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Ionization Mechanism of Positive-Ion Direct Analysis in Real Time: A Transient Microenvironment Concept

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A transient microenvironment mechanism (TMEM) is proposed to address matrix effects for direct analysis in real time (DART). When the DART gas stream is in contact with the sample, a transient microenvironment (TME), which can shield analytes from direct ionization, may be generated through the desorption of the matrix containing the analyte. The DART gas stream can directly ionize the matrix molecules, but the analytes will be ionized primarily through gas-phase ion/molecule reactions with the matrix ions. Experimental results showed that as little as 10 nL of liquid or 10 μg of solid was able to generate an efficient TME. Generated TMEs were able to control the ionization of an analyte below an analyte-to-matrix ratio that was dependent on the DART temperature and the boiling points of the analyte and matrix. TMEs generated by common solvents were studied in detail. The ionization of both polar and nonpolar compounds, present in a solvent or another analyte below a ratio of 1:100, were found to be mainly controlled by the generated TMEs at a DART temperature of 300 °C.

The ionization process for mass spectrometry (MS) has been traditionally accomplished in a vacuum environment. This process has been moved into the atmospheric environment by the development of atmospheric pressure ionization (API) methods, i.e. electrospray ionization (ESI),[1] atmospheric pressure chemical ionization (APCI),[2] and atmospheric pressure photoionization (APPI).[3,4] Recently, the introduction of desorption electrospray ionization (DESI)[5] by Cooks and co-workers in late 2004, followed by direct analysis in real time (DART)[6] by Cody et al. in early 2005, further moved the ionization process for MS into the open-air environment, where the samples are present in native forms. Since then, there has been an explosive emergence of these types of ionization techniques, including atmospheric solid analysis probe (ASAP),[7] electrospray laser desorption ionization (ELDI),[8] desorption atmospheric pressure chemical ionization (DAPCI),[9] desorption sonic spray ionization (DeSSI),[10] MALDI (matrix-assisted laser desorption ionization) assisted electrospray ionization (MALDESI),[11] neutral desorption extractive electrospray ionization (ND-EESI),[12] desorption atmospheric pressure photoionization (DAPPI),[13] dielectric barrier discharge ionization (DBDI),[14] laser ablation electrospray ionization (LAESI),[15] plasma-assisted desorption ionization (PADI),[16] and flowing afterglow atmospheric pressure glow discharge (APGD) ionization,[17] all of which accordingly established a new subfield of MS, i.e., open-air desorption/ionization mass spectrometry (OADI-MS).[18−20]

Most of the OADI techniques can be related to an API technique by an ionization process (i.e., ESI, APCI, and APPI), and, as such, they generate similar mass spectra for the same compounds. ESI-related OADI techniques include DESI,[5] ELDI,[8] DeSSI,[10] MALDESI,[11] ND-EESI,[12] and LAESI.[15] APCI-related OADI techniques include ASAP[7] and DAPCI.[9] Although DART,[6] DBDI,[14] PADI,[16] and flowing afterglow APGD[17] may have a substantially different source design from APCI, they are still related because their ionization is initiated by electrical discharge in a gas. An APPI-related OADI technique is DAPPI.[13] However,

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the key difference between the OADI and API techniques resides in the desorption process, which gives OADI the ability to interrogate samples in their native state, which has been proven to be extremely useful in many areas such as homeland security, counterfeit tablet detection, food quality monitoring, art conservation, tissue imaging, forensic analysis, and drug discovery.

Several ionization mechanisms were initially proposed for DESI, but recent investigations support a droplet pick-up mechanism.[21,22] It is believed that the surface of a condensed sample is prewetted by initial droplets of an ESI jet to form a thin surface liquid film that subsequently dissolves analytes from the sample. Subsequent droplets break up this thin surface liquid film and create numerous offspring droplets that contain the analytes, which are further ionized by the ESI mechanism. Although the DART ionization mechanisms are not yet fully understood, it has been proposed[6] that, in the positive-ion mode, metastable He atoms induce Penning ionization of ambient water in the open air, generating protonated water clusters, mostly $H_5O_2^+$, which further ionize analytes through gas-phase ion/molecule reactions. Because of the appearance of multiple charged ions in the spectra, DESI enables the analysis of molecules with high masses, e.g., proteins, but is limited to moderately polar to highly polar compounds. On the other hand, DART can analyze less-polar compounds,[6,23−36] but is limited to small organic molecules, because singly charged ions are usually observed.

So far, DART has demonstrated intriguing success in the analysis of many samples in their native forms, e.g., perfumery raw materials deposited on smelling strips,[27] counterfeit Cialis tablets,[37] strobilurin fungicides in the ethyl acetate extract of wheat,[38] fatty acid methyl esters from bacterial whole cells,[32] self-assembled monolayers of dodecanethiol on gold surfaces,[28] taxoids from cell cultures of *Taxus wallichiana*,[23] alkaloids from the intact hairy roots of *Atropa acuminate*,[39] and cuticular hydrocarbons from an awake behaving fly.[33] Recent studies have even demonstrated successful quantitative analysis without sample cleanup or chromatography using DART with an AutoDART HTL PAL autosampler (Leap Technologies, Carrboro, NC) and Dip-it tips (IonSense, Danvers, MA).[34,35,38] An inexpensive autosampler has also been developed and is able to rapidly analyze hundreds of cotton swab wipe samples from a simulated chemical dispersal event.[24−26]

DART is especially useful in monitoring synthetic reaction mixtures. With traditional API methods, the commonly used infusion technique requires internal calibration and tedious cleanup procedures to avoid contamination. With DART, sample introduction can be easily accomplished within seconds, using disposable melting-point capillaries. Instantaneous external calibration is good enough to fulfill the requirement of accurate mass measurement. Furthermore, source contamination is minimized because the sampling area is in an open-air environment and only ~1 μL of sample is required. Recently, DART has been demonstrated to be a complementary tool to LC/ESI-MS for reaction monitoring in drug discovery.[31] Sample zones from high-performance thin-layer chromatography (HPTLC)[30] have also been directly analyzed by DART. In our laboratory, DART is routinely used to analyze synthetic organic compounds and the previously proposed ionization mechanism of DART by Cody et al.[6] is used to interpret DART mass spectra. However, this interpretation method does not specifically address matrix effects. To account for these matrix effects, a transient microenvironment mechanism (TMEM), which is supported by a scheme consisting of nine gas-phase reactions and thermodynamic data, is proposed and able to better interpret the observed mass spectra. Furthermore, the TMEM can also better interpret previously published data where dimethyl sulfoxide (DMSO) was determined to be an unfavorable solvent to DART ionization.[31]

EXPERIMENTAL SECTION

Reagents. Acetonitrile, chloroform, methylene chloride, methanol, hexanes, heptane, iso-octane, tetrahydrofuran (THF), chlorobenzene, and toluene were high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Suwanee, GA). 2-Propanol, ethyl acetate, cyclohexane, acetone, benzene, fluorobenzene, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethylbenzene, *o*-xylene, *p*-xylene, and anisole were certified ACS grade and purchased from Aldrich Chemical (St. Louis, MO). Ethanol was 200 proof and purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY). Hexafluorobenzene was purchased from Lancaster Synthesis (Ward Hill, MA). These compounds were analyzed as liquids and solvents of analytes by positive-ion DART. Their boiling points (bp) and ionization energies (IE), which were obtained from the NIST Chemistry WebBook (http://webbook.nist.gov),[40] are listed in Table S-1 in the Supporting Information. Their proton affinity (PA) in different forms such as monomer, dimer, and (S−H) radicals are listed in Table S-2 in the Supporting Information. The PA values of their monomers were also obtained from the NIST Chemistry WebBook.[40] The PA values of their dimers and (S−H) radicals were thermochemically estimated, which is described in the Supporting Information.

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Naphthalene, 1,2,4,5-tetramethylbenzene (1,2,4,5-TMB), decanoic acid, 1-naphthol, anthracene, 1,3-dimethoxybenzene (1,3-DMOB), 9-methylanthracene, 12-Crown-4, *N*,*N*-dimethylaniline ($PhNMe_2$), and tributylamine were purchased from Aldrich Chemical (St. Louis, MO). These compounds were analyzed as solids and analytes in solutions of different solvents by positive-ion DART mass spectrometry. Their bp values, IEs, and PAs are listed in Table S-3 in the Supporting Information. Most of the IE and PA values were obtained from the NIST Chemistry WebBook.[40] Some of these values were thermochemically estimated by the method which has been described in the Supporting Information. The chemical structures for the compounds analyzed are shown in Figure S-1 in the Supporting Information.

Reserpine and polyethylene glycols (PEG 200 and PEG 600) were purchased from Aldrich Chemical (St. Louis, MO). They are used to calibrate the mass spectrometer.

Apparatus. All experiments were performed using a JEOL Model JMS-T100LC (AccuTOF) orthogonal time-of-flight (TOF) mass spectrometer (Peabody, MA) with an IonSense (Danvers, MA) DART source. The DART source used helium gas at a flow rate of 4 L/min, with a flow factor of 0.3. The following gas heater, needle voltage, grid electrode voltage, and discharge electrode voltage settings of the DART source were used: 300 °C, 3000 V, 250 V, and 150 V, respectively. The general controlling parameters for the AccuTOF were as follows: temperature at orifice 1, 80 °C; voltage at orifice 1, 20 V; voltage at orifice 2, 3 V; ring lens voltage, 3 V; and peak voltage, 200 V. The distance between the outlet of the DART gas and the inlet of orifice 1 of the AccuTOF was ~1 cm. The DART ionization and AccuTOF settings were chosen to produce as little in-source fragmentation as possible. Liquid or solid samples were deposited onto the closed end of a melting point capillary by dipping it directly into the sample. This technique usually samples ~1 μL of liquid or 0.1 mg of solid. Sample introduction was accomplished by moving the closed end of a melting point capillary across the helium gas stream in a slow and consistent motion, equidistant from the DART source and orifice 1 of the AccuTOF. Sample ionization was instantaneous after the DART gas stream contacted the sample. The AccuTOF system was tuned using electrospray ionization (ESI) with reserpine to a resolving power of over 6000 (fwhm). Calibration was performed using DART with a mixture solution of 5 μL/mL PEG 200 and 10 μL/mL PEG 600 in a solvent of methanol and methylene chloride (1:1), using the $[M + H]^+$ ion series. However, PEG spectra were not acquired in the same data file of sample spectra for every acquisition because the analytes were authentic. The spectra recording interval was 0.5 s. The mass acquisition range was 10−300 m/z for solvents. However, to avoid having the intense peaks of most solvent ions overshadow the analyte ions, the acquisition range was 120−200 m/z for analyte solutions. Sample introduction was repeated six times to generate six reconstructed total ion current (RTIC) profile peaks in each analysis. The spectra shown in Figures 1 and 2 represent the mass spectra corresponding to the maximum of the RTIC profile peak.

RESULTS AND DISCUSSION

Matrix Effects on DART. Sample preparation is usually not required by DART. Instead, samples in their native states (i.e.,

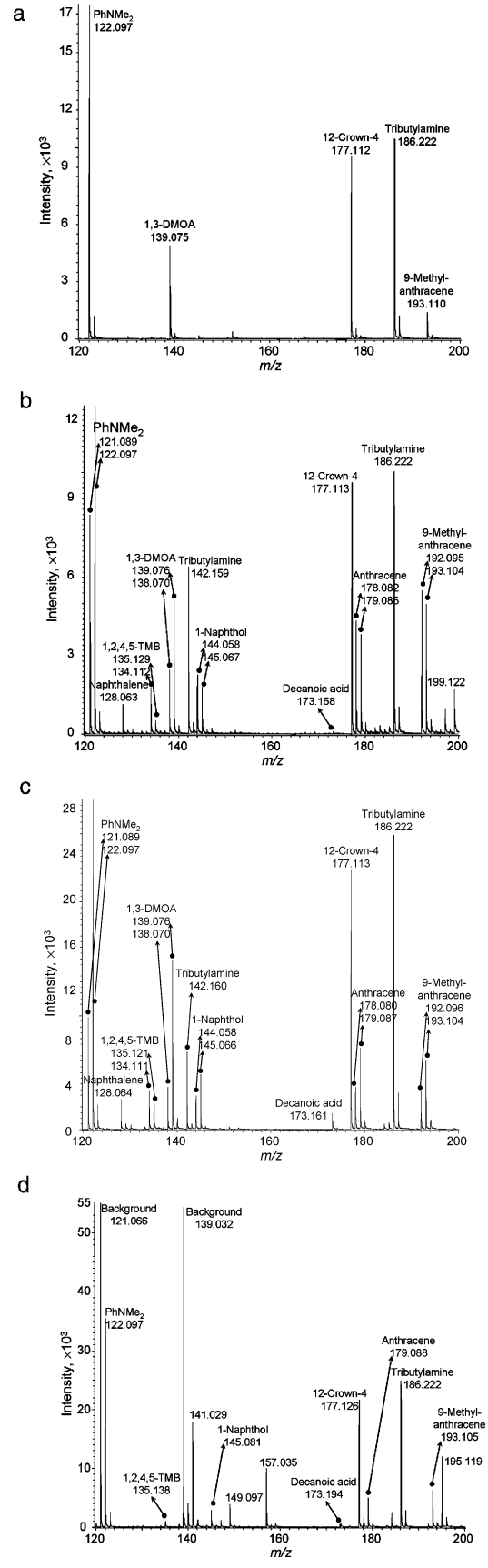

Figure 1. Direct analysis in real time (DART) spectrum of ~1 μL of naphthalene, 1,2,4,5-TMB, decanoic acid, 1-naphthol, anthracene, 1,3-DMOB, 9-methylanthracene, 12-Crown-4, $PhNMe_2$, and tributylamine at a respective concentration of 1 μg/mL in (a) methanol, (b) toluene, (c) hexanes, and (d) chloroform.

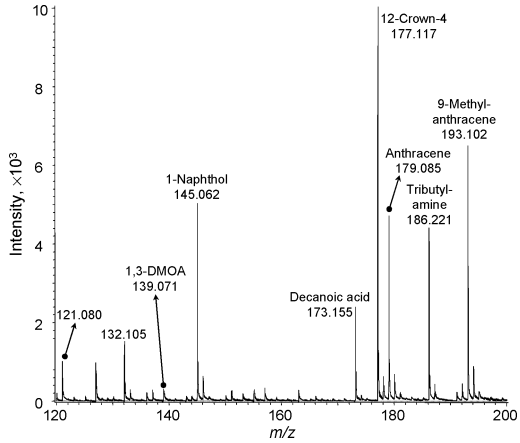

Figure 2. DART spectrum of ~1 ng of naphthalene, 1,2,4,5-TMB, decanoic acid, 1-naphthol, anthracene, 1,3-DMOB, 9-methylanthracene, 12-Crown-4, $PhNMe_2$, and tributylamine.

liquids or solids and solutions) can be directly analyzed by DART. However, typical analytes usually exist in small amounts in a matrix (e.g., synthetic compounds in solvents, impurities in chemicals, drugs in tablets, creams, solutions or foams, pollutants in water, sludge, or soil, and metabolites in tissue or body fluids). Therefore, the matrix composition will certainly influence DART ionization. This is demonstrated in Figure 1, where 1 μL of naphthalene, 1,2,4,5-TMB, decanoic acid, 1-naphthol, anthracene, 1,3-DMOB, 9-methylanthracene, 12-Crown-4, $PhNMe_2$, and tributylamine was ionized by DART at a respective concentration of 1 μg/mL in methanol, toluene, hexanes, or chloroform.

The Transient Microenvironment Mechanism (TMEM). DART ionization begins with a stream of gas (usually helium), which is passed through an electrical discharge to produce ions, electrons, and metastable species. After heating and the removal of charged particles, this stream of gas exits the DART source into the open air and is able to ionize samples by instant contact. In the positive-ion mode, Cody et al.[6] proposed that metastable He atoms induce Penning ionization of ambient water in the open air, generating protonated water clusters, mostly $H_5O_2^+$, which further ionize analytes through gas-phase ion/molecule reactions. That mechanism does not specifically address the matrix effects; therefore, here, we propose a Transient Micro-Environment Mechanism (TMEM). The TMEM states that, when the DART gas stream, which contains a significant amount of both metastable He atoms and $H_5O_2^+$, is in contact with the sample, a TME can shield the analytes from direct ionization by the DART gas stream. The TME may be generated through desorption of the volatile matrix of the analyte. The DART gas stream will directly ionize the volatile matrix molecules in the TME, and then those matrix ions are the species that ionize the analytes via gas-phase ion/molecule reactions.

Scheme 1 shows a series of reactions that describe what can happen when a solution is analyzed. There are three steps:

(1) When the helium gas stream, which contains metastable atoms, is in contact with the atmosphere, molecular ions of water are formed (reaction A in Scheme 1), which, in turn, produce protonated water clusters (reaction B in Scheme 1).

(2) When the stream of He metastable atoms is in contact with the solvent molecules that constitute a TME, reaction C in Scheme 1 will occur, resulting in solvent molecular ions, which, in turn, react with other solvent molecules to produce protonated solvent molecules (reaction D in Scheme 1). Protonated water clusters can also react with solvent molecules to produce protonated solvent molecules (reaction E in Scheme 1).

(3) The analyte molecules are ionized to form protonated molecules through gas-phase ion/molecule reactions with protonated solvent molecules (reaction F in Scheme 1). Solvent molecular ions can react with analyte molecules to produce both protonated analyte molecules and analyte molecular ions (reactions G and H in Scheme 1).

Henceforth, pure solvent and analyte molecules will be denoted as "S" and "M," respectively.

Note that the TMEM is an extension and clarification of the ionization mechanism of positive-ion DART that was proposed by Cody et al.[6,36] In the original DART article,[6] molecular ions of toluene were shown, which is an observation that is consistent with the TMEM. In the follow-up article,[36] fluorobenzene was used as a dopant, which is, in fact, a clear example of a TME. The TMEM provides a more complete list of possible gas-phase ion/molecule reactions in the positive-ion DART process, especially when a complex matrix is present.

Analysis of Liquids/Solvents. Analysis of solvents by positive-ion DART can provide useful information about the TME involved, because the TME is generated by desorption of the volatile matrix of a sample. Approximately 1 μL each of individual solvents was analyzed, and the observed ions are listed in Table 1. Representative mass spectra of the background air and solvents are shown in Figure S-2 in the Supporting Information.

In Table 1, the solvents are organized in four groups: proton acceptors, benzene derivatives, alkanes, and chlorinated methanes. The proton acceptor solvents are listed in Table 1 in increasing order of their PA values, include methanol, ethanol, acetonitrile, 2-propanol, acetone, THF, ethyl acetate, DMF, and DMSO. Because all of these solvents have IE values (see Table S-1 in the Supporting Information) that are less than helium's metastable energy (19.8 eV), they can be ionized through reaction C in Scheme 1 to generate $S^{+\bullet}$ ions. These should further undergo reaction D in Scheme 1 to become $[S + H]^+$ ions, because they have PA values stronger than their (S − H) radicals (see Table S-2 in the Supporting Information). Note that these solvents can form clusters, mostly dimers, possessing PA values stronger than the corresponding monomers. These clusters may be ionized through reactions C and D in Scheme 1. In addition, the PA values of these solvents (monomer and dimer) are also stronger than the PA values of water (monomer and dimer); these solvents may be ionized through reaction E in Scheme 1. For the alcoholic solvents (i.e., methanol, ethanol, and 2-propanol), $[S_2 + H - H_2O]^+$ ions were also observed. They are produced by the condensation reaction of a protonated alcohol with a neutral molecule to form a protonated ether ion plus water.[41] However, we believe that these ions do not play a dominant role in the TME, because of their low abundance. Overall, the mass spectra of the proton acceptor solvents were dominated by $[S_2 + H]^+$ and/or $[S + H]^+$ ions.

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Scheme 1. Main Reactions in Positive-Ion DART[a]

$$He^* + H_2O \rightarrow He + H_2O^{+\cdot} + e^-, \quad \text{as ME(He)>IE}(H_2O) \quad (A)$$

$$H_2O^{+\cdot} + (H_2O)_m \rightarrow HO^{\cdot} + [(H_2O)_m + H]^+, \quad \text{as PA}((H_2O)_m)\text{>PA}(HO^{\cdot}) \quad (B)$$

$$He^* + S \rightarrow He + S^{+\cdot} + e^-, \quad \text{as ME(He)>IE(S)} \quad (C)$$

$$S^{+\cdot} + S_n \rightarrow [S - H]^{\cdot} + [S_n + H]^+, \quad \text{if PA}(S_n)\text{>PA}([S\text{-}H]^{\cdot}) \quad (D)$$

$$[(H_2O)_m + H]^+ + S_n \rightarrow (H_2O)_m + [S_n + H]^+, \quad \text{if PA}(S_n)\text{>PA}((H_2O)_m) \quad (E)$$

$$[S_n + H]^+ + M \rightarrow S_n + [M + H]^+, \quad \text{if PA(M)>PA}(S_n)\text{>PA}([S\text{-}H]^{\cdot}) \quad (F)$$

$$S^{+\cdot} + M \rightarrow [S - H]^{\cdot} + [M + H]^+, \quad \text{if PA(M)>PA}([S\text{-}H]^{\cdot})\text{>PA}(S_n) \quad (G)$$

$$S^{+\cdot} + M \rightarrow S + M^{+\cdot}, \quad \text{if PA}([S\text{-}H]^{\cdot})\text{>PA}(S_n) \text{ and IE(S)>IE(M)} \quad (H)$$

$$[(H_2O)_m + H]^+ + M \rightarrow (H_2O)_m + [M + H]^+, \quad \text{if the TME is thin} \quad (I)$$

[a] ME(He) is helium's metastable energy, 19.8 eV; $m = 1$, 2, or 3; $n = 1$ or 2. Reaction D has a few variants for alkanes and chlorinated methanes, as described in the text.

The benzene derivative solvents, which are listed in Table 1 in increasing order of their PA values, include hexafluorobenzene, benzene, chlorobenzene, fluorobenzene, toluene, ethyl benzene, *p*-xylene, *o*-xylene, and anisole. All of these solvents have IE values (see Table S-1 in the Supporting Information) lower than helium's metastable energy and may be ionized through reaction C in Scheme 1 to generate $S^{+\bullet}$ ions. However, the $S^{+\bullet}$ ions cannot undergo reaction D in Scheme 1 to become $[S + H]^+$ ions, because the PA values are weaker than their (M − H) radicals (see Table S-2 in the Supporting Information). Conversely, these solvents, with the sole exception of hexafluorobenzene, possess PA values stronger than the monomer of water and may also be ionized by reaction E in Scheme 1 to generate $[S + H]^+$ ions. As the PA values of these solvents increase, the abundance of the $[S + H]^+$ ions should increase, but the abundance of the $S^{+\bullet}$ ions may decrease. This is confirmed in Table 1. Additional ions were also observed for C_6F_6 and C_6H_5F. The $[S - F + OH]^+$ ions are thought to arise from a nucleophilic aromatic substitution reaction of $S^{+\bullet}$ with H_2O. This reaction is exothermic by ca. −84 kJ/mol for both C_6F_6 and C_6H_5F, which is driven by the extremely strong bond strength of the neutral HF product. The presence of $[S + H]^+$ ions for C_6H_5F but not C_6F_6 is consistent with the PA value for the former being 756 kJ/mol, which is 65 kJ/mol stronger than that of water, and 648 kJ/mol for the latter, which is 43 kJ/mol weaker than that of water (see Table S-2 in the Supporting Information). Similarly, the $[S - F + H_2O]^+$ ions, which are seen with C_6H_5F but not C_6F_6, probably arise from the reaction of the protonated C_6H_5F with water, losing HF. Similarly, the presence of the $[2S - 2F + H_2O]^+$ ions with C_6H_5F but not C_6F_6 implies that this likely arises ultimately from the $[S + H]^+$ ion. For ethyl benzene, a $[S - H]^+$ ion was also observed, which could be interpreted similarly to the alkanes in the next paragraph. Note that the $H_5O_2^+$ ion (i.e., m/z 37.1) was also observed for most of the benzene derivatives (data not shown in Table 1). Overall, the mass spectra of the benzene derivative solvents were dominated by $S^{+\bullet}$ and $[S + H]^+$ ions.

Alkane solvents listed in Table 1 include hexanes, heptane, iso-octane, and cyclohexane. Because all of these solvents have IE values (see Table S-1 in the Supporting Information) lower than helium's metastable energy, they can be ionized through reaction C in Scheme 1 to generate $S^{+\bullet}$ ions. However, the $S^{+\bullet}$ ions of alkanes are known to be able to undergo a hydride/alkide abstraction reaction to form $[S - (CH_2)_n - H]^+$ ions:[42]

$$S^{+\bullet} + S \rightarrow [S - H]^{\bullet} + C_nH_{2(n+1)} + [S - (CH_2)_n - H]^+ \quad (n = 0, 1, 2, ...)$$

Such $[S - (CH_2)_n - H]^+$ ($n = 0$, 1, 2, ...) ions can be considered as $[S + H]^+$ ions of the corresponding alkenes. Note that the $H_5O_2^+$ ion (i.e., m/z 37.1) also appeared in their mass spectra (data not shown in Table 1). Overall, the mass spectra of the alkane solvents were dominated by $[S - (CH_2)_n - H]^+$ ($n = 0$, 1, 2, ...) ions.

Chlorinated methane solvents in Table 1 include methylene chloride and chloroform. Because both methylene chloride and chloroform have IE values lower than helium's metastable energy (see Table S-1 in the Supporting Information), they can be ionized through reaction C in Scheme 1 to generate $S^{+\bullet}$ ions. It is possible that the $S^{+\bullet}$ ions of methylene chloride and chloroform undergo a hydride/HCl abstraction reaction to form $[S - H]^+$ and/or $[S - Cl]^+$ ions:

$$CH_2Cl_2^+ + CH_2Cl_2 \rightarrow CH_2Cl_2^{+\bullet} + H_2 + CHCl_2^+$$

$$CHCl_3^{+\bullet} + CHCl_3 \rightarrow CCl_3^{\bullet} + HCl + CHCl_2^+$$

$$CHCl_3^{+\bullet} + CHCl_3 \rightarrow CCl_3^{\bullet} + H_2 + CCl_3^+$$

Table 1. Observed Ion Peaks with Relative Intensity over 5% in the DART Mass Spectra[a]

solvent	Relative Intensity (%) $[S - H]^+$	$S^{+\bullet}$	$[S + H]^+$	$[S_2 + H]^+$	other detected ions and their relative intensity
Proton Acceptors					
MeOH			72	100	$[S_2 + H - H_2O]^+$, 15%
EtOH			74	100	$[S_2 + H - H_2O]^+$, 16%
MeCN			100	63	
i-PrOH			14	100	$[S_2 + H - H_2O]^+$, 16%
acetone			90	100	
THF			46	100	
EtOAc			31	100	
DMF				100	
DMSO				100	
Benzene Derivatives					
C_6F_6		90			$[S - F + OH]^+$, 68%
benzene		100	30		
PhCl		100	18		
PhF		100	39		$[S - F + OH]^+$, 63%; $[S - F + H_2O]^+$, 11% $[2S - 2F + H_2O]^+$, 14%
$PhCH_3$		72	100		
PhC_2H_5	18	34	100		
p-xylene		26	100		
o-xylene		49	100		
$PhOCH_3$		14	100		
Alkanes					
hexanes	100				$C_4H_9^+$, 11%; $C_5H_{11}^+$, 10% $[S - 4H]^+$, 11%; $[S - 3H]^+$, 62% $[S - 2H]^+$, 15%
heptane	11				$C_4H_9^+$, 100%; $C_5H_9^+$, 14% $C_5H_{11}^+$, 52%; $[S - 3H]^+$, 25% $[S - 2H]^+$, 4%
iso-octane					$C_4H_9^+$, 100%
CyHex[b]	100				
Chlorinated Methanes					
CH_2Cl_2[c]					$C_3H_7^+$, 7%; $C_4H_7^+$, 6% $C_5H_{11}^+$, 60%; $CHCl_2^+$, 9%
$CHCl_3$[d]					$C_3H_7^+$, 11%; $C_5H_9^+$, 26% $C_5H_{11}^+$, 100%; $CHCl_2^+$, 45% $C_6H_{11}^+$, 12%; CCl_3^+, 2% $C_6H_{11}Cl^+$, 11%
$CHCl_3$[e]					$[C_2H_5OH + H]^+$, 100%; $[(C_2H_5OH)_2 + H]^+$, 46% $CHCl_2^+$, 5%

[a] Contribution from the isotopic peak of $[m/z - 1]$ was subtracted. [b] Cyclohexane. [c] 15−100 ppm amylene or 40−100 ppm cyclohexene is present as a preservative. [d] 50 ppm pentene is present as a preservative. [e] 0.75% ethanol is present as a preservative.

However, the appearance energy for $CHCl_2^+$ from $CHCl_3$ is only 0.1 eV above the IE,[40] and 0.8 eV above the IE for CH_2Cl_2 as the source. It may be that the $[S - H]^+$ and/or $[S - Cl]^+$ ions are simply fragment ions formed upon ionization of the halogenated matrices. Note that preservatives such as 15−100 ppm "amylene" (pentenes) or 40−100 ppm cyclohexene are usually added to commercial methylene chloride for stabilization. Similarly, 50 ppm pentene or 0.75% ethanol is usually added to commercial chloroform. These preservatives can be ionized through the TMEs of methylene chloride and chloroform. Therefore, corresponding peaks that represent the preservatives' ions are observed in the mass spectra of methylene chloride and chloroform, as shown in Table 1 (please refer to the next section for the interpretation of the observed ions).

Analysis of Solutions. Approximately 1 μL of solutions of naphthalene, 1,2,4,5-TMB, decanoic acid, 1-naphthol, anthracene, 1,3-DMOB, 9-methylanthracene, 12-Crown-4, $PhNMe_2$ and tributylamine, each at a concentration of 1 μg/mL, in methanol, acetonitrile, 2-propanol, acetone, THF, ethyl acetate, DMF, DMSO, anisole, *o*-xylene, toluene, chlorobenzene, fluorobenzene, hexanes, heptane, iso-octane, methylene chloride, or chloroform were ionized by positive-ion DART. The ions observed are listed in Table 2. Representative mass spectra of both the solvent and the analyte solutions are shown in Figure S-3 in the Supporting Information. These mass spectra show the appearance of the solvent ion(s) in the mass spectrum of the analyte solution, which is evidence of the TME.

(42) Harrison, A. G. *Chemical Ionization Mass Spectrometry*; CRC Press: Boca Raton, FL, 1983.

Table 2. Observed Ion Peaks in the DART Mass Spectra of Analytes in Solutions[a]

	$PhNMe_2$		Naphthalene		1,2,4,5-TMB		1,3-DMOB		1-Naphthol	
solvent[b]	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$
MeOH		49						18		
MeCN		13								
i-PrOH		B						B		
acetone		12								
THF		5							B	B
EtOAc		2								
$PhOCH_3$	25	36						8		
o-xylene	B	B			5	4	11	9	6	18
$PhCH_3$	27	35	4		7	2	8	17	9	6
PhCl	34	27	B	B	15	6	16	17	12	4
PhF	51	63	3		5	3	10	28	8	5
hexanes	31	78	9	1	11	8	13	46	12	16
heptane	20	52	8	8	10	6	10	37	10	15
iso-octane	12	56	2	B	4	3	4	33	4	8
CH_2Cl_2	B	36				3		B		3
[d]$CHCl_3$	B	100				5		B		12

	Decanoic Acid		12-Crown-4		Anthracene		Tributyl-amine		9-Methyl-anthracene	
solvent[b]	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$	$M^{+\bullet}$	$[M + H]^+$
MeOH				31				34		7
MeCN				27				21		
i-PrOH				18				17		
acetone				18				19		
THF				6				3		
EtOAc				B	B	B		0		
$PhOCH_3$		3		12	5		19	15	5	0
o-xylene		0		18	11	1	17	20	13	3
$PhCH_3$		0		32	16	12	20	33	19	15
PhCl		5		17	15	8	20	21	15	9
PhF	B	5		36	13	8	19	63	13	12
hexanes		7		72	13	22	20	80	12	19
heptane		9		49	14	24	11	42	16	28
iso-octane		3		41	6	17	5	36	9	16
CH_2Cl_2		5		30		6		27		9
[d]$CHCl_3$		6		70		19		76		23

[a] Ion intensity was normalized to the most intensive one as a percentage. The contribution of ion intensity from the isotopic peak of $[m/z - 1]$ was subtracted. The symbol "B" that is used in this table represents a background ion. [b] No relevant peaks were observed when DMF and DMSO were used. [c] A m/z 142.16 ion, which may be $(C_4H_9)_2NCH_2^{+\bullet}$, was observed. It may be a fragment from an unstable $M^{+\bullet}$ ion. [d] 0.75% ethanol is present as a preservative.

When analytes are dissolved in proton acceptor solvents (i.e., methanol, acetonitrile, 2-propanol, acetone, THF, ethyl acetate, DMF, and DMSO), they should be ionized through reaction F in Scheme 1, because the TMEs of theses solvents are dominated by $[S_2 + H]^+$ ions. Therefore, only $[M + H]^+$ ions can be observed and their intensities should be dependent on $PA(M) - PA(S_2)$ values (see Tables S-2 and S-3 in the Supporting Information). Methanol should be the best solvent for the ionization of all the analytes, because it has the weakest $PA(S_2)$ among the proton acceptor solvents. DMF and DMSO should be the worst solvents for the ionization of all the analytes, because they have the strongest $PA(S_2)$. Both conclusions are confirmed in Table 2. Even with methanol, half of the analytes (including naphthalene, 1,2,4,5-TMB, 1-naphthnol, decanoic acid, and anthracene) were still not ionized, as shown in Figure 1A. This is because the corresponding $PA(M) - PA(S_2)$ values are negative. For both DMF and DMSO, none of the analytes were ionized, also because the corresponding $PA(M) - PA(S_2)$ values are negative. Note that unfavorable solvents for DART ionization such as THF, ethyl acetate, DMF, and DMSO were used in the analysis of organic synthetic products from drug discovery.[31] While DMSO was found to be unfavorable for DART ionization, no significant differences were reported among methanol, acetonitrile, THF, ethyl acetate, and DMF,[31] which might be the results of using an analyte with high proton affinity (i.e., warfarin) and high concentration (i.e., 100 μg/mL).

When analytes are dissolved in benzene derivative solvents (i.e., anisole, *o*-xylene, toluene, chlorobenzene, fluorobenzene), they should be ionized through reactions F, G and/or H in Scheme 1, because the TMEs of these solvents are dominated by $S^{+\bullet}$ and $[S + H]^+$ ions. Therefore, $[M + H]^+$ ions can be observed when $PA(M) - PA(S)$ values (see Tables S-2 and S-3 in the Supporting Information) are positive. $M^{+\bullet}$ ions can also be observed when $IE(M) - IE(S)$ values are negative (see Tables S-2 and S-3 in the Supporting Information). This is confirmed in Table 2. No significant $M^{+\bullet}$ ions were observed for decanoic acid, because of its high IE; and anisole was the worst solvent to ionize the analytes via their $M^{+\bullet}$ ions, because of its lowest IE among the solvents. The most favorable benzene derivative solvent to ionize this study's selection of analytes is toluene; the corresponding mass spectrum is shown in Figure 1B.

Table 3. Observed Ion Peaks with Relative Intensity over 10% in the DART Mass Spectra[a]

analyte	Relative Intensity (%) $[M - H]^+$	$M^{+\bullet}$	$[M + H]^+$	$[M_2 + H]^+$	other detected ions
naphthalene		68	100		
1,2,4,5-TMB	11	91	100		
decanoic acid			61	100	$[M - H_2O + H]^+$, 58%
1-naphthol		43	100		
anthracene		51	100		
1,3-DMOB		17	100		$[M - H + CH_3]^+$, 20%
9-methylanthracene		78	100		$[M + O]^+$, 12% $[M + O_2 + H]^+$, 27%
12-Crown-4			100		
$PhNMe_2$	44	36	100		$[M + CH_3]^+$, 20% $[M - CH_3 + 2H]^+$, <10%
tributylamine	24		100		$[M - C_3H_7]^+$, 83%

[a] The contribution of ion intensity from the isotopic peak of $[m/z - 1]$ was subtracted.

When analytes are dissolved in alkane solvents (i.e., hexanes, heptane, and iso-octane), they should be ionized first by reaction F in Scheme 1, because the TMEs of these solvents are dominated by $[S - (CH_2)_n - H]^+$ (for $n = 0, 1, 2, ...$) ions, which can be considered as $[S + H]^+$ ions of the corresponding alkenes. Therefore, $[M + H]^+$ ions can be observed for all the analytes, because the PA(M)−PA(S) values are positive (see Tables S-2 and S-3 in the Supporting Information). This is confirmed in Table 2. In addition, most of the analytes were also ionized as $M^{+\bullet}$ ions (please refer to the next section for the interpretation of the absence of $M^{+\bullet}$ ions from decanoic acid and 12-Crown-4). This should occur through reaction H in Scheme 1 and requires both the existence of $S^{+\bullet}$ ions and the IE(M)−IE(S) values to be negative (see Tables S-2 and S-3 in the Supporting Information). Although peaks representing $S^{+\bullet}$ ions of alkane solvents were not observed, they did exist as the precursors of $[S - (CH_2)_n - H]^+$ ($n = 0, 1, 2, ...$) ions. Such alkane radical cations are thermochemically higher in energy as reactants for reaction D in Scheme 1 than benzene derivative radical cations, and thus may have a shorter lifetime in the source, such that they are not observed. There was no significant difference among the alkane solvents in the ionization of all the analytes. Figure 1C shows the corresponding mass spectrum when hexanes were used.

When methylene chloride and chloroform were used as solvents, the ionization of the analytes seemed to be similar to that of the alkanes. However, no significant $M^{+\bullet}$ ions were observed, possibly implying a greater reactivity of $S^{+\bullet}$ ions from methylene chloride and chloroform than alkanes. This is consistent with the IE of methylene chloride and chloroform being higher than the alkanes (see Table S-1 in the Supporting Information). In addition, the ionization of the analytes and the stabilizers in the solvents seemed similar. With 15−100 ppm amylene (presumably a pentene mixture) as a stabilizer in methylene chloride, protonated pentene was observed. With 0.75% ethanol as a stabilizer in chloroform, the protonated monomer and dimer of ethanol were observed. With 50 ppm pentene as a stabilizer in chloroform, protonated pentene was observed, along with other pentene fragment ions (similar to the $[S - (CH_2)_n - H]^+$ ($n = 0, 1, 2, ...$) ions of alkanes) and $C_6H_{10}Cl^+$of unknown provenance.

Analysis of Solids. A TME can also consist of vapors from solids that can be desorbed by the DART gas stream and further ionized by DART. Therefore, an analysis of solids without a liquid matrix present can also provide us with useful information about the TME involved in the DART ionization mechanism. Most of the analytes used in this study are solids, so they are analyzed for that purpose.

First, the analytes were sampled by dipping the closed end of a melting point capillary directly into the solid. Approximately 0.1 mg of solid was sampled this way, and TMEs similar to those when ~1- μL solvents were analyzed were observed. Next, the amount of solid sample was reduced to assess the changes in the TME. The analytes were dissolved in a solvent (e.g., toluene) at individual concentrations of 10 mg/mL, 100 μg/mL, and 1 μg/mL. They were sampled by dipping the closed end of a melting point capillary directly into the solutions of the analytes and then were air-dried for ~3 min. Approximately 10 μg, 100 ng, and 1 ng of analytes, which were dried from ~1 μL of solution, were analyzed. The results indicated that ~10 μg of solid was required to generate an efficient TME; i.e., both $M^{+\bullet}$ and $[M + H]^+$ ions are abundant for naphthalene. If liquid instead of solid was used, the required volume should be 10 nL, assuming a density of 1 mg/mL.

Table 3 lists the observed ions by positive-ion DART for ~10 μg of individual analyte. The generation of $[M - H]^+$, $M^{+\bullet}$, and $[M + H]^+$ ions occurred mostly through reactions C, D, and E in Scheme 1, which were also used to interpret the generation of similar ions from the solvents. Note that no $M^{+\bullet}$ ion was observed for decanoic acid and 12-Crown-4, which is probably due to reaction D in Scheme 1, although the PAs of the corresponding (M − H) radicals were not available. Other ions were also detected, as shown in Table 3, because of gas-phase ion/molecule reactions; however, interpretation of their formation is beyond the scope of this study. Nevertheless, note that gas-phase reactions for the analytes seemed more complicated than the solvents most of the time.

Figure 2 shows a mass spectrum of 1 ng of all the analytes analyzed by positive-ion DART. The analytes were presumably ionized through reaction I in Scheme 1, so only $[M + H]^+$ ions were observed. Note that no ions for naphthalene, 1,2,4,5-TMB, and $PhNMe_2$ were observed. Although the absence of protonated naphthalene could be due to its weaker PA than the dimer of water, the absence of protonated 1,2,4,5-TMB and $PhNMe_2$ was puzzling. This may be due to unknown gas-phase ion/molecule reactions. However, it does suggest that direct

analysis of analytes in solid states is not always a better choice than analysis of analytes in a solution.

Analysis of Impurities in Solids. Vapors of solids can generate an efficient TME under DART conditions. The ionization of impurities in these solids should occur through gas-phase ion/molecule reactions with the ions of these solids. Two such samples, i.e., 1 ng of naphthol in 10 μg of naphthalene and 1 ng of naphthalene in 10 μg of naphthol (1:10,000), were analyzed. Abundant $M^{+\bullet}$ and $[M + H]^+$ ions of naphthol were observed for the sample of 1 ng of naphthol in 10 μg of naphthalene. As shown in Table 3, the TME from 10 μg of naphthalene consisted of its $M^{+\bullet}$ and $[M + H]^+$ ions, which would ionize naphthol through reactions F and H in Scheme 1, because naphthol possesses a lower IE and stronger PA value than naphthalene (see Table S-3 in the Supporting Information). No ions of naphthalene were observed for the sample of 1 ng of naphthalene in 10 μg of naphthol. As shown in Table 3, the TME from 10 μg of naphthol consisted of its $M^{+\bullet}$ and $[M + H]^+$ ions, which would not ionize naphthalene through reactions F and H in Scheme 1, because naphthalene possesses a higher IE and weaker PA than naphthol (see Table S-3 in the Supporting Information).

A critical analyte:matrix ratio is explored to better predict the effect of TME. When the analyte:matrix ratio is lower than the critical ratio value, DART ionization will be controlled by the TME. Three more samples were analyzed: 10 ng of naphthalene in 10 μg of naphthol (1:1000), 100 ng of naphthalene in 10 μg of naphthol (1:100), and 1 μg of naphthalene in 10 μg of naphthol (1:10). $M^{+\bullet}$ and $[M + H]^+$ ions of naphthalene were observed when naphthalene is in excess of 100 ng, which indicated that the DART ionization was not controlled by the TME anymore. Therefore, naphthalene ionization in a naphthol matrix was mainly controlled by the TME in ratios below 1:100. Note that the critical ratio should be dependent on the DART temperature and the boiling points of the analyte and matrix.

CONCLUSION

The ionization mechanism of direct analysis in real time (DART) previously proposed by Cody et al.[6] has been expanded in this study by specifically addressing the matrix effect with a Transient Microenvironment Mechanism (TMEM). The TMEM is supported by a scheme that consists of nine gas-phase ion/molecule reactions. Simulated samples of liquids, solids, and solutions were analyzed and the mass spectra were interpreted. The relevant transient microenvironments (TMEs) generated from most of the common solvents in four groups (i.e., proton acceptors, benzene derivatives, alkanes, and chlorinated methanes) were studied in detail. Methanol, toluene, hexanes, and chloroform were determined to be the best representatives and provide complementary data. It is important to clarify that tetrahydrofuran (THF), ethyl acetate, dimethyl formamide (DMF), and dimethyl sulfonate (DMSO) are unfavorable solvents for DART ionization. More complicated DART TMEs and ionization mechanisms can be expected when a sample contains multiple matrix components; however, the ionization mechanisms should still be interpretable through the TMEM. Because DART is the premiere APCI-related OADI method, this study may provide useful fundamentals on the ionization mechanism of other APCI-related OADI methods, especially when solvent is involved in the ionization.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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