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# Negative Ion-Atmospheric Pressure Photoionization: Electron Capture, Dissociative Electron Capture, Proton Transfer, and Anion Attachment

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To better guide the development of liquid chromatography/electron capture-atmospheric pressure photoionization-mass spectrometry (LC/EC-APPI-MS) in analysis of low polarity compounds, the ionization mechanism of 19 compounds was studied using dopant assisted negative ion-APPI. Four ionization mechanisms, i.e., EC, dissociative EC, proton transfer, and anion attachment, were identified as being responsible for the ionization of the studied compounds. The mechanisms were found to sometimes compete with each other, resulting in multiple ionization products from the same molecule. However, dissociative EC and proton transfer could also combine to generate the same  $[M - H]^-$  ions. Experimental evidence suggests that  $O_2^-$ , which was directly observed in the APPI source, plays a key role in the formation of  $[M - H]^-$  ions by way of proton transfer. Introduction of anions more basic than  $O_2^-$ , i.e.,  $C_6H_5CH_2^-$ , into the APPI source, via addition of di-*tert*-butyl peroxide in the solvent and/or dopant, i.e., toluene, enhanced the deprotonation ability of negative ion-APPI. Although the use of halogenated solvents could hinder efficient EC, dissociative EC, and proton transfer of negative ion-APPI due to their EC ability, the subsequently generated halide anions promoted halide attachment to compounds that otherwise could not be efficiently ionized. With the four available ionization mechanisms, it becomes obvious that negative ion-APPI is capable of ionizing a wider range of compounds than negative ion chemical ionization (NICI), negative ion-atmospheric pressure chemical ionization (negative ion-APCI) or negative ion-electrospray ionization (negative ion-ESI). (J Am Soc Mass Spectrom 2007, 18, 1789–1798) © 2007 American Society for Mass Spectrometry

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It is well known that liquid chromatography mass spectrometry (LC/MS) favors the analysis of polar compounds, as acid-base solution reactions are the most commonly observed ionization mechanism when using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Traditionally, gas chromatography mass spectrometry (GC/MS) using both electron ionization (EI) and chemical ionization (CI) is employed for the analysis of volatile and thermo-stable nonpolar compounds. However, it can become difficult to analyze nonvolatile and/or thermo-labile nonpolar compounds via hyphenated techniques of chromatography and MS.

In the year 2000, atmospheric pressure photoionization (APPI) [1] was developed as a complement to LC/ESI-MS and LC/APCI-MS. Presently, two fundamentally different APPI sources are commercially available [1, 2]. Syngen Technology (Tustin, CA) produces PhotoMate, and Applied Biosystems/MDS SCIEX (Concord, Ontario, Canada) markets PhotoSpray. In

both designs, photons emitted by a krypton discharge lamp are used to initiate a series of gas-phase reactions, which finally lead to analyte ionization. However, they differ in that PhotoMate utilizes a design to enhance direct APPI, while PhotoSpray implements a design using a dopant, usually toluene, to enhance ion-molecule reactions. Due to a different ionization process as compared to ESI and APCI, LC/APPI-MS is anticipated to expand the polarity range of analytes amenable to LC/MS to include low polarity compounds [3, 4]. Unfortunately, there has been little progress reported thus far [5].

Low polarity compounds with positive electron affinities (EA) can be ionized via an electron capture (EC) mechanism. Traditionally, gas chromatography electron capture-negative ion chemical ionization-mass spectrometry (GC/EC-NICI-MS) has been one of the most sensitive and specific analytical methods, and has been widely used in the analysis of natural electrophores and other compounds after electrophoric derivatization [6]. Recently, LC/EC-APCI-MS methodology [7] was developed and used to analyze some low polarity compounds. In our most recent study [8], an EC mechanism was found to be highly efficient in the

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analysis of fullerenes and perfluorinated compounds when dopant-assisted negative ion-APPI was employed. In addition, a dissociative EC mechanism was also found to be highly efficient in the analysis of pentafluorobenzyl derivatives. These results suggest that LC/EC-APPI-MS may permit the analysis of non-volatile, low polarity compounds with the sensitivity and selectivity that GC/EC-NICI-MS delivers for volatile nonpolar compounds. However, unlike in a NICI source, a series of gas-phase reactions are expected in an APPI source, especially when using a dopant. These reactions may compete with the EC and/or dissociative EC ionization and result in alterations of the expected sensitivity and selectivity. The aim of this work is to study, in detail, the relevant reactions so that LC/EC-APPI-MS development can be better guided toward analysis of low polarity compounds in the future.

The basic principle of APPI is the formation of a molecular radical cation caused by the absorption of a photon by a molecule, and is accompanied by the formation of a thermal electron [9]. In dopant assisted negative ion-APPI, the ionization process is initiated by thermal electrons. Currently, subsequent ionization mechanisms including EC, dissociative EC, and proton transfer are suggested to be responsible for the formation of negative ions [8, 10, 11]. In this study, nitroaromatics were preliminarily chosen as analytes because previous studies demonstrated multiple ionization products by both EC-NICI [12, 13] and EC-APCI [14–19]. Toluene was exclusively used as both dopant and solvent so that chemical reactions in the APPI source could be kept at a minimum and the formation of ionization products could be better interpreted. Other aromatics with similar structures to the nitroaromatics used were also selected and studied to better understand the proton transfer mechanism of negative ion-APPI. Anion attachment was identified as a novel ionization mechanism for negative ion-APPI. It offers the ability to ionize compounds that cannot be efficiently ionized by EC, dissociative EC, and proton transfer. The effect of solvents and buffer reagents on ionization was not the focus of this study, as it is predictable according to the ionization mechanisms and has been studied extensively in previous work [8, 11].

## Experimental

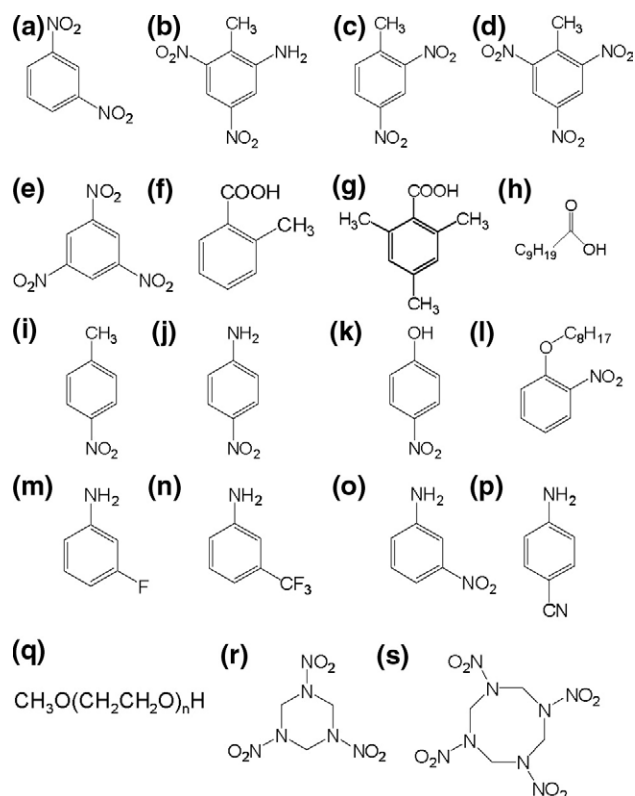
### Reagents

Toluene was HPLC grade and purchased from Fisher Scientific (Suwanee, GA). Methylene chloride, bromoethane, iodoethane, 2,4,6-trimethylbenzoic acid (2,4,6-TMBA), decanoic acid, 4-nitroaniline, 4-nitrophenol, 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and poly(ethylene glycol)methyl ether with average M.W. 750 (PEG methyl ether 750) were purchased from Aldrich Chemical (St. Louis, MO). 1,3-Dinitrobenzene (1,3-DNB), 2-amino-4,6-dinitrotoluene (2-amino-4,6-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,4,6-trinitrotoluene (TNT),

1,3,5-trinitrobenzene(1,3,5-TNB), RDX, and HMX were purchased from AccuStandard (New Haven, CT). 2-Methylbenzoic acid (2-MBA) was purchased from TCI (Wellesley Hills, MA). 4-Nitrotoluene was purchased from Eastman Kodak (Rochester, NY). 2-Nitrophenyl octyl ether, 4-cyanoaniline, and di-*tert*-butyl peroxide were purchased from Fluka (St. Louis, MO). The structures of the studied compounds are shown in Figure 1. Their thermodynamic data is given in Table 1. The thermodynamic data of oxygen and nitrogen dioxide from the atmosphere is also given in Table 1. The existence of oxygen in an APPI source plays a key role in negative ion-APPI [11].

### Apparatus

All experiments were performed using a MDS SCIEX QSTAR XL triple quadrupole time-of-flight (QTOF) mass spectrometer (Concord, Ontario, Canada) using an APPI source with a 10 eV krypton discharge lamp. High purity nitrogen was used for nebulizer, auxiliary, curtain, and lamp gas. A micro syringe pump (Harvard Apparatus, Holliston, MA) was used to deliver the analyte solution at a flow rate of 20  $\mu$ L/min. HPLC



**Figure 1.** Structures of studied compounds: (a) 1,3-dinitrobenzene; (b) 2-amino-4,6-dinitrotoluene; (c) 2,4-dinitrotoluene; (d) 2,4,6-trinitrotoluene; (e) 1,3,5-trinitrobenzene; (f) 2-methylbenzoic acid; (g) 2,4,6-trimethylbenzoic acid; (h) decanoic acid; (i) 4-nitrotoluene; (j) 4-nitroaniline; (k) 4-nitrophenol; (l) 2-nitrophenyl octyl ether; (m) 3-fluoroaniline; (n) 3-trifluoromethylaniline; (o) 3-nitroaniline; (p) 4-cyanoaniline; (q) poly(ethylene glycol) methyl ether with average M.W. 750; (r) RDX; (s) HMX.

**Table 1.** Thermodynamic data for studied compounds<sup>a</sup>

Name	MW	M – H	EA (eV)	$\Delta H_{\text{acid}}$ (kcal/mol)
Oxygen	31.9898	/	0.45 <sup>b</sup>	/
Nitrogen dioxide	45.9929	/	2.27 <sup>c</sup>	/
1,3-Dinitrobenzene	168.0171	167.0093	1.66 <sup>b</sup>	~356 <sup>c</sup>
2-Amino-4,6-dinitrotoluene	197.0437	196.0358	~1.6 <sup>c</sup>	~329 <sup>c</sup>
2,4-Dinitrotoluene	182.0328	181.0249	~1.6 <sup>c</sup>	~328 <sup>c</sup>
2,4,6-Trinitrotoluene	227.0178	226.0100	~2.5 <sup>c</sup>	315.6 <sup>b</sup>
1,3,5-Trinitrobenzene	213.0022	211.9944	~2.6 <sup>c</sup>	~340 <sup>c</sup>
2-Methylbenzoic acid	136.0524	135.0446	~0 <sup>c</sup>	339.2 <sup>b</sup>
2,4,6-Trimethylbenzoic acid	164.0837	163.0759	~0 <sup>c</sup>	339.0 <sup>b</sup>
Decanoic Acid	172.1763	171.1385	<0 <sup>c</sup>	338.8 <sup>b</sup>
4-Nitrotoluene	137.0477	136.0399	0.95 <sup>b</sup>	352.5 <sup>b</sup>
4-Nitroaniline	138.0429	137.0351	0.92 <sup>b</sup>	343.5 <sup>b</sup>
4-Nitrophenol	139.0269	138.0191	~0.9 <sup>c</sup>	327.8 <sup>b</sup>
2-Nitrophenyl octyl ether	251.1521	250.1443	~1.0 <sup>c</sup>	~377 <sup>c</sup>
3-Fluoroaniline	111.0484	110.0406	<0 <sup>c</sup>	361.2 <sup>b</sup>
3-Trifluoromethylaniline	161.0452	160.0374	~0.0 <sup>c</sup>	356.9 <sup>b</sup>
3-Nitroaniline	138.0429	137.0351	0.95 <sup>b</sup>	352.3 <sup>b</sup>
4-Cyanoaniline	118.0531	117.0453	~0.2 <sup>c</sup>	348.8 <sup>b</sup>
CH <sub>3</sub> O(CH <sub>2</sub> CH <sub>2</sub> O) <sub>11</sub> H	516.3146	/	<0 <sup>c</sup>	~373 <sup>c</sup>
RDX	222.0349	/	<0 <sup>c</sup>	>400 <sup>c</sup>
HMX	296.0465	/	<0 <sup>c</sup>	>400 <sup>c</sup>

<sup>a</sup>EA, electron affinity;  $\Delta H_{\text{acid}}$ , gas-phase acidity.

<sup>b</sup>Data obtained from reference [27].

<sup>c</sup>Data estimated using experimental data for structural analogs, with data from reference [27]. Uncertainties are estimated as being  $\pm 5$  kcal/mol for acidities, and  $\pm 0.2$  eV for electron affinities.

grade toluene was used as the analyte solvent, and simultaneously served as the dopant. Therefore, the dopant delivery port of the APPI source was sealed. However, some auxiliary gas flow was necessary to achieve the best sensitivity. The general controlling parameters and settings for negative ion-APPI MS using TOFMS scan were GS1 (gas 1 or nebulizer gas), 30; GS2 (gas 2 or auxiliary gas), 30; CUR (curtain gas), 40; IS (ion spray voltage),  $-1500$  V; TEM (temperature),  $250$  °C; DP (decluster potential),  $-30$  V; FP (focusing potential),  $-80$  V; DP2 (decluster potential 2),  $-12$  V; and CAD (collision gas), 3. The general controlling parameters for negative ion-APPI MS/MS using product ion scan were identical to the TOFMS scan with an extra parameter, collision energy (CE). The acquisition time for one spectrum was 1 s. Although spectra of analyte mixtures are used for a concise presentation in this article, spectra of individual analytes were acquired with varying in-source fragmentation parameters, i.e., DP and TEM, so that the formation of ionization products could be correctly interpreted. In addition, the QTOF mass spectrometer offered accurate mass measurement such that we were able to investigate the elemental composition of interesting peaks in each spectrum.

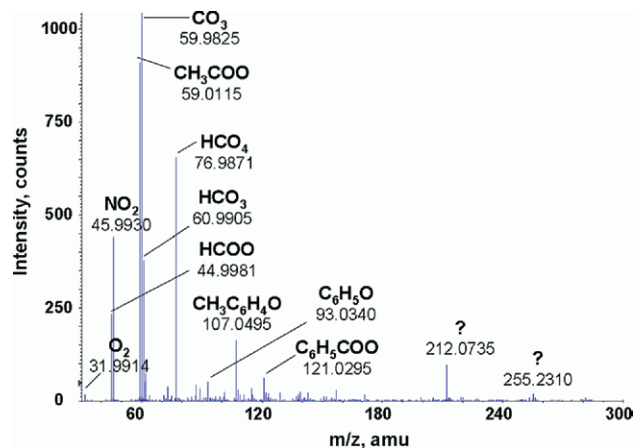
## Results and Discussion

### Background Ions

A background spectrum of toluene using negative ion-APPI is demonstrated in Figure 2. Making use of the accurate mass measurement offered by the QTOF mass

spectrometer, we were able to investigate the elemental composition of background ions. The probable structure of most background ions and the gas-phase acidity of their corresponding acids are listed in Table 2.

Previously, Kauppila et al. [11] reported the  $m/z$  46, 59, 60, 61, and 77 background anions from toluene using a triple quadrupole mass spectrometer. They interpreted the  $m/z$  46, 60, and 61 anions to be  $\text{NO}_2^-$ ,  $\text{CO}_3^-$ , and  $\text{HCO}_3^-$ . Furthermore, although  $\text{O}_2^-$  was not observed directly in a spectrum, its existence was assumed by the interpretation of ionization products of some analytes. The existence of  $\text{O}_2$  and  $\text{NO}_2$  anions was easily inferred by the ionization of atmospheric gases



**Figure 2.** Background spectrum of toluene using negative ion-APPI.

**Table 2.** Probable structure and thermodynamic data for background ions<sup>a</sup>

Background ions ( <i>m/z</i> )	Probable structure	Theoretical <i>m/z</i>	$\Delta H_{\text{acid}}$ (kcal/mol)
31.9914	O <sub>2</sub> <sup>-</sup>	31.9898	353.0 <sup>b</sup>
44.9981	HCOO <sup>-</sup>	44.9977	346.2 <sup>b</sup>
45.9930	NO <sub>2</sub> <sup>-</sup>	45.9929	340 <sup>b</sup>
59.0115	CH <sub>3</sub> COO <sup>-</sup>	59.0133	348.6 <sup>b</sup>
59.9825	CO <sub>3</sub> <sup>-</sup>	59.9847	~324 <sup>c</sup>
60.9905	HCO <sub>3</sub> <sup>-</sup>	60.9926	334.6 <sup>b</sup>
76.9871	O <sub>2</sub> +HCOO <sup>-</sup>	76.9875	/
93.0340	C <sub>6</sub> H <sub>5</sub> O <sup>-</sup>	93.0340	349.2 <sup>b</sup>
107.0495	CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> O <sup>-</sup>	107.0497	~350.3 <sup>c</sup>
121.0295	C <sub>6</sub> H <sub>5</sub> COO <sup>-</sup>	121.0290	340.1 <sup>b</sup>
57.0334 <sup>d</sup>	CH <sub>3</sub> COCH <sub>2</sub> <sup>-</sup>	57.0340	369.0 <sup>b</sup>
91.0530 <sup>d</sup>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> <sup>-</sup>	91.0548	382.4 <sup>b</sup>

<sup>a</sup> $\Delta H_{\text{acid}}$ , gas-phase acidity for the corresponding acids of background ions.

<sup>b</sup>Data obtained from reference [27].

<sup>c</sup>Data estimated using experimental data for structural analogs, with data from reference [27]. Uncertainties are estimated as being  $\pm 5$  kcal/mol for acidities, and  $\pm 0.2$  eV for electron affinities.

<sup>d</sup>Both anions only existed when 1% (vol/vol) di-tert-butyl peroxide was used as an additive in toluene.

through an EC mechanism. Both O<sub>2</sub> and NO<sub>2</sub> possess positive EAs as shown in Table 1.

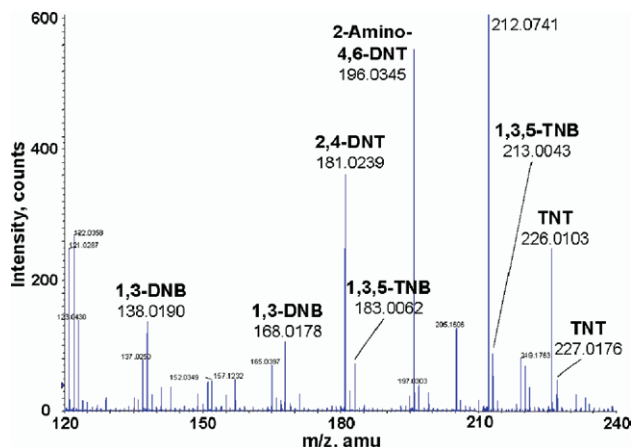
Although the mass spectrometer used in this study discriminates against low *m/z* ions, the existence of O<sub>2</sub><sup>-</sup> was still directly observed in a spectrum as shown in Figure 2. This is very important because O<sub>2</sub><sup>-</sup> possesses the strongest gas-phase basicity of all background ions, as shown in Table 1, when toluene was exclusively used as both the solvent and dopant. The existence of other nonatmospheric gas ions in Figure 1 can possibly be interpreted through gas-phase reactions as previously reported by Traldi and coworkers [20]. However, we were unable to assign the *m/z* 212.0735 and 255.2310 ions probable chemical structures. The closest match to the *m/z* 212.0735 ion has an elemental composition of C<sub>13</sub>H<sub>10</sub>NO<sub>2</sub> with 11 ppm mass error. A product ion scan of the *m/z* 212.0735 ion generated more than eight fragment ions (data not shown). There is no elemental composition containing exclusively C, H, O, and N for identification of the *m/z* 255.2310 ion with less than 250 ppm mass error. A product ion scan of the *m/z* 255.2310 ion generated only one small product ion by loss of a water molecule.

### EC and Dissociative EC

Five nitro-aromatic compounds, including 1,3-DNB, 2-amino-4,6-DNT, 2,4-DNT, TNT, and 1,3,5-TNB, were analyzed using negative ion-APPI. The negative ion-APPI spectrum of a mixture of these five compounds produced from the consumption of 3.3 pg (pg) of each analyte is illustrated in Figure 3. It can be seen that the ionization of 1,3-DNB and 1,3,5-TNB generated exclusively two types of ions, M<sup>-</sup> and [M - NO]<sup>-</sup>. It is noted that in Figure 3 the M<sup>-</sup> ion of 1,3,5-TNB is well

separated from the isotopic peak of the background ion with *m/z* 212.0741.

The formation of M<sup>-</sup> ions can be easily attributed to an EC mechanism since 1,3-DNB and 1,3,5-TNB possess positive EAs as shown in Table 1. However, the formation of [M - NO]<sup>-</sup> ions can be interpreted as the result of either a dissociative EC mechanism or in-source fragmentation of the M<sup>-</sup> ions. If the latter was responsible, alteration of the in-source fragmentation parameters, such as source temperature and DP value, would result in an increase of the [M - NO]<sup>-</sup> ion intensity and a simultaneous decrease of the M<sup>-</sup> ion intensity. By altering the source temperature (using values of 150, 250, 350, and 450 °C), the relative ion intensity between an M<sup>-</sup> ion and its corresponding [M - NO]<sup>-</sup> ion did not change significantly. In contrast, altering the DP value (using values of 20, 30, 40, 50, and 70 V) changed the relative ion intensity between an [M - NO]<sup>-</sup> ion and its corresponding M<sup>-</sup> ion from 1.0, 1.3, 2.1, 4.3 to 22.8 for 1,3-DNB, and 0.5, 0.6, 1.0, 1.9 to 17.2 for 1,3-DNB, respectively. We, therefore, concluded that formation of the [M - NO]<sup>-</sup> ions were due to in-source fragmentation. This was further proven by collisionally induced dissociation (CID) of the M<sup>-</sup> ions. Even when the collision energy (CE) was as low as 5 V, the intensity of the [M - NO]<sup>-</sup> ions for 1,3-DNB and 1,3,5-TNB reached 2% and 1% that of the M<sup>-</sup> ions, respectively. The intensity of the [M - NO]<sup>-</sup> ions for 1,3-DNB and 1,3,5-TNB reached a maximum when CE was increased to be 12 and 15 V, respectively.

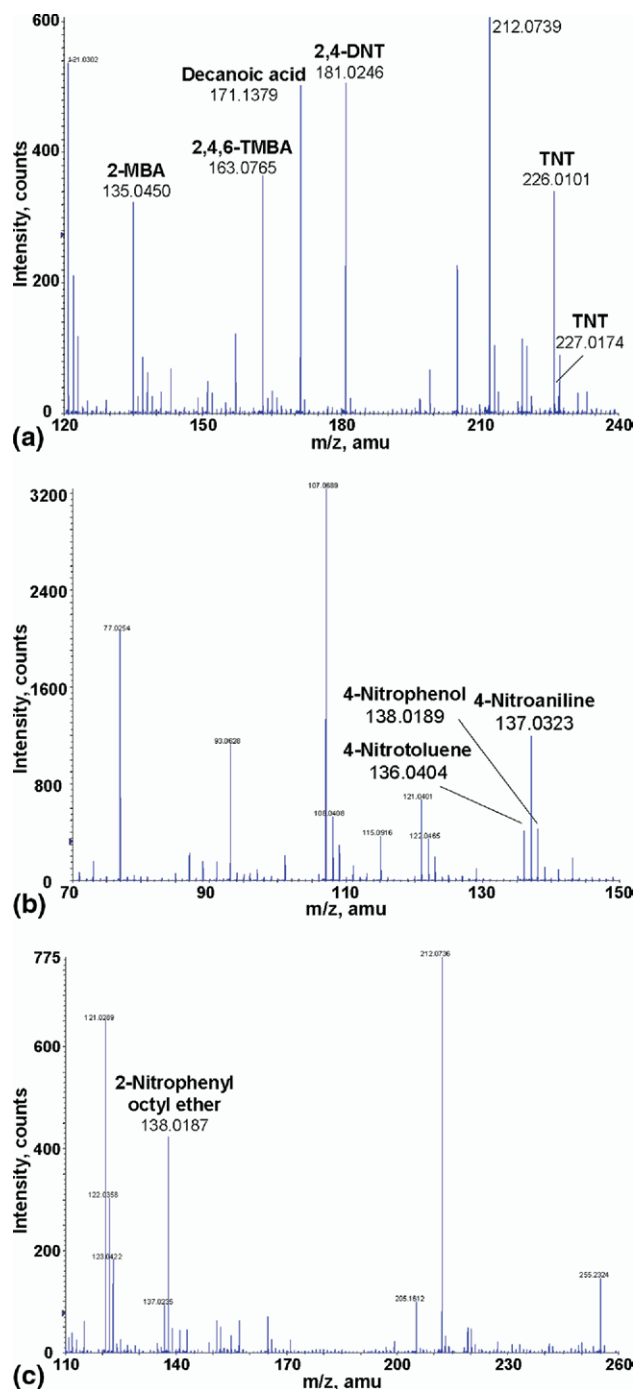


**Figure 3.** Negative ion-APPI spectrum of a mixture of 1,3-DNB, 2-amino-4,6-DNT, 2,4-DNT, TNT, and 1,3,5-TNB produced from the consumption of 3.3 pg of each analyte (concentration 0.01 ng/ $\mu$ L for each analyte, flow rate 20  $\mu$ L/min, acquisition time 1 s). Mass spectrometric conditions are the same as described in the Experimental section, with the exception of source temperature maintained at 300 °C. Ions of *m/z* 138.0190 and 168.0178 are interpreted as [M - NO]<sup>-</sup> and M<sup>-</sup> ions, respectively, from 1,3-DNB. Ions of *m/z* 183.0062 and 213.0043 are interpreted as [M - NO]<sup>-</sup> and M<sup>-</sup> ions, respectively, from 1,3,5-TNB. Ions of *m/z* 181.0239, 196.0345 and 226.0103 are interpreted as [M - H]<sup>-</sup> ions from 2,4-DNT, 2-amino-4,6-DNT, and TNT, respectively. Ion of *m/z* 227.0176 is interpreted as M<sup>-</sup> ion from TNT.

Previously, Holmgren et al. [19] reported an EC-APCI spectrum of 1,3-DNB with the following ions and relative ion intensity: 168 (46%), 138 (100%), and 108 (4%). Holmgren et al. [19] also reported an EC-APCI spectrum of 1,3,5-DNB with the following ions and relative ion intensity: 213 (86%), 183 (100%), 167 (2%), and 125 (4%).

### EC and Proton Transfer

It can be seen from Figure 3 that the ionization of 2,4-DNT, TNT, and 2-amino-4,6-DNT generated mostly



[M – H]<sup>−</sup> ions. Of the three analytes, the molecular ion, i.e., M<sup>−</sup>, of only TNT was observed, with an intensity of ~10% that of its respective [M – H]<sup>−</sup> ion. Figure 3 also shows no obvious [M – NO]<sup>−</sup> ion for 2-amino-4,6-DNT. It is noted, however, that the ion at *m/z* 151 in Figure 3 is a background ion rather than a [M – NO]<sup>−</sup> ion from 2,4-DNT. Figure 4a shows no obvious [M – NO]<sup>−</sup> ion for TNT, either.

Again, the formation of M<sup>−</sup> ion from TNT can be easily attributed to an EC mechanism because TNT possesses a positive EA, as shown in Table 1. The formation of [M – H]<sup>−</sup> ions should involve proton transfer between the analytes and the most basic background ion, i.e., O<sub>2</sub><sup>−</sup>. It can be seen from Table 2 that O<sub>2</sub><sup>−</sup> is a relatively strong gas-phase base because the gas-phase acidity of HO<sub>2</sub> is equal to 353.0 kcal/mol. Proton transfer is possible for the analytes possessing gas-phase acidity stronger than 353.0 kcal/mol, which is true for 2,4-DNT, TNT, and 2-amino-4,6-DNT, as shown in Table 1. Although 1,3,5-TNB also possesses gas-phase acidity stronger than 353.0 kcal/mol, steric hindrance may have prevented the loss of a proton.

Previous studies using EC-NICI or EC-APCI for the ionization of TNT typically generated complicated spectra. For example, Sigman et al. [12] reported an EC-NICI spectrum of TNT using methane as reagent gas with the following ions and relative ion intensity: *m/z* 229 (2%), 228 (10%), *m/z* 227 (100%), 210 (16%), 198 (3%), 197 (22%), 195 (2%), and 167 (3%). Fu et al. [18] reported an EC-APCI spectrum of TNT with the following ions and relative ion intensity: *m/z* 245 (12.7%), 227 (100%), and 197 (59.1%). Holmgren et al. [19] reported an EC-APCI spectrum of TNT with the following ions and relative ion intensity: *m/z* 227 (50%), 226 (50%), 210 (100%), 197 (69%), 180 (5%), 167 (15%), 152 (13%), 137 (9%), 124 (3%), and 109 (3%). Negative ion-APPI offers an obvious advantage over EC-NICI and EC-APCI with fewer ionization products.

**Figure 4.** (a) Negative ion-APPI spectrum of a mixture of 2-MBA, 2,4,6-TMBA, decanoic acid, 2,4-DNT, and TNT produced from the consumption of 13.3 pg each of 2-MBA, 2,4,6-TMBA, decanoic acid, plus 6.7 pg each of 2,4-DNT and TNT (concentration 0.02 or 0.01 ng/μL for each analyte, flow rate 20 μL/min, acquisition time 1 s); (b) negative ion-APPI spectrum of a mixture of 4-nitrotoluene, 4-nitroaniline, and 4-nitrophenol produced from the consumption of 330 pg 4-nitrotoluene, 3.3 pg 4-nitroaniline, and 0.67 pg 4-nitrophenol (concentration 1, 0.01, and 0.002 ng/μL for each analyte, flow rate 20 μL/min, acquisition time 1 s); (c) negative ion-APPI spectrum of 2-nitrophenyl octyl ether produced from the consumption of 16.7 pg analyte (concentration 0.05 ng/μL, flow rate 20 μL/min, acquisition time 1 s). Mass spectrometric conditions are the same as described in the Experimental section. Ions of *m/z* 135.0450, 163.0765, 171.1376, 181.0239, and 226.0101 in (a) are interpreted as [M – H]<sup>−</sup> ions from 2-MBA, 2,4,6-TMBA, decanoic acid, 2,4-DNT, and TNT, respectively. Ion of *m/z* 227.0174 in (a) is interpreted as M<sup>−</sup> ion from TNT. Ions of *m/z* 136.0404, 137.0323, and 138.0189 in (b) are interpreted as [M – H]<sup>−</sup> ions from 4-nitrotoluene, 4-nitroaniline, and 4-nitrophenol, respectively. Ion of *m/z* 138.0187 in (c) is interpreted as 2-nitrophenol anion from 2-nitrophenyl octyl ether.

The ionization efficiency of negative ion-APPI for TNT is also very high. The limit of detection (LOD), defined by detection of its  $[M - H]^-$  ion at a signal to noise ratio (S/N) of 3, is about 90 fg or 0.4 fmol. However, no direct comparison of negative ion-APPI with EC-NICI, EC-APCI or negative ion-ESI was performed in this study. In previously published studies, Sigman et al. [12] reported a LOD of 190 pg using GC/EC-NICI-MS and Holmgren et al. [19] reported a LOD of 4.0 ng using LC/EC-APCI-MS.

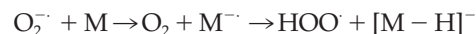
### Dissociative EC and Proton Transfer

In Figure 3, the formation of  $[M - H]^-$  ions from 2,4-DNT, TNT, and 2-amino-4,6-DNT may not only involve proton transfer as discussed above, but also possibly a dissociative EC mechanism. Dissociative EC with thermal electrons via a hydrogen atom loss to form the  $[M - H]^-$  ion should only be thermochemically accessible for acids stronger than  $\Delta H_{\text{acid}} = 313.6$  kcal/mol, the ionization energy of the hydrogen atom. Only TNT qualifies for this. However, excess energy in the free electrons in the source could possibly promote it for weaker acids. Three experiments were performed to investigate this possibility.

Figure 4a illustrates the analysis of 2-MBA, 2,4,6-TMBA, decanoic acid, 2,4-DNT, and TNT using negative ion-APPI. In contrast, with 2,4-DNT and TNT, the carboxylic acids 2-MBA, 2,4,6-TMBA, and decanoic acid possess zero or negative EAs, and are still acidic enough to be deprotonated by  $O_2^-$ , but not enough to qualify for dissociative EC. Therefore, only a proton transfer ionization mechanism should be responsible for the formation of their  $[M - H]^-$  ions. After normalization to the same amount of material, the higher ion intensity of  $[M - H]^-$  ions produced by 2,4-DNT and TNT may be a result of their positive EAs, and, therefore, a dissociative EC mechanism.

Figure 4b depicts the analysis of 4-nitrotoluene, 4-nitroaniline, and 4-nitrophenol using negative ion-APPI. 4-Nitrotoluene is comparable to  $O_2^-$  for proton transfer, so is expected to be the least sensitive to ionization of the three analytes. 4-Nitroaniline has a similar gas-phase acidity to 2-MBA, 2,4,6-TMBA, and decanoic acid, but its ion intensity is more than an order of magnitude higher after normalization to the same amount of material upon comparison of Figure 4a and b. In addition, it can be seen from Figure 4b that when the functional groups of the nitroaromatics are changed from methyl-, to amino-, and finally to hydroxyl-, the intensity of  $[M - H]^-$  ions was greatly enhanced after normalization to the same amount of material. All three of these nitroaromatics, though, are far too weak as acids for dissociative EC with thermal electrons to be possible. There must be some reason that analytes with positive EAs yield much stronger  $[M - H]^-$  signals than analytes of comparable acidity but with zero or negative EAs. A possibility is a mechanism involving an

alternate intermediate to that for simple proton transfer, but which yields the same product ions:



This involves electron-transfer, followed by hydrogen atom transfer. Such a single electron transfer (SET) mechanism is known, but not common, in solution [21]. The first step would be exothermic for analytes with EAs more positive than that of  $O_2$  at 0.45 eV, thus providing a driving force, and possibly a rate constant favored over simple proton transfer. The existence of other ionization products from 4-nitrotoluene, 4-nitroaniline, and 4-nitrophenol in Figure 5b was also examined individually and appeared to be insignificant after background subtraction.

Figure 4c demonstrates the analysis of 2-nitrophenyl octyl ether using negative ion-APPI. A 2-nitrophenolate anion was exclusively observed. In this case a dissociative EC mechanism would yield a primary carbon-centered radical, plus the ion indicated. Such a mechanism should be ca.  $-10$  kcal/mol favored in this case, based on thermochemistry in reference [26]. An alternate mechanism, involving  $O_2^-$  acting as a base in an E2 elimination reaction to give 1-octene plus the phenoxide, cannot be ruled out. Previously, a dissociative EC mechanism was exclusively observed in the analysis of pentafluorobenzyl derivatized phenols [8]. Therefore, a dissociative EC mechanism may also be expected for other phenyl ethers with sufficiently positive EAs.

### Manipulating Proton Transfer

Although a previous study by Kauppila et al. [11] implied the key role of  $O_2^-$  in negative ion-APPI with regard to the

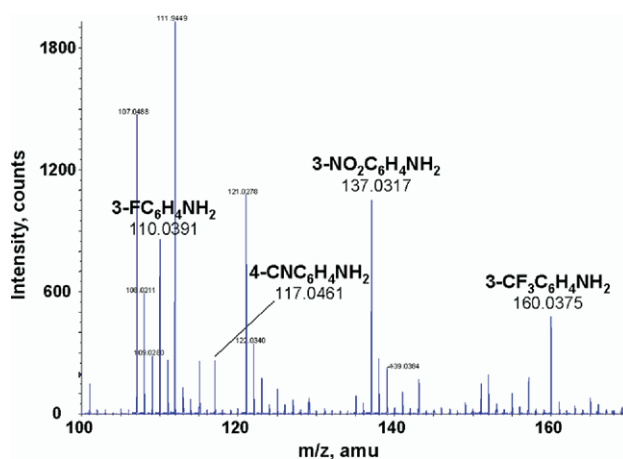


Figure 5. Negative ion-APPI spectrum of a mixture of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline produced from the consumption of 13.3 ng, 1.33 ng, 133 pg, and 13.3 pg of each respective analyte (concentration 40, 4, 0.4, and 0.04 ng/ $\mu$ L for each analyte, flow rate 20  $\mu$ L/min, acquisition time 1 s). Ions of  $m/z$  110.0391, 117.0461, 137.0317, and 160.0375 are interpreted as  $[M - H]^-$  ions from 3-fluoroaniline, 4-cyanoaniline, 3-nitroaniline and 3-trifluoromethylaniline, respectively.

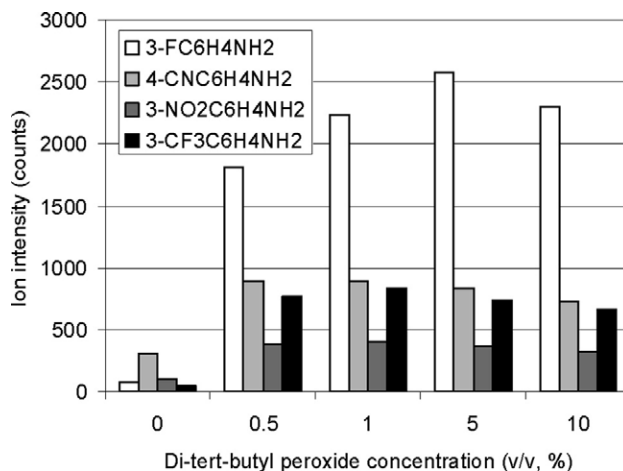
proton transfer mechanism, no direct experimental evidence was provided. Here, we selected four aromatics (3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline) to fulfill this task. The gas-phase acidities of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline are 361.2, 356.9, 352.3, and 348.8 kcal/mol, respectively, as shown in Table 1. Since the gas-phase acidity of HO<sub>2</sub> is 353.0 kcal/mol, [M – H]<sup>–</sup> ions of 3-fluoroaniline and 3-trifluoromethylaniline should not theoretically be expected on reaction with O<sub>2</sub><sup>–</sup>; if only exothermic reactions are observed. Figure 5 displays a negative ion-APPI spectrum of a mixture of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline produced from the consumption of 13.3 ng, 1.33 ng, 133 pg, and 13.3 pg of each respective analyte. After being normalized to the same amount of material, a significant decrease in ion intensity was revealed when the gas-phase acidity of the analytes changed from 348.8, 352.3, 356.9 to 361.2 kcal/mol, which is in good agreement with the above theoretical prediction.

The existence of M<sup>–</sup> ions in Figure 5 was also examined after background subtraction since many low intensity background ions were observed in the studied mass range (data not shown). Apparently no significant M<sup>–</sup> ions were detected.

Because even 1 ppm of oxygen as an impurity in the nebulizer and/or auxiliary gas is enough to affect negative ion-APPI, it is almost impossible to totally eliminate O<sub>2</sub><sup>–</sup> from the APPI source. However, manipulation of the proton transfer reactions in an APPI source can still be achieved by introduction of anions with stronger gas-phase basicity than O<sub>2</sub><sup>–</sup>. Di-*tert*-butyl peroxide may be able to provide such an anion, i.e., (CH<sub>3</sub>)<sub>3</sub>CO<sup>–</sup> (the gas-phase acidity of its corresponding acid is 374.6 kcal/mol [27]), as the peroxide is able to undergo a dissociative EC reaction [22]. Therefore, the analysis of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline by negative ion-APPI was also performed with HPLC grade toluene as the solvent and 1% (vol/vol) di-*tert*-butyl peroxide as an additive. The ion intensity of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline increased ~29, 18, 4, and 3 times, respectively, after being normalized to the same amount of material.

The effect of di-*tert*-butyl peroxide concentration on negative ion-APPI efficiency was also examined and demonstrated in Figure 6. When the concentration was decreased from 1% to 0.5% and 0.1% (vol/vol), the analyte ion intensity was also decreased. However, when the concentration of di-*tert*-butyl peroxide was increased from 1% to 5% and 10% (vol/vol), significant enhancement of the ion intensity of the analytes was not observed.

The background spectrum of toluene with 1% (vol/vol) di-*tert*-butyl peroxide by negative ion-APPI was examined and compared with Figure 1. Surprisingly, there was no obvious increase in the ion intensity of *m/z* 73 ion, which existed in Figure 1 with low intensity.



**Figure 6.** Effect of di-*tert*-butyl peroxide concentration on ionization efficiency of 3-fluoroaniline, 3-trifluoromethylaniline, 3-nitroaniline, and 4-cyanoaniline (normalized respective consumption of each analyte: 1.33 ng, 133 pg, 13.3 pg, and 13.3 pg).

Therefore, there is no evidence corresponding to the existence of (CH<sub>3</sub>)<sub>3</sub>CO<sup>–</sup> anion, as expected. However, an obvious additional *m/z* 91.0530 ion with an ion intensity of 374 counts was observed, whose closest elemental match is C<sub>7</sub>H<sub>7</sub> with 19.5 ppm mass error. This anion can be interpreted as deprotonated toluene, i.e., C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub><sup>–</sup>. Toluene has a gas-phase acidity of 382.4 kcal/mol. Therefore, the existence of C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub><sup>–</sup> may be responsible for the enhanced deprotonation ability of negative-ion APPI. Since the gas-phase acidity of toluene is 7.8 kcal/mol weaker than that of (CH<sub>3</sub>)<sub>3</sub>COH, its dominant concentration in the APPI source may be responsible for its deprotonation. As shown in Figure 5, 3-fluoroaniline can still be deprotonated at a concentration of 40 ng/μL, even though its gas-phase acidity is 9.1 kcal/mol weaker than that of HO<sub>2</sub>. In addition to the *m/z* 91.0530 ion, another obvious additional background ion was *m/z* 57.0334. Though its intensity was only 63 counts, it had increased from ~1 count in Figure 1. The closest elemental match for this anion is C<sub>3</sub>H<sub>5</sub>O, with 11.2 ppm mass error. This anion can be interpreted as deprotonated acetone, i.e., CH<sub>3</sub>COCH<sub>2</sub><sup>–</sup>. Acetone can be a decomposition product of di-*tert*-butyl peroxide under moderate temperature. Its gas-phase acidity is 369.0 kcal/mol, 5.6 kcal/mol stronger than that of (CH<sub>3</sub>)<sub>3</sub>COH. The existence of CH<sub>3</sub>COCH<sub>2</sub><sup>–</sup> in the APPI source is, therefore, understandable.

### Anion Attachment

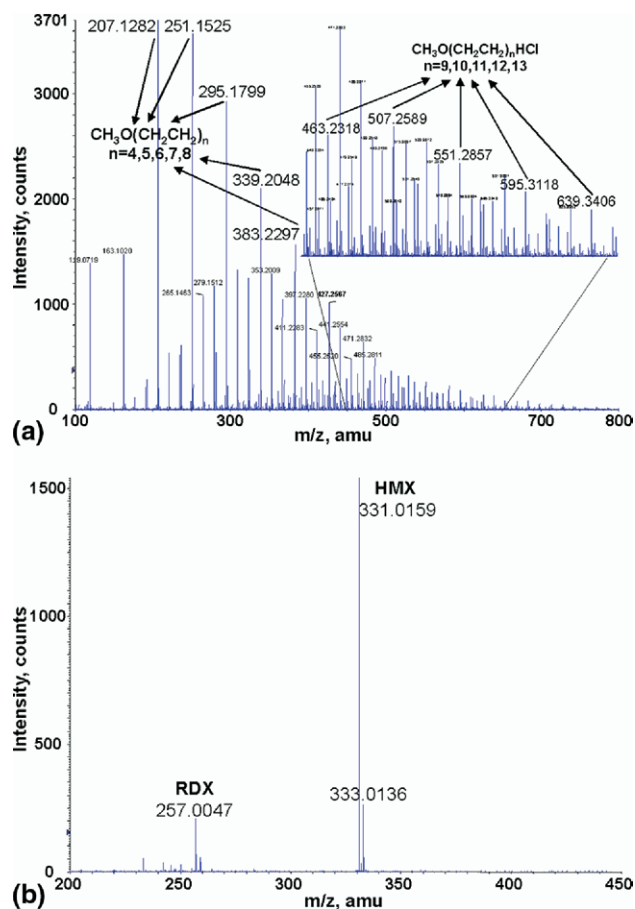
Anion attachment was primarily observed and studied in NICI using halide ions as the reactant. Dougherty et al. [23] showed that a variety of organic compounds, including aromatic and aliphatic carboxylic acids, amides, amino acids, aromatic amines, and phenols, displayed strong chloride attachment. Caldwell et al. [24] demonstrated that the highest sensitivity of halide

attachment was for strong acids with  $\Delta H_{\text{acid}}$  values stronger than 350 kcal/mol.

With negative ion-APPI, our previous study [8] indicated that halogenated solvents were inappropriate for use. Due to their positive EAs, they can capture thermal electrons and, consequently, prevent ionization of analytes through EC, dissociative EC, and/or proton transfer mechanisms. In the present study, the background toluene spectra were acquired after 1% (vol/vol) methylene chloride, bromoethane, or iodoethane were added (data not shown). Highly abundant halide ions were observed, as were clusters of halide ions for bromoethane and iodoethane. Meanwhile, the abundance of the anions observed in Figure 2 was significantly decreased. However, halide attachment to the acids responsible for the anions observed in Figure 2 were not found in the background spectra, indicating inefficient halide attachment to strong acids with  $\Delta H_{\text{acid}}$  values stronger than 350 kcal/mol for negative ion-APPI. This differs from NICI as reported by Caldwell et al. [24]. This was further proven by the analysis of 2-MBA, 2,4,6-TMBA, and decanoic acid using toluene with 1% (vol/vol) methylene chloride as solvent (data not shown). For all three of the carboxylic acids,  $[M - H]^-$  ions were primarily observed without significant  $[M + Cl]^-$  ions. In addition, the intensity of the  $[M - H]^-$  ions was decreased more than a decade in comparison to that in Figure 4a after intensity normalization for the same amount of material.

The competition between deprotonation and halide attachment was observed in the analysis of PEG methyl ether 750. When pure toluene was used as the solvent, the negative ion-APPI spectrum of PEG methyl ether 750 showed  $[\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n]^-$  ions (data not shown). When toluene with 1% methylene chloride was used as the solvent, the  $[\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n]^-$  ions were still observed, but with slightly enhanced intensity, as shown in Figure 7a. In addition, a series of  $[\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{HCl}]^-$  ions were also observed for  $n \geq 6$ , most likely representing the molecular adducts of PEG methyl ether 750. For  $n = 11$ , the ion intensity ratio between the  $[\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{HCl}]^-$  and  $[\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n]^-$  ions was about 1:1. For  $n > 11$ , the attachment/deprotonation ratio was even larger.

The analytical potential of an anion attachment ionization mechanism in negative ion-APPI is demonstrated in Figure 7b for the analysis of a mixture of RDX and HMX. As shown in Table 1, RDX and HMX do not have positive EAs or sufficient gas-phase acidity to be ionized by EC, dissociative EC, or proton transfer. However, their chloride attachment was so efficient that the limit of detection (LOD), defined by detection of their  $[M + Cl]^-$  ions at a signal to noise ratio (S/N) of 3, was estimated to be 424 and 64 fg or 1.9 and 0.2 fmol, respectively. Previously, Holmgren et al. [19] reported the analysis of RDX and HMX by LC/negative ion-APCI-MS through chloride attachment with LOD of 3.3 and 1.7 ng, respectively. HMX was also analyzed via anion attachment by Pan et al. [25] using LC/negative



**Figure 7.** (a) Negative ion-APPI spectrum of poly(ethylene glycol) methyl ether with average M.W. 750 from the consumption of 33  $\mu\text{L}$  of the analyte (concentration 0.1  $\text{nL}/\mu\text{L}$ , flow rate 20  $\mu\text{L}/\text{min}$ , acquisition time 1 s); (b) negative ion-APPI spectrum of a mixture of RDX and HMX produced from the consumption of 33 pg of each analyte (concentration 0.1  $\text{ng}/\mu\text{L}$  for each analyte, flow rate 20  $\mu\text{L}/\text{min}$ , acquisition time 1 s). Mass spectrometric conditions are GS1, 30; GS2, 30; CUR, 40; IS,  $-1900$  V; TEM,  $350^\circ\text{C}$ ; DP,  $-25$  V; FP,  $-80$  V; DP2,  $-12$  V; and CAD (collision gas), 3. Ions of  $m/z$  257.0047 and 331.0159 in (b) are interpreted as  $[M + Cl]^-$  ions from RDX and HMX, respectively.

ion-ESI-MS. Results indicated that acetate attachment was more efficient than formate, propionate, nitrate, chloride, nitrite, and nitrate with a LOD of 0.78 pg.

Four concentrations of methylene chloride [0.1, 0.5, 1.0, and 2.0% (vol/vol)] in toluene were tested to determine the optimum conditions for chloride attachment in the analysis of RDX and HMX. Highly abundant  $[M + Cl]^-$  ions were observed even with 0.1% (vol/vol) methylene chloride. The intensity of the  $[M + Cl]^-$  ions increased with the concentration of methylene chloride up to 1% (vol/vol).

In comparison to 1% (vol/vol) methylene chloride, the intensity of the  $[M + \text{Br}]^-$  ions of RDX and HMX became lower with 1% (vol/vol) bromoethane, and that of the  $[M + \text{I}]^-$  ions became even lower with 1% (vol/vol) iodoethane.



## Conclusions

Three ionization mechanisms were shown to be involved in negative ion-APPI of nitroaromatics; EC, dissociative EC, and proton transfer. These ionization mechanisms may compete with each other and result in multiple ionization products from the same molecule. Moreover, dissociative EC and proton transfer can combine to generate the same  $[M - H]^-$  ions. There is also a possibility, in need of further investigation, that an alternate electron/hydrogen transfer mechanism, rather than a simple proton transfer, is involved in the formation of  $[M - H]^-$  ions from analytes with both gas-phase acidity and positive EAs. For ease of deprotonation with nitroaromatics, the order was found to be methyl- < amino- < hydroxyl- functional groups attaching to the benzene ring. A dissociative EC mechanism is likely for 2-nitrophenyl octyl ether and expected for other phenyl ethers with sufficient positive EAs.

It is well known that the EC ability of an electrophoric compound is determined by its EA value. It is currently impossible to predict the dissociative EC ability of an electrophoric compound. However, this study certainly provides guidelines. It can be especially useful to guide the design of electrophoric derivatization reagents. Nitroaromatics have been used as electrophoric derivatization reagents in LC/EC-APCI-MS for the analysis of amines, aldehydes, ketones, 25-hydroxylvitamin D-3, and neurosteroids [14–17].

This study provided experimental evidence that  $O_2^-$  plays a key role in dopant assisted negative ion-APPI for the formation of  $[M - H]^-$  ions through proton transfer. The existence of  $O_2^-$  in an APPI source was directly observed in a spectrum of background ions when toluene was used exclusively as solvent and/or dopant. While analytes with gas-phase acidity, i.e.,  $\Delta H_{acid}$ , a few kcal/mol lower than  $HO_2^-$  could be efficiently deprotonated, analytes with gas-phase acidity a few kcal/mol higher than  $HO_2^-$  could not be efficiently deprotonated. Introduction of anions more basic than  $O_2^-$ , i.e.,  $C_6H_5CH_2^-$ , into the APPI source through the addition of di-*tert*-butyl peroxide in the solvent and/or dopant enhanced the deprotonation ability of negative ion-APPI. Better deprotonation ability for negative ion-APPI cannot be expected if the solvents and/or buffer reagents have gas-phase acidity higher than toluene.

Anion attachment is a novel ionization mechanism discovered for negative ion-APPI in this study. It offers great analytical potential because it may lead to efficient ionization of compounds, which cannot be ionized by EC, dissociative EC, or proton transfer ionization mechanisms. These compounds usually lack polarity and may be thermo-labile and, therefore, difficult to be ionized by any other available ionization methods. Anion attachment to these compounds may be a result of hydrogen bonding, dipole attraction, and/or polarization, which will be studied in detail in the future.

Nevertheless, with the available four ionization mechanisms, it becomes obvious that negative ion-APPI is able to ionize a wider range of compounds than NICI, negative ion-APCI or negative ion-ESI. This is especially true for low polarity compounds.

Although the effect of solvents and buffer reagents on negative ion-APPI is not the focus of this study, it can be predicted according to the ionization mechanisms. Halogenated solvents and/or buffer reagents possessing positive EAs will block EC, dissociative EC, and proton transfer because they will consume thermal electrons. Solvents and/or buffers possessing gas-phase acidity stronger than thermal electrons, i.e.,  $\Delta H_{acid} < 313.6$  kcal/mol, will hinder EC, dissociative EC, and proton transfer for the same reason. The efficiency of proton transfer will be decreased when solvents or buffer reagents possess gas-phase acidities stronger than  $HO_2^-$ . Therefore, the commonly used buffer reagents in negative ion-ESI and APCI, such as formic acid, acetic acid, trifluoroacetic acid, and their ammonium salts, are inappropriate for negative ion-APPI. This has also been proven by previous studies [8, 11].

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