points of comparison between questioned samples.

The sodium salt of naproxen was used as a pharmaceutical exemplar for negative mode analysis. The spectrum, shown in figure 2.2d, shows matrix species similar to the pseudoephedrine tablet, but the negative ion dimer is the most abundant species. The observation of the monomer, dimer, and trimer is of interest considering the nature of the starting material as a sodium salt. No sodium-bound species are observed in negative mode, suggesting that ions are not derived directly from the solid, but rather are generated post desorption in the gas phase. Additional negative mode data for maleic acid, also showing formation of the monomer, dimer, and trimer, are presented in the Supplemental Information.



Figure 2.2. a) Positive mode spectrum of an ibuprofen tablet b) Negative mode spectrum of an ibuprofen tablet c) Positive mode spectrum of a pseudoephedrine tablet. d) Negative mode spectrum of a naproxen sodium tablet. The 513 Da adduct peak in the naproxen sodium spectrum is attributed to magnesium stearate present in the tablet. Temperatures shown refer to the heater temperature of the EZ-DART source.

For all the above experiments no changes were made to the settings of the EZ-DART source between the positive and negative mode samplings. These results show that the EZ-DART source can identify a variety of different pharmaceutical tablets with either positive or negative analyte ions without the need for adjustment of operating conditions external to the mass spectrometer. However, the presence of matrix ions indicates that the formation of adducts of the analytes of interest with other sample molecules needs to be taken into consideration for the analysis of complex multi-component organic samples.

2.4.2 Species of Forensic Interest

DART-MS has proven useful for forensic applications, including the analysis of illicit drugs, condom and sexual lubricants, and explosives.^{21, 49, 61, 62} Cody et al. analyzed currency to find cocaine by placing a bank note directly into the sampling region.⁴⁵ We reproduced that test as shown in figure 2.3. A well-circulated twenty dollar bill was placed in the sample area. The resultant mass spectrum had major peaks corresponding to the [M+H]⁺ species for cocaine and methamphetamine, with the other species corresponding to a polymer series spaced by 58 Da, all of which are ammonia adducts. These adducts were confirmed by observation of a 17 Da loss in CID, corresponding to the loss of an ammonia adduct. A second twenty dollar bill and a fifty dollar bill showed similar results. Cocaine and methamphetamine were confirmed by CID, similar to the structural confirmation performed in other DART experiments,⁶³ the data for which are included in the Supplemental Information. Prior analyses of bank notes have given similar results,^{64, 65} showing that small amounts of illicit substances can be detected readily with no sample preparation using the

EZ-DART. With the success of this sampling, further testing is warranted for the EZ-DART on forensic samples, such as swabs from buildings contaminated by illicit drug manufacture.⁴⁹



Figure 2.3. Spectrum of a 2009 issue bank note taken at 250°C heater temperature where 304.4 m/z corresponds to protonated cocaine. Inset shows region in which methamphetamine was detected.

The forensic interest in trace analysis of chemicals spreads beyond illicit substances, with explosives detection being one of the earliest experimental targets. DART has been effective at trace detection of different explosives, such as peroxides and nitroaromatics.^{20,} ^{21, 45} The ability of DART to determine trace amounts of the majority of these explosives under qualitative screening conditions was found to be comparable to or improved from common determination methodology, with faster analysis times. With the proven success of

DART in this application, the ability of the EZ-DART source to detect comparably trace amounts of explosives was tested using RDX solutions from 0.25 ng/µl to 1.75 ng/µl, examined in triplicate by depositing 2 µl (providing for deposition amounts of 0.5 ng to 3.5ng) onto a glass surface, with a new surface being used after each deposition. A representative spectrum for these data is shown in figure 2.4. Since the $[M-H]^-$ peak was not seen, the nitrite adduct was monitored for each deposition. This peak was ratioed to the reliable background peak of 255 Da (palmitic acid from fingerprint contamination). A lower limit of detection of 1.1 ng was determined, with a linear range of detection extending to 2.5 ng. Details are given in the Supporting Information. The LOD result is twice the lower limit of detection of 0.5 ng reported previously using a commercial DART source.²⁰ The result suggests that the EZ-DART source should be able to detect similar levels of explosive residue, even if they are detected only as adducts. While the limited linear range of the source would preclude its use for quantitative assessments, the DART study noted for comparison focused on qualitative screening conditions.²⁰



Figure 2.4. Average of three spectra of a drop of acetonitrile containing 1.5 ng of RDX on glass. The RDX is seen as the nitrite adduct. The 255 Da and 283 Da are palmitic and steric acid respectively, and were seen in each spectrum due to fingerprint contamination.

2.4.3 TPD Based Experiments

Complex organic mixtures, comprising compounds of varying molecular weight and structural complexity, can be difficult to analyze with ambient ionization methods and often require extensive sample preparation to obtain a more complete analysis.^{59,64,66} Applications adjusting the DART gas heater temperature have been used to characterize pyrolysis product evolution,⁶⁷⁻⁶⁹ complex sample differentiation,⁷⁰⁻⁷² and to induce fragmentation of known molecules at higher temperatures (150-400°C).⁷³⁻⁷⁵ Additionally, temperature programmed desorption (TPD) has been used in petroleomics studies with success at helping to simplify analysis.⁶⁵ Using the EZ-DART source we examined the TPD analysis of a PPG sample (described in the Supplemental Information) and a viscous liquid asphalt, shown here,

demonstrating that methods requiring DART heater adjustment can be used with the EZ-DART source.

A viscous liquid asphalt sample from the La Brea Tar Pits was chosen as a representative complex organic mixture relevant to samples of interest in petroleum analysis. The sample was examined at both 100°C and at 250°C (figure 2.5). The spectrum shown in figure 2.5b at 250°C shows peaks from 200-400 Da, a region for which peaks have been observed for other petroleum oil samples examined using DART.^{45, 75} Of particular interest is the shifting of mass envelopes noted in comparing the two scans, with many of the low mass species visible at 100°C decreasing at 250°C. An additional envelope of even higher mass was observed at 250°C, accompanied by the change in the calculated average mass from 222 to 400 Da. Peaks in this higher mass envelope are not observed in the lower temperature spectrum. The 100°C spectrum appears to have a single mass envelope centered at around 250 Da. In contrast, the 250°C spectrum has a high mass envelope centered around 550 Da, in addition to two lower intensity mass envelopes, centered around 280 Da and 300 Da, respectively.



Figure 2.5. EZ-DART mass spectrum of La Brea Tar Pits asphalt taken at a) 100°C b) 250°C. An asterisk indicates background ions.

These EZ-DART spectra were compared to an ESI spectrum of the same sample, the procedure and spectrum for which are provided in the Supplemental Information. Interestingly, the peaks detected in the EZ-DART spectrum were mostly even mass ions, indicating incorporation of an odd number of nitrogen, whereas the ESI peaks were odd masses, possibly indicating the detection of different components or adducts in the sample with each method. The resultant CID spectra for the EZ-DART data did not display losses of 17 or 18 mass units, which would have been indicative of ammonia or water adducts, respectively. This suggests that the detection of species with nitrogen is not related to adduct formation during EZ-DART analysis, but instead a fundamental difference in species detected between ESI and DART.

The difference in the mass envelopes is another indication of deviation between species detected by ESI and EZ-DART. The ESI spectrum shows a more continuous increase in mass over the entire region, with only a few peaks dominating the spectrum. The EZ-DART spectrum shows two distinct envelopes, for which the lower mass envelope at 250°C shows smaller envelopes with $\Delta m/z$ of 14 or 16 Da, which suggests oxidized species with varying numbers of carbonyl and hydroxyl groups. These factors are suggestive that ESI and EZ-DART combined could provide a much more thorough and complete characterization of a complex organic mixture than either technique alone.

These data show that the EZ-DART allows for the ionization and detection of species with varying volatilities present in complex organic mixtures through TPD analysis. The changes with increasing temperature in the observed mass spectra are consistent with increasingly lower volatility compounds being introduced into the vapor phase, in agreement with previous TPD DART studies.⁷⁰ TPD-based DART characterization is a technique readily and effectively utilized with the EZ-DART source.

2.4.4 Reagent Assisted Desorption Ionization

The use of gas phase chemical modification has been a part of DART analyses since the original work by Cody et al in 2005. In these studies dichloromethane and trifluoroacetic acid were used as dopants to enhance the negative ion signal for analytes of interest.⁴⁵ Other studies have used chloroform to similar effect.⁴⁸ Studies of alcohols by DART utilized an adduct with superoxide produced during sampling.⁷⁶ The commonly observed ammonia adduct has been enhanced to advantage by the introduction of ammonia vapor. These types of chemical modification with DART have focused on adducts created through the introduction of gas phase vapors.

The formation of chemically modified analytes during sampling has also been extensively used in the case of reactive DESI. These experiments can differ from those seen with DART with the involvement of either covalent derivatization, such as fixed charge derivatization with betaine aldehyde⁵⁰ and ketone modification with hydroxylamine,⁵⁴ or non-covalent complexation, such as dicationic pairing^{51, 52} and other supramolecular host-guest complexes.⁵³ DART detection of organometallic compounds have involved some similar experiments by mixing the ligand and metal together prior to sampling,^{77, 78} and gas phase chemical reactions have been induced by low temperature plasma,^{79, 80} showing the potential for host-guest supramolecular chemistry with DART. Drawing from these experiments, we introduce the concept of using host-guest based supramolecular complexation chemistry directly in the gas phase, termed here reagent assisted desorption ionization (RADI), specifically for the enhanced detection of neurotransmitters.

Several neurotransmitters were chosen for the initial testing of RADI, all of which contain a primary amine functionality along with considerable interest in detecting these molecules in neurological and other tissues. These include serotonin, norepinephrine, dopamine, and gamma-aminobutyric acid (GABA). These small molecules are usually detected through covalent derivatization in MALDI analysis of tissues⁸¹ and chromatographic separation techniques^{82, 83}. A host-guest chemical system was chosen to examine the detection of all four neurotransmitters alone as well as simultaneously from the same sample.

All four neurotransmitters have a primary amine group that is sterically available, making an ideal target for complexation. 18-crown-6 ether is a proven host for primary amines,⁸³⁻⁸⁵ with three of the oxygen atoms forming strong hydrogen bonds to a protonated amine group, which would stabilize the charge and increase the ease of detection with mass spectrometry. Additionally this interaction is known to be much stronger in the gas phase compared to the solution phase, making it an ideal host to test RADI.⁸⁶

This stronger gas phase interaction is key to the mechanism of RADI. The 18-crown-6 ether and neurotransmitter position in the EZ-DART gas stream were interchangeable; either could be ionized first. This is suggestive that the assistance provided by the 18-crown-6 ether does not occur in the solid phase. The neurotransmitter and the 18-crown-6 ether are most likely both desorbed from the solid into the gas phase. At this point ionization occurs by the typical proposed proton transfer mechanism. Since ionization is occurring in ambient conditions, molecules with higher proton affinity can scavenge protons from the neurotransmitter under typical conditions. The 18-crown-6 ether prevents this behavior by forming a strong complex to the protonated primary amine directly in the gas phase, protecting it from any proton scavengers prior to detection. As such, RADI can expand the applicability of the EZ-DART source for what may otherwise be difficult analyses

Initially all four neurotransmitters were tested individually and together to determine their respective spectra and relative intensity without the 18-crown-6 ether. These are shown in figure 2.6a-e. The spectra for the serotonin and norepinephrine samples have a high background signal, with a few background peaks observed for the dopamine sample and none for the GABA sample. The protonated species is identifiable above background in all individual neurotransmitters except for norepinephrine, which shows a water loss as the major peak. Products of dehydration and ammonia elimination reactions are observed with the DART analysis of neurotransmitters; this supports the analogy to APCI ionization mechanisms. While these losses are understood, they would complicate the analysis of neurotransmitters in a biological matrix.

The testing of the four neurotransmitters mixed as a solid, shown in figure 2.6e, shows that the sampling of multiple neurotransmitters simultaneously also presents problems. Protonated GABA appears to be the most intense species, likely due to its small size and simple functionality relative to the other neurotransmitters. While background ions and an extremely small intensity signal for dopamine are seen, there is no evidence of serotonin or norepinephrine in the sample. The competition from both protonated and dehydrated GABA when examining the mixture shows that DART alone is not sufficient to perform a complete analysis. Discrimination may be a serious problem when comparing analytes with slightly varying volatilities and proton affinities.

Next the 18-crown-6 ether host-guest interaction was tested with the four neurotransmitters individually and in a solid mixture, shown in figure 2.6f-j. The introduction of the 18-crown-6 ether separately from the neurotransmitter allowed for the confirmation of the complex formation in the gas phase. The abundance of ammonia seen in gas phase DART spectra combined with its affinity for complexation with 18-crown-6 ether makes it a competitive guest for the crown ether relative to the neurotransmitter. This competition could decrease formation of the complex of interest. While ammonia adduction was observed in each case, adduction of the 18-crown-6 ether to the neurotransmitters was still seen. Since both the neurotransmitter and 18-crown-6 ether are desorbed as neutrals, the formation of the complex would require the neurotransmitter to become ionized through

the typical proton transfer mechanisms. While that process is occurring, ambient ammonia is also being protonated, with which the 18-crown-6 ether forms an extremely stable complex. The concurrent nature of the ionization helps to explain the prevalence of the ammonia complex compared to the analyte.

The protonated and ammoniated 18-crown-6 ether was the most abundant species for serotonin, norepinephrine and dopamine, while the adducted neurotransmitter was most abundant for GABA. Complexation was seen exclusively to the protonated ion, with no water or ammonia losses observed for any of the adducted neurotransmitters. The mechanisms impacting the relative abundance for each species is likely related to their overall structure. While all four neurotransmitters contain a primary amine, the steric hindrance around that primary amine differs for each molecule. GABA contains the most accessible primary amine, with dopamine being the next most accessible, and norepinephrine being the least with the hydroxyl group in close proximity. While this explains the relative abundance trend for these three neurotransmitters, serotonin displays a different behavior even though the primary amine has similar accessibility to dopamine. The presence of a secondary amine in the ring for serotonin presents an additional protonation site less likely to complex to the 18-crown-6 ether, decreasing the chance for complex formation.

All four neurotransmitters also display a signal enhancement when compared to the unfragmented and uncomplexed ion. This enhancement trends differently than the overall intensities. GABA and serotonin both only display an increase of one order of magnitude in the signal/noise ratio from the uncomplexed spectrum. Dopamine shows an increase of three orders of magnitude and norepinephrine an increase of two orders of magnitude. While not all of these increases are significant, the increase in the mixed sample is incredibly pronounced, where both serotonin and norepinephrine are indistinguishable from the noise without the 18-crown-6 ether complexation. Thus we can see that RADI using 18-crown-6 ether increases detection relative to background, simplifies the spectra, and allows for the simultaneous detection of all four transmitters from the same sample.



Figure 2.6. a-e: Spectra of each neurotransmitter and mixture of all four neurotransmitters. A single asterisk refers to background ions while a double asterisk refers to sample impurities. f-j: Spectra of each neurotransmitter and mixture of all four with 18-crown-6 ether sampled separately in the EZ-DART gas stream. All spectra were taken at 250°C EZ-DART gas temperature.

More host-guest systems will need to be tested for the application of RADI to the detection of other functional groups. The current method using 18-crown-6 ether could be applied to the detection of neurotransmitters and other difficult to detect analytes with primary amines in a multitude of different samples of biological relevance. Since the 18-crown-6 ether host guest system simplifies the spectra and enhances the detection of targeted samples, it is reasonable to think that this method could be applied to imaging applications, considering that reactive DESI has been successfully applied in this way before.⁸⁷

2.5 Conclusions

An inexpensive and versatile home-built DART source has been developed as an easily assembled device enabling versatile capabilities. The ability to interface the EZ-DART to mass spectrometers equipped with an atmospheric pressure inlet and the enhanced analytical possibilities from CID allow for its application to multiple relevant applications by a variety of user groups. Additionally the use of reagent assisted desorption ionization (RADI) opens up the use of DART sampling to other complex organic mixtures and new analytical applications.

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2.8 Supplemental Information

2.8.1 Maleic Acid Data



Figure 2.7 Spectrum of maleic acid in negative mode showing the deprotonated monomer, dimer, and trimer. The temperature refers to the measured heating tape temperature.

2.8.2 CID Data for Currency

An EZ-DART spectrum was taken for both fifty (figure 2.8a) and twenty (figure 2.3 and figure 2.8c) dollar notes, both of which had been in circulation prior to testing. The methamphetamine identification was supported by the CID data for the 150.1 m/z peak,¹ shown in figure 2.8b, from the fifty dollar note. The cocaine identification was supported by the CID data for the 304.4 m/z peak,² shown in figure 2.8d, from the 20 dollar note. Structures for both are shown in figure 2.8c and their respective CID spectra. While the both notes displayed each peak, identification required separate CID spectra due to the relative intensities being insufficient to obtain CID data from a single currency note. Due to the large number of peaks and high amount of background signal observed from the 50 dollar note, additional identifications were not pursued.



Figure 2.8 a) The overall spectrum of the 50 dollar bill. b) CID spectrum for protonated methamphetamine from a 50 dollar bill. The loss of 31 corresponds to a loss of CH_3NH_2 and the loss of 59 to a loss of C_3H_8NH c) The overall spectrum for the 20 dollar bill as shown in figure 2.3. d) CID spectrum for protonated cocaine from the 20 dollar bill. The loss of 122 corresponds to a loss of benzoic acid. The targeted m/z values for each CID spectra are indicated on the side.



Figure 2.9 Calibration curve of RDX used for the determination of the LOD for RDX. Signal response was normalized to the background peak for palmitic acid.

The lower limit of detection calculation for RDX with an R2 of 0.915. The LOD of 1.1ng was calculated using linear regression analysis.³ 2.5ng was found to be the limit of linearity for the calibration curve.

The calibration's linearity is observed to drop at higher concentrations, possibly due to the signal for the RDX in these data being normalized to the highest background signal. The drop in linearity may be related to increasing ionization of the background normalization ion with the higher concentrations due to increasing amounts of solvent during standard deposition, as well as the overlap between the sample and the DART region of desorption and ionization. A combination of these two effects could account for the observed loss of linearity. While cluster ion formation has been observed with RDX, such clusters were not observed at higher mass for any amount of RDX used in our experiments.

2.8.4 PPG Data and Discussion

In addition to the La Brea Tar Pit hydrocarbon sample described in the text, Poly(propylene glycol) (PPG) was used for a TPD study, due to the high number of possible ion species which follow an easily interpreted pattern. The average mass of desorbed and ionized PPG molecules detected in the EZ-DART spectrum was calculated for a temperature ramped from 60°C to 250°C, showing an overall linear relationship. Since there were few noticeable changes in average mass at and below 60°C, the lower temperatures were not examined in detail. The mass spectra of PPG at 60°C, 150°C, and 250°C are shown in Figure 2.10, highlighting the temperature-dependent shifting of the mass envelope. Identification of the observed ion species was performed using MS3, shown below.

The stability of the ion current during the temperature ramp suggests that the overall ionization efficiency is not significantly impacted by temperature for a more complex sample. Additionally, these data show that even at elevated temperatures, the only observed species are of mass less than 1000 Da. For this PPG sample, where the average mass is 2700 amu, analysis by EZ-DART does not seem to be appropriate for characterization of the entire polymer, but does provide characterization for the lower molecular weight components in the distribution. The effect of temperature on the detected average mass for PPG has implications for the analysis of other complex organic mixtures with varying volatility. The

near complete loss of species under 200 Da at a typical DART operating temperature of 250°C indicates that when performing analysis with the EZ-DART on a complex organic mixture, the temperature must carefully be considered if characterization of both low and mid mass species is desired. From these results, it appears that having the ability to perform TPD analysis enables the possible targeting of compounds with variable volatility when performing the analysis of a complex organic mixture with the EZ-DART.



Figure 2.10. Spectrum of PPG with subunit count labeled as M_X and relevant adducts labeled.

The identification of each species present in the PPG spectra was further supported by the presence of adduct species. The additional possible in-source dehydration reactions and heterogeneous dimerization can make the identification of the specific monomer involved in a peak difficult, especially with unit-mass resolution mass spectral data. To assist with data interpretation the most abundant species in the spectra were targeted for CID analysis. An automated multistep CID program was used, targeting the most abundant species for CID. Initial CID removed the adducting molecule or separated a dimer, and subsequent CID allowed for the confirmation of the species involved, sample spectra for which are shown below. The use of the software based CID procedure allowed for simple identification of each species with changes in temperature.



Figure 2.11 a) The CID spectrum of 308 m/z at 150°C showing only the loss of ammonia b) The subsequent CID of 291.1 from a, showing multiple losses. These losses correspond to the losses of different monomer units. The targeted m/z values for each CID spectra are indicated on the side.

2.8.5 ESI Spectrum and Method Discussion for La Brea Tar Pits Sample

Approximately 2 grams of tar was mostly dissolved in 20 mL of 50/50 methanol/toluene solution. The solubilized fraction was then diluted by 1:10 in 100% methanol twice serially. This solution was then electrosprayed, providing the spectrum observed below.



Figure 2.12 The electrospray mass spectrum for dissolved tar.

2.8.6 Supplemental Information References

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