

Atmospheric Pressure Photoionization Mass Spectrometry. Ionization Mechanism and the Effect of Solvent on the Ionization of Naphthalenes

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The ionization mechanism in dopant-assisted atmospheric pressure photoionization and the effect of solvent on the ionization efficiency was studied using 7 naphthalenes and 13 different solvent systems. The ionization efficiency was 1–2 orders of magnitude higher with dopant than without, indicating that the photoionization of the dopant initiates the ionization process. In positive ion mode, the analytes were ionized either by charge exchange or by proton transfer. Charge exchange was favored for low proton affinity solvents (water, hexane, chloroform), whereas the addition of methanol or acetonitrile to the solvent initiated proton transfer. In negative ion mode, the compounds with high electron affinity were ionized by electron capture or by charge exchange and the compounds with high gas-phase acidity were ionized by proton transfer. In addition, some oxidation reactions were observed. All the reactions leading to ionization of analytes in negative ion mode are initiated by thermal electrons formed in photoionization of toluene. The testing of different solvents showed that addition of buffers such as ammonium acetate, ammonium hydroxide, or acetic acid may suppress ionization in APPI. The reactions are discussed in detail in light of thermodynamic data.

The two most significant atmospheric pressure ionization (API) techniques used to date have been electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). In ESI the ionization is induced by an electric field and in APCI by various chemical reactions in the gas phase. The development of these two ionization methods has made the analysis of labile, highly polar and ionic compounds possible by LC/MS. However, it has not been possible to fully analyze nonpolar compounds with these techniques.

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Recently, Bruins and co-workers introduced a new LC/MS ionization method: dopant-assisted atmospheric pressure photoionization (APPI).¹ The photoionization detector (PID) has long been known to be used as a detection method for gas chromatography (GC),^{2–4} and it has also been used as a detection method for LC,^{5–8} although in the early LC-PID techniques, the conditions in the ionization chamber were very similar to the GC-PID conditions—the liquid was vaporized prior to the ionization that occurs in a vacuum. Photoionization in atmospheric pressure had earlier been carried out in connection with ion mobility spectrometry (IMS)^{9–11} and recently as a mass spectrometric ionization method without a dopant.^{12,13}

In dopant-assisted APPI, the ionization is initiated by 10-eV photons emitted by a krypton discharge lamp. The photoionization lamp replaces the discharge needle used in APCI—otherwise the body of the APPI ion source is very similar to the heated nebulizer body in APCI. A substance of favorable ionization energy (IE) called a dopant (e.g., toluene) is introduced into the vaporizer coaxially at a flow rate of ~1/10 of the solvent's flow rate. The APPI interface has been described in detail by Bruins et al.¹

The initial reaction in APPI is the formation of a radical cation of the dopant by 10-eV photons. For this reaction to occur, the ionization energy of the dopant has to be lower than the energy of the photons. The dopant radical cations formed may then ionize the analyte through charge exchange. Alternatively, the dopant radical cation can ionize solvent molecules by proton transfer; the protonated solvent molecules can then protonate the analyte

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molecules. Eventually, either a radical cation or a protonated molecule of the analyte is detected. In negative ion mode, the ionization can occur by either deprotonation, electron capture, or charge exchange. The formation of a radical cation makes it possible to ionize nonpolar molecules that cannot be efficiently analyzed by ESI or APCI.

The introduction of APPI-MS arose interest among mass spectrometry enthusiasts, and some commercial applications have emerged.^{13,14} To date, APPI-MS has been successfully used in the analysis of flavonoids,¹⁵ steroids,^{16,17} vitamins,¹⁸ antioxidants,¹⁹ pharmaceuticals,^{20–22} polyaromatic hydrocarbons,^{23,24} agrochemicals,²⁵ and aromatic imines and amines.²⁶ In addition to reversed-phase liquid chromatography (RP-LC), APPI has been used in preparative LC¹⁷ and normal-phase liquid chromatography (NP-LC).^{16,20,23}

The ionization process in APPI has been studied by Bruins²⁷ and Kostiainen,²⁴ but because of its complicated nature, it has been only partly explained. Therefore, the aim of this study is to investigate the ionization process and mechanism in positive and negative ion APPI by analysis of 7 naphthalenes in 13 different

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Table 1. Solvents Used in the Experiment

1	hexane
2	chloroform
3	water
4	methanol
5	acetonitrile
6	water/methanol (50:50%)
7	water/acetonitrile (50:50%)
8	water/methanol/acetic acid (50:50:0.1%)
9	water/methanol/ammonium acetate (50:50:0.1%)
10	water/methanol/ammonium hydroxide (50:50:0.1%)
11	water/acetonitrile/acetic acid (50:50:0.1%)
12	water/acetonitrile/ammonium acetate (50:50:0.1%)
13	water/acetonitrile/ammonium hydroxide (50:50:0.1%)

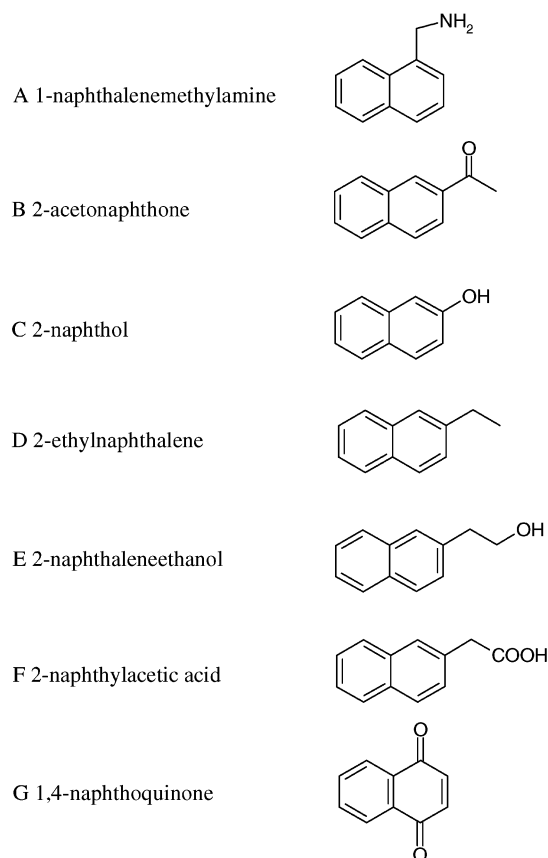


Figure 1. Structures of the studied naphthalenes.

solvent systems. Our interest was especially directed to the formation of reactant ions and their effect on the different routes of ionization in APPI: charge exchange, proton transfer, and electron capture. We will first examine reactant ions formed in different solvent systems and then discuss the ionization of the naphthalenes in positive and negative ion modes in light of the observed reactant ions.

EXPERIMENTAL SECTION

The solvents used in the study are presented in Table 1. The structures of the naphthalenes are shown in Figure 1, and thermodynamical data for the solvents and naphthalenes is given in Table 2. The naphthalenes, hexane, chloroform, and toluene were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile and acetic acid were purchased from Rathburn (Walkerburn, Scotland), methanol and ammonium hydroxide were

Table 2. Ion Energetics of the Studied Solvents and Naphthalenes^{33 a}

compound	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
ammonia	10.07	853.6		
benzyl radical	7.2	831.4		
toluene	8.83	784.0		1567
acetic acid	10.65	783.7		1429
acetonitrile	12.2	779.2	0.01	1528
methanol	10.84	754.3		1565
water	12.6	691.0		1607
hexane	10.13			
chloroform	11.37		0.622	1464
oxygen	12.1	421.0	0.451	
1-naphthalenemethylamine	8.05	961.2 ^b		
2-acetonaphthone	8.31	909.0 ^b		
2-naphthol	7.87	865.2 ^b		
2-ethylnaphthalene	7.95	835.9 ^b	<0.195	
2-naphthaleneethanol		826.3 ^b		
2-naphthaleneacetic acid	8.05	823.4 ^c		
1,4-naphthoquinone	9.5		1.813	

^a IE, ionization energy; PA, proton affinity; EA, electron affinity; $\Delta_{\text{acid}}G$ gas-phase acidity. ^b The PAs of the naphthalenes were estimated from the PAs of the corresponding phenyl compounds.³³ The substituted naphthalene ring was estimated to have a 47.9 kJ/mol higher PA than a substituted benzene ring. This was calculated from the PAs of toluene (784 kJ/mol) and 2-methylnaphthalene (831.9 kJ/mol). ^c The PA of naphthaleneacetic acid was estimated by comparing the PAs of acetic acid (783.7 kJ/mol), propanol (786.5 kJ/mol), and naphthalene-ethanol (826.3 kJ/mol) as the PA of the phenylacetic acid could not be found in the literature.³³

from J. T. Baker (Deventer, Holland), and ammonium acetate was from Merck (Darmstadt, Germany). All the chemicals used were of analytical or chromatographic grade. The water was purified in a Milli-Q water purification system (Millipore, Molsheim, France).

Stock solutions (10 $\mu\text{mol/mL}$) of the naphthalenes were prepared in hexane, chloroform, water/methanol (1:1), methanol, and acetonitrile. The stock solutions were diluted in the studied solvent systems (Table 1) to final concentrations of 10 nmol/mL. 2-Ethylnaphthalene could not be dissolved in water, nor could 2-naphthylacetic acid be dissolved in hexane, so neither was measured in these solvents.

The 10 nmol/mL samples were injected by using a 50- μL loop into a continuous solvent stream. Two microsyringe pumps (Harvard Apparatus Inc., Holliston, MA) were used to deliver the dopant and solvent at flow rates of 0.02 and 0.2 mL/min, respectively. HPLC-grade toluene was used as the dopant. The mass spectrometer was a PE Sciex API 3000 triple quadrupole mass spectrometer (Sciex, Concord, Canada) using an APPI interface (Machine Shop, University of Groningen, The Netherlands). The photoionization lamp used was a 10-eV Cathodeon Ltd. model PKS 100 krypton discharge lamp. The temperature of the nebulizer was 350 °C. Nitrogen produced by a Whatman 75–720 nitrogen generator (Whatman Inc., Haverhill, MA) was used as curtain and nebulizer gases and high-purity nitrogen (99.999%, Oy Woikoski Ab, Woikoski, Finland) as lamp gas in both positive and negative ion modes and as auxiliary gas in the negative ion mode. In the positive ion mode, compressed air filtered by an Atlas Copco air-dryer (Wilrijk, Belgium) was used as the auxiliary gas. Data were collected by using a Dell Optiplex computer and PE Sciex Analyst software (version 1.1). The instrument was operated in positive and negative ion modes. For the positive ion

mode, the mounting bracket voltage was +1300 V and for the negative ion mode, –1500 V. The declustering potentials used in the positive and negative ion modes were 20 and 27 V, respectively. Reactant ion spectra were recorded using a declustering potential of 0 V. The scan range was m/z 10–500 (1.5 s/scan). Tandem mass spectrometric (MS/MS) measurements were carried out in the product ion scan mode, using the same apparatus. The scan range was m/z 10 – m/z of the precursor ion + 10. The collision energy was varied between 10 and 50 V, depending on the stability of the precursor ion.

The gas-phase selected ion–molecule reactions were performed via MS/MS experiments run on an Extrel (Pittsburgh, PA) pentaquadrupole ($Q_1Q_2Q_3Q_4Q_5$) mass spectrometer, which is described in detail elsewhere.²⁸ Toluene radical cation was formed by 70-eV electron ionization (EI) of the corresponding neutral molecule. For the MS/MS ion–molecule reactions,^{29,30} Q1 was used to select m/z 92 (C_7H_8^+) for further reactions in q2 with a selected neutral reagent. Ion translational energies were set to near 0 eV as calibrated by the m/z 39:41 ratio in neutral ethylene/ionized ethylene reactions.³¹ Product ion mass spectra were acquired by scanning Q5, while operating Q3 and q4 in the broadband rf-only mode. Multiple collision conditions were used in q2, as indicated by typical beam attenuations of 50–70%. The indicated pressures in each differentially pumped region were typically 2×10^{-6} (ionsource) and 8×10^{-6} (q2) Torr.

RESULTS AND DISCUSSION

The ionization process in APPI directly depends on the reactant ion composition, which in turn depends on solvent, dopant, nebulizing gas, auxiliary gas, and impurities as well as components of the surrounding air. Ion–molecule reactions in the gas phase play a very important role in the formation of the reactant ions, since the free pathway of ions at atmospheric pressure is only 6.5×10^{-6} cm ($L = 0.66/p$, $T = 300$ K) and ions undergo several collisions with neutral molecules before mass analysis. As the optimum flow rate in APPI is between 0.1 and 0.3 mL/min, the solvent produces the main fraction of the reactant ions in the APPI system and therefore solvent composition has a significant effect on the ionization process.

The formation of reactant ions was studied by recording the spectra of 13 different solvent systems commonly used in LC (Table 1). The effect of the reactant ion composition on the APPI ionization process was studied with seven naphthalenes with varying functional groups (Figure 1). This series provides a good basis for understanding the ionization mechanism in APPI, since proton transfer is dependent on the proton affinity (PA) of the attached functional group, whereas the low IEs of the naphthalenes also enable ionization through charge exchange (Table 2). 1,4-Naphthoquinone was chosen because of its high electron affinity (EA) and its ability to form negative ions by either electron capture or charge exchange. However, full gas-phase energetics data for all the compounds studied were not available and some estimates had to be made. Furthermore, 10-eV photons may excite

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Table 3. Main Ions in the Positive Ion APPI Spectra of 13 Solvents^a.

solvent	<i>m/z</i> (rel abund) ^b		
	C ₇ H ₈ ^{•+}	S _{<i>n</i>} H ⁺	observed ions
toluene ^c	92 (100)		95 (74), 96 (39), 101 (34), 107 (30), 108 (47), 109 (98), 111 (97), 112 (34)
1 (hexane)	92 (52)		84 (88), 94 (62), 95 (31), 99 (100), 101 (34), 108 (17), 109 (40), 111 (35), 115 (23)
2 (chloroform) ^d	92 (87)		108 (100), 91 (72), 107 (57), 93 (56), 109 (38), 105 (34), 74 (29), 106 (18), 111 (14)
3 (water)	92 (100)		83 (15), 95 (21), 96 (21), 101 (22), 107 (23), 108 (42), 109 (26), 111 (59), 112 (18)
4 (methanol)		51 (7.9) [32 + 18a + H] ⁺ , 65 (100) [2 × 32 + H] ⁺ , 97 (6.5) [3 × 32 + H] ⁺	59 (9.5), 60 (9.4), 73 (14), 74 (60), 87 (36), 101 (10), 115 (7.7)
5 (acetonitrile)		42 (5.1) [41 + H] ⁺ , 83 (100) [2 × 41 + H] ⁺	59 (21), 60 (8.3), 84 (17), 100 (5.9), 101 (22), 115 (4.8), 142 (3.8), 148 (10)
6 (water/methanol)		65 (100) [2 × 32 + H] ⁺ , 97 (27) [3 × 32 + H] ⁺	60 (15), 73 (12), 74 (83), 87 (33), 101 (9.6), 106 (26), 115 (9.7), 127 (4.2)
7 (water/acetonitrile)		83 (100) [2 × 41 + H] ⁺	59 (51), 60 (7.4), 100 (15), 101 (15), 102 (3.5), 105 (5.6), 108 (3.7), 109 (4.3), 148 (4.3)
8 (water/methanol/HAc)		65 (100) [2 × 32 + H] ⁺ , 97 (27) [3 × 32 + H] ⁺	59 (6.6), 60 (15), 74 (53), 106 (16), 109 (6.7), 11 (5.9), 115 (7.6), 127 (6.9)
9 (water/methanol/NH ₄ Ac)	92 (5.7)		50 (3.4), 59 (100), 60 (42), 77 (41), 82 (6.0), 87 (6.8), 100 (3.7), 118 (8.4), 119 (21)
10 (water/methanol/NH ₄ OH)		51 (8.4) [32 + 18b + H] ⁺ , 65 (100) [2 × 32 + H] ⁺ , 97 (18) [3 × 32 + H] ⁺	59 (17), 60 (21), 74 (41), 106 (9.5), 108 (6.0), 109 (8.2), 115 (6.4)
11 (water/acetonitrile/HAc)		59 (100) [41 + 18b] ⁺ , 83 (5.1) [2 × 41 + H] ⁺	64 (5.3), 80 (5.3), 100 (33), 105 (76), 106 (5.2), 109 (5.3), 146 (5.9), 148 (5.3)
12 (water/acetonitrile/NH ₄ Ac)		59 (100) [41 + 18b] ⁺	64 (2.3), 77 (1.5), 80 (2.2), 100 (35), 101 (5.2), 105 (32), 106 (2.1), 144 (1.4), 146 (2.1)
13 (water/acetonitrile/NH ₄ OH)		59 (100) [41 + 18b] ⁺	100 (14), 144 (3.1), 136 (1.9), 145 (1.7), 133 (1.3), 115 (1.3), 74 (1.2), 112 (1.2), 148 (1.2)

^a The measurements were done using an orifice voltage of 0 V. S, solvent; HAc, acetic acid; NH₄Ac, ammonium acetate. ^b 92 = C₇H₈^{•+}; 41 = CH₃CN; 32 = CH₃OH; 18a = H₂O; 18b = NH₄. ^c The toluene spectrum was overloaded and therefore the relative abundances of the ions should be taken as being suggestive. ^d Chloroform spectrum was recorded by using a declustering potential of 20 V.

aromatic compounds such as naphthalenes to an excited state that is more reactive than the ground state of the molecule.³² Therefore, the thermodynamic values for the naphthalenes should be considered as being suggestive in some cases. Since the API source used discriminates against lower mass/charge values, the APPI-MS spectra showed no ions at low-mass numbers. The cutoff value is between *m/z* 30 and 40.

Formation of Positive Reactant Ions. In dopant-assisted APPI, 10-eV photons emitted by the photoionization lamp first ionize the dopant (the IE of the dopant must be below 10 eV; toluene in our experiments) producing a radical cation of the dopant (D^{•+}), which either remains in the system or reacts further with neutral solvent molecules.^{1,24} The APPI-MS spectra of the solvent systems studied (Table 3) showed that the radical cation of toluene (C₇H₈^{•+}, *m/z* 92) remained in the system containing low-PA solvents (water, hexane, and chloroform; solvents 1–3, respectively) but disappeared from the system when solvents containing methanol or acetonitrile (solvents 4–13) were used.

Instead of C₇H₈^{•+}, protonated solvent molecules or their dimers, trimers, or solvent–water clusters were observed in these solvents, indicating that C₇H₈^{•+} donates a proton to the solvent molecule.

The proton transfer between C₇H₈^{•+} and the solvent molecule is only possible if the PA of the solvent is higher than that of the benzyl radical, C₇H₇[•] (831.4 kJ/mol, Table 2). This is not the case with water, hexane, and chloroform, and therefore, in these solvents C₇H₈^{•+} remained in the system (Table 3). Interestingly, according to the literature, the PAs of methanol and acetonitrile are also lower than that of benzyl radical³³ (Table 2), but still C₇H₈^{•+} disappeared from the spectra and protonated solvent species were observed (Table 3). This phenomenon may be explained by the presence of solvent and solvent/water clusters that have PAs significantly higher than those of the individual monomers.^{34,35} It is apparent that the PAs of the clusters exceed

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Table 4. Ions Formed in the Ion–Molecule Reactions between $C_7H_8^{*+}$ (m/z 92) and Solvent Molecules

solvent	m/z (rel abund) ^a	
	product ions derived from toluene	other observed ions
hexane	91 (49), 92 (100)	83 (2.8), 85 (57), 93 (9.0)
chloroform	91 (100), 92 (29)	83 (28), 85 (16), 139 (15) 141 (2.5)
water	65 (37), 66 (3.9), 91 (100), 92 (50)	
methanol	91 (100), 92 (52), 93 (7.7)	59 (57), 65 (14), 67 (6.7), 68 (3.1), 73 (41), 80 (44), 117 (51)
acetonitrile	65 (38), 66 (3.1), 91 (100), 92 (20)	39 (13), 42 (25) 106 (5.5), 132 (3.4)
ammonia	65 (45), 66 (6.9), 91 (100), 92 (46)	

^a 91 = $[C_7H_8^* - H]^+$; 85 = $[C_6H_{14} - H]^+$; 139, 141 = $[C_7H_7 + CHCl]^+$; 42 = $[CH_3CN + H]^+$.

that of benzyl radical and the proton transfer between $C_7H_8^{*+}$ and solvent clusters is thermodynamically possible. The same conclusion has also been made by Bruins and co-workers.²⁷ The observation of cluster ions in the solvent spectra, even when the declustering potential was as high as 20 V, suggests that higher degree clusters are also present in the system and may affect the ionization process. The formation of methanol monomer, dimer, and trimer was observed to be dependent on the temperature of the nebulizer. The relative abundance of the monomer increased whereas the relative abundances of dimer and trimer decreased when the temperature was raised from 200 to 500 °C. Therefore, the temperature of the nebulizer also has an effect on the ionization process.

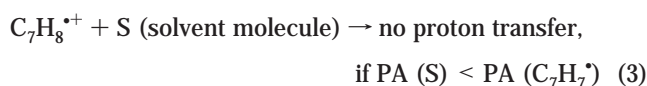
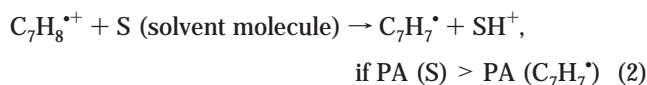
The formation of possible reactant ions was studied further by selected ion–molecule reactions between $C_7H_8^{*+}$ and solvent molecules using MS/MS (Table 4). Reactions of $C_7H_8^{*+}$ with methanol produced ion m/z 65, which can be $[(MeOH)_2 + H]^+$, a fragment of $C_7H_8^{*+}$, or a mixture of these two entities. Reactions of $C_7H_8^{*+}$ with acetonitrile mainly produced m/z 42 $[ACN + H]^+$. Bruins et al. performed similar experiments, and they observed abundant $[(MeOH)_3 + H]^+$ and weak $[(MeOH)_2 + H]^+$ and $[(MeOH)_4 + H]^+$ ions with methanol and abundant $[(ACN)_2 + H]^+$ with acetonitrile.²⁷ The differences between our results and those of Bruins can be explained by the different experimental conditions used, such as collision energy and pressure. These results prove that $C_7H_8^{*+}$ upon low-energy collisions can donate a proton to dimers and higher clusters of methanol and acetonitrile, although direct proton transfer from $C_7H_8^{*+}$ is thermodynamically unfavorable. This situation is also possible in the APPI process, which explains the disappearance of $C_7H_8^{*+}$ in the APPI-MS spectra also with solvents containing methanol or acetonitrile. For methanol, the PA of methanol clusters is at least 77 kJ/mol (PA ($C_7H_7^*$) – PA(MeOH), Table 2) higher than that of the methanol monomer, and in the case of acetonitrile, the difference in PAs is at least 52 kJ/mol (PA ($C_7H_7^*$) – PA(ACN), Table 2).

The selected ion–molecule reactions between $C_7H_8^{*+}$ and water, hexane, and chloroform showed no proton transfer (Table 4), as the PAs of these solvents and their possible clusters are too low. This is in agreement with the observation made in the APPI experiments that the ionized toluene fails to transfer a proton to these solvents and instead remains in the APPI process. In the experiments with water, only product ions formed by collisionally activated dissociation of $C_7H_8^{*+}$ were detected, whereas collisions between $C_7H_8^{*+}$ and hexane produced an ion of m/z 85 formed by hydride abstraction from hexane. The reaction of $C_7H_8^{*+}$ with chloroform produced ions $CHCl_2^+$ of m/z 83 and $C_7H_7CHCl^+$ of

m/z 139. Although only ions of m/z 139 could be found in the respective APPI-MS spectra, the results give reason to assume that reactions other than charge exchange or proton transfer can occur between $C_7H_8^{*+}$ and the solvent molecules in the APPI system.

The APPI-MS spectra of the solvents also contained many background ions (Table 3). For example, the ion of m/z 108, which was very intensive in most of the solvent spectra as well as in the spectrum of pure toluene, was recognized to be cresol, which can be either an impurity present in toluene or a product of a gas-phase reaction between ionized toluene and water. Such impurities and products of possible gas-phase reactions can react further with other compounds in the ion source and thus complicate any predictions made for the ionization process.

In summary, the main reactant ions in toluene-assisted APPI are $C_7H_8^{*+}$ with the low-PA solvents (in our experiments, lower than or as low as 691 kJ/mol = PA of water) and protonated solvent clusters with the high-PA solvents (in our experiments, higher than or as high as 754 kJ/mol = PA of methanol). In solvent mixtures, the reactant ion of highest probability is formed; i.e., the protons are transferred to the component of highest PA, and in charge exchange, the charge is transferred to the component of lowest IE. However, impurities and secondary reaction products may also act as reactant ions. The formation of main reactant ions in positive ion mode can be summarized by the following reactions:



Ionization of Naphthalenes in Positive Ion Mode. Next, the ionization efficiency and mechanism in APPI was studied with 7 naphthalenes in the same 13 solvent compositions. The effect of solvent on the overall ionization efficiency was studied by recording the absolute abundances (peak area) of the total ion currents of the naphthalenes (Figure 2). The relative abundances of protonated molecule (MH^+) and molecular ion (M^{*+}) indicate the mechanism by which the naphthalenes are ionized (Figure 2). The spectra of the naphthalenes in water/methanol presented

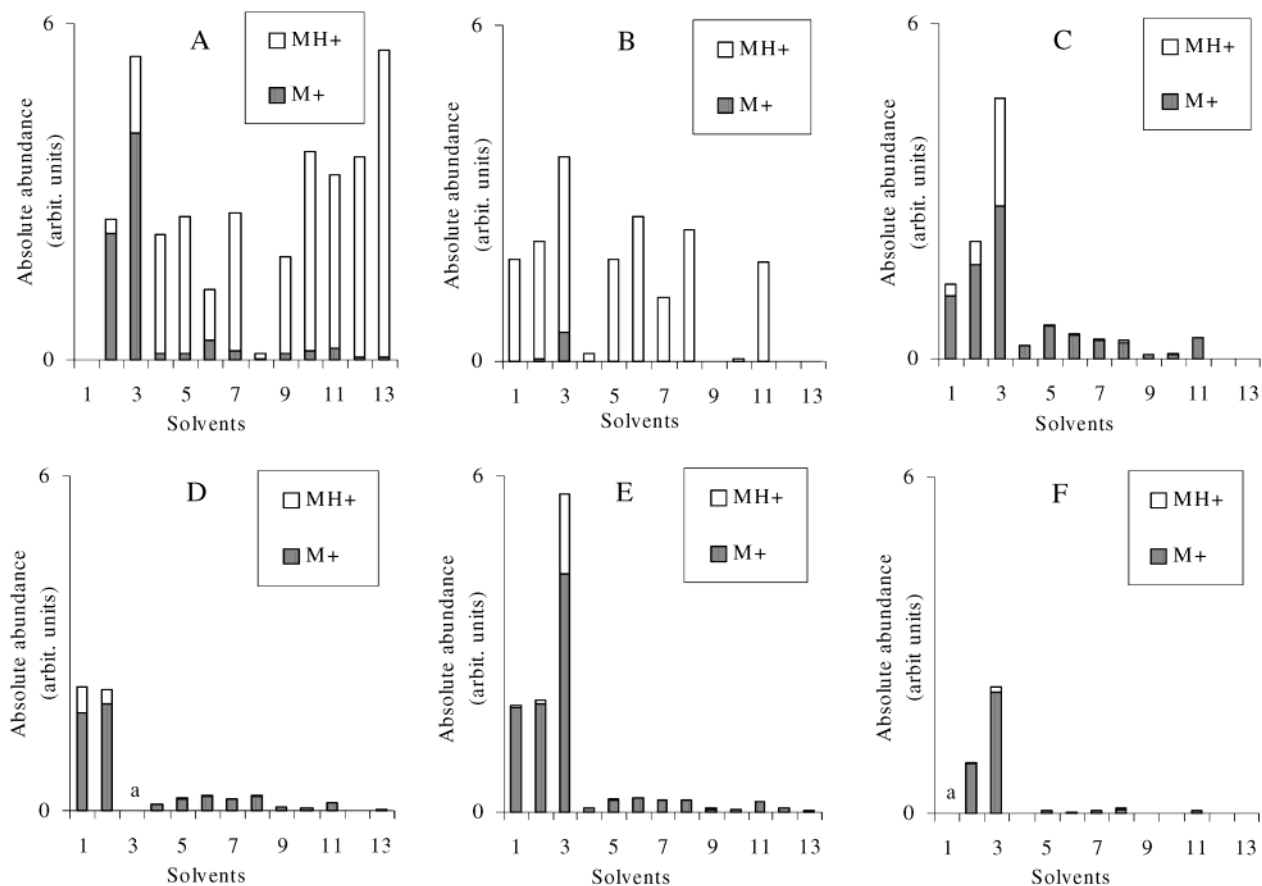


Figure 2. Absolute abundance of the total ion currents and relative proportions of M^+ and $[M + H]^+$ for the 6 naphthalenes in 13 different solvent compositions (Table 1) in the positive ion mode. The letters indicate the studied compounds: (A) 1-naphthalenemethylamine, (B) 2-acetonaphthone, (C) 2-naphthol, (D) 2-ethylnaphthalene, (E) 2-naphthaleneethanol, and (F) 2-naphthaleneacetic acid. (a) The naphthalene could not be dissolved in the solvent in question and was therefore left out of the study.

Table 5. Positive Ion APPI-MS Spectra of the Naphthalenes Measured in Water/Methanol (Solvent 6)

	<i>m/z</i> (rel abund)		
	$[M + H]^+$	M^+	fragments and adducts
2-naphthalenemethylamine	158 (37)	157 (13)	141 (100) $[M + H - NH_3]^+$, 156 (38) $[M + H - 2H]^+$
2-acetonaphthone	171 (100)		
2-naphthol	145 (8.0)	144 (100)	
2-ethylnaphthalene		156 (100)	141 (14) $[M - CH_3]^+$
2-naphthaleneethanol		172 (100)	155 (67) $[M + H - H_2O]^+$, 141 (28) $[M - CH_2OH]^+$
2-naphthaleneacetic acid		186 (52)	141 (25) $[M - COOH]^+$

as an example in Table 5 showed M^+ , MH^+ , and some fragment ions. The MH^+ ions in APPI are most likely formed by proton transfer from the protonated solvent species to the analyte, whereas M^+ ions are formed by charge exchange between $C_7H_8^{*+}$ and the analyte. The formation of M^+ directly by 10-eV photons is, at least in our experiments, not the main mechanism, since the ionization efficiency decreased ~ 2 orders of magnitude when toluene was removed.

Efficient charge exchange can only be expected if $C_7H_8^{*+}$ remains in the APPI source, which is only possible for low-PA solvents, such as water, hexane, and chloroform. Also, the compound to be ionized should have an IE lower than that of toluene. This is in good agreement with the results presented in Figure 2. The formation of the abundant M^+ ion of the naphthalenes (Figure 2) was obtained only with water, hexane, and chloroform (solvents 1–3). Note that 2-ethylnaphthalene is

insoluble in water and 2-naphthylacetic acid in hexane. Charge exchange may occur also with other solvents (4–13), but with significantly lower ionization efficiency than with solvents 1–3 (Figure 2).

It is worth noting that the molecular ion can be deprotonated by dissociative proton transfer described earlier by Syage.^{36,37} The reaction $M^+ + S \rightarrow [M - H]^{\bullet} + SH^+$ is possible if the PA of the solvent (S) is higher than that of $[M - H]^{\bullet}$. Unfortunately, the full ion energetics data of the naphthalenes are not available. However, it can be estimated with the respective phenyls that the PAs of the naphthalene radicals, $[M - H]^{\bullet}$, are about 30–60 kJ/mol higher than those of the respective neutral molecules, M (Table 2).³³ Since the PAs of the solvents (Table 2) are lower than the PAs of $[M - H]^{\bullet}$ of the naphthalenes studied, the dissociative

(36) Syage, J. A. *J. Phys. Chem.* **1989**, *93*, 170–179.

(37) Steadman, J.; Syage, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 6786–6795.

Table 6. Main Ions in the Negative Ion APPI Spectra of 13 Solvents^a

solvent	m/z (rel abund)	
	identified ions	other ions
toluene		46 (100), 59 (16), 60 (71), 61 (57), 76 (26), 77 (23), 96 (45), 107 (18), 117 (14), 121 (14)
1 (hexane)		45 (10), 46 (34), 59 (23), 60 (95), 61 (100), 77 (37), 84 (11), 113 (12), 117 (32), 135 (13)
2 (chloroform)	35 (93) [Cl] ⁻ , 153 (80) [CHCl ₃ + Cl] ⁻ , 155 (100) [CHCl ₃ + Cl] ⁻ , 157 (69) [CHCl ₃ + Cl] ⁻ , 159 (95) [CHCl ₃ + Cl] ⁻	62 (98), 93 (86), 95 (97), 109 (64), 117 (43)
3 (water)		45 (100), 46 (35), 59 (44), 60 (6.8), 81 (6.5), 91 (55), 92 (6.4), 97 (7.8), 105 (9.0), 117 (11)
4 (methanol)		45 (100), 46 (67), 59 (29), 61 (13), 75 (32), 76 (9.5), 77 (33), 96 (17), 113 (28), 117 (18), 121 (19)
5 (acetonitrile)	40 (74) [CH ₃ CN - H] ^{-b}	42 (27), 45 (19), 46 (50), 59 (22), 60 (26), 61 (25), 77 (18), 84 (100), 117 (22)
6 (water/methanol)		45 (41), 46 (100), 59 (57), 75 (89), 76 (26), 77 (86), 95 (16), 96 (37), 111 (38), 137 (31)
7 (water/acetonitrile)	40 (74) [CH ₃ CN - H] ^{-b}	42 (17), 45 (100), 46 (20), 84 (25), 91 (68), 94 (15), 97 (13), 107 (27), 113 (15), 117 (28)
8 (water/methanol/HAc)		45 (100), 46 (27), 75 (5.1), 77 (11), 91 (79), 92 (6.8), 96 (6.0), 97 (5.5), 111 (11), 113 (9.4)
9 (water/methanol/NH ₄ Ac)	59 (58) [CH ₃ COOH - H] ⁻ , 119 (100) [2 × CH ₃ COOH - H] ⁻	89 (6.6), 95 (9.1), 105 (9.5), 113 (5.5), 117 (8.7), 120 (14), 121 (4.8), 141 (29)
10 (water/methanol/NH ₄ OH)		45 (100), 46 (68), 77 (10), 89 (9.9), 91 (68), 92 (6.7), 97 (4.9), 111 (7.7), 113 (28), 117 (20)
11 (water/acetonitrile/HAc)	59 (51) [CH ₃ COOH - H] ⁻ , 119 (100) [2 × CH ₃ COOH - H] ⁻	95 (11), 97 (6.2), 105 (5.8), 117 (14), 120 (15), 121 (7.9), 139 (12), 141 (48)
12 (water/acetonitrile/NH ₄ Ac)	59 (57) [CH ₃ COOH - H] ⁻ , 119 (100) [2 × CH ₃ COOH - H] ⁻	62 (6.3), 95 (11), 97 (5.5), 105 (11), 113 (7.0), 117 (13), 120 (13), 141 (26)
13 (water/acetonitrile/NH ₄ OH)		45 (100), 84 (87), 59 (80), 42 (45), 46 (43), 77 (36), 61 (30), 117 (25), 89 (19), 97 (17)

^a The measurements were done using an orifice voltage of 0 V. ^b Ion observed in orifice voltage of 27 V.

proton transfer with solvent monomers is unlikely. However, the reaction may take place if the solvent exists as a dimer, since the PA of the dimer is higher than that of the respective monomer.

Another possible ionization pathway of the naphthalenes is proton transfer, for the cases in which the PA of the naphthalene in question is higher than that of reactant molecule. The results showed that the PAs of 1-naphthalenemethylamine and 2-acetonaphthone were high enough, whereas the PAs of the other naphthalenes were too low for efficient protonation (Figure 2, Table 5). Efficient ionization by proton transfer was obtained with solvent systems capable of producing protonated reactant ions as a result of proton transfer from C₇H₈⁺ to the solvent molecules (solvents 4–13). On the contrary, the ionization efficiency of the low-PA naphthalenes can be enhanced by the use of low-PA solvent systems (solvents 1–3) and thereby promoting charge exchange. These results showed that nonpolar compounds can also be ionized efficiently by APPI, which may not be possible with conventional API sources (APCI and ESI).

Interestingly, 2-acetonaphthone was readily ionized by proton transfer also with solvents 1–3 (Figure 2), although protonated reactant molecules are not formed from the solvent molecules in these solvents. This might be explained by background ions, protonated reaction products (see Formation of Positive Reactant

Ions) or water clusters from traces of water in the surrounding air that can act as reactant ions. However, the PA of 1-naphthalenemethylamine is higher than that of 2-acetonaphthone, but 1-naphthalenemethylamine did not produce MH⁺, but instead was ionized mainly by charge exchange in solvents 1–3 (Figure 2). The behavior of 2-acetonaphthone might be explained by the fact that charge exchange between C₇H₈⁺ and 2-acetonaphthone is less exothermic than with other naphthalenes, since the IE of 2-acetonaphthone (8.31 eV) is higher than the IEs of the other naphthalenes (7.87–8.05 eV)³³ (Table 2). As a consequence, proton transfer may be thermodynamically more favorable for 2-acetonaphthone than charge exchange.

The addition of ammonium acetate or ammonium hydroxide (solvents 9, 10, 12, and 13) deteriorated significantly the ionization efficiency of all the naphthalenes except for 1-naphthalenemethylamine (Figure 2). Even small additions of ammonium ions may change the reactant ion system efficiently toward ammonium-based reactant ions, due to the high PA of ammonia.³⁸ Obviously, the PAs of ammonium-based reactant ions (NH₄⁺, NH₄⁺(solvent)) are higher than the PAs of all the other naphthalenes except that of naphthalenemethylamine. Note that the PA of ammonia as

(38) Rudewicz, P.; Munson, B. *Anal. Chem.* **1986**, *58*, 2903–2907.

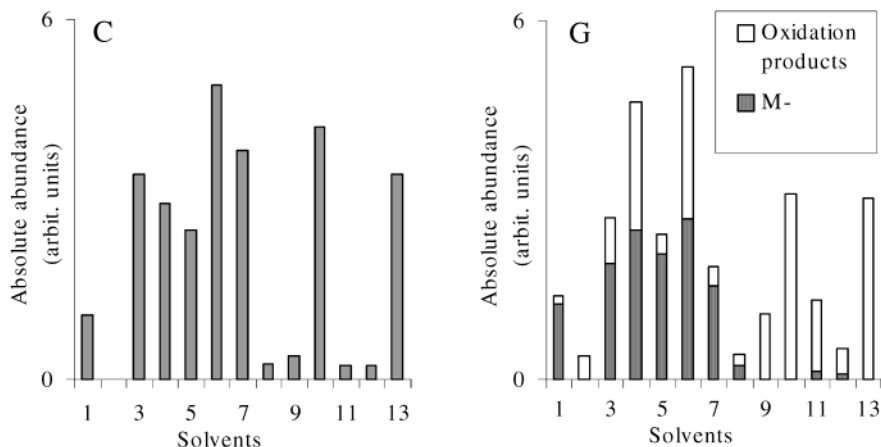
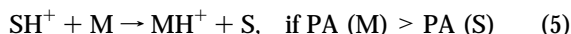
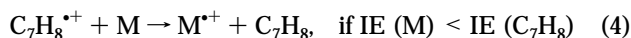


Figure 3. Absolute abundance of the total ion current for 2-naphthol and 1,4-naphthoquinone in 13 different solvent compositions (Table 1) in the negative ion mode. The relative abundances of M^- and the recognized oxidation products are presented for 1,4-naphthoquinone. The letters indicate the studied compounds: (C) 2-naphthol; (G) 1,4-naphthoquinone.

Table 7. Negative Ion APPI-MS Spectra of the Naphthalenes Measured in Water/Methanol (Solvent 6)

	m/z (rel abund)		
	$[M - H]^-$	M^-	fragments and adducts
2-naphthol	143 (100)	144 (25)	158 (11) $[M - 2H + O]^-$, 159 (8.2) $[M - H + O]^-$
2-naphthaleneacetic acid			171 (100), 141 (35) $[M - COOH]^-$, 172 (4.9)
1,4-naphthoquinone		158 (100)	173 (57) $[M - H + O]^-$, 188 (38) $[M - 2H + 2O]^-$, 145 (33), 159 (9), 191 (13)

reported in ref 33 is lower than the estimated proton affinities of 2-acetonaphthone and 2-naphthol. However, the PAs of ammonia clusters are higher than that of ammonia and may exceed that of 2-acetonaphthone. The ionization of the naphthalenes can be summarized by the following reactions:



Ionization of Naphthalenes in Negative Ion Mode. There are several possible ionization mechanisms in negative ion APPI: interactions between electron and molecule, charge exchange, proton transfer, and association and displacement reactions. The ionization in the negative ion mode is expected to be initiated by thermal electrons formed in the photoionization of toluene. All the components with positive EA, including analytes, can capture thermal electrons and thus form negative molecular anions, which via further reactions can form deprotonated molecules. To predict the possible ionization mechanisms in the negative ion mode, the APPI-MS spectra of different solvent systems were measured (Table 6). The background spectra showed ions derived from toluene and deprotonated solvent molecules, for example, $[CH_3CN - H]^-$, as well as CH_3COO^- and its dimer. However, background spectra contained several ions that were not identified. Furthermore, some of the ions derived from the solvents or background could not be detected by using our mass spectrometer, because it discriminates against masses below m/z 30–40. This excludes detection of many ions that are expected to have an important role in the ionization process, such as OH^- , O_2^{*-} , NH_2^- , and CH_3O^- .

The naphthalene spectra were measured in negative ion mode with the same solvents as in positive ion mode (Table 1). The

spectra showed ion formation only for 1,4-naphthoquinone, 2-naphthol, and 2-naphthaleneacetic acid. 1,4-Naphthoquinone has a high positive EA (1.813 eV),³³ and it formed abundant molecular anion M^- , but also oxidation products $[M - H + O]^-$ of m/z 173, $[M - 2H + O]^-$ of m/z 172, and $[M - 2H + 2O]^-$ of m/z 188 (Figure 3, Table 7). 2-Naphthol contains an acidic group and formed an abundant deprotonated molecule $[M - H]^-$ of m/z 143 in all the solvents (Table 7). Although 2-naphthylacetic acid also contains an acidic group, the deprotonated molecule was not abundant; instead, fragment ions of m/z 171 and 141 were detected (Table 7), but in such low and undefined intensity that 2-naphthylacetic acid was largely left out of the discussion in the negative ion mode. The other naphthalenes were not ionized in the negative ion mode, since they lack acidic groups or groups with sufficiently high EA. The measurements made without toluene in the negative ion mode showed a dramatic decrease in sensitivity for 1,4-naphthoquinone, 2-naphthol, and 2-naphthaleneacetic acid, indicating that the formation of negative ions (M^- , $[M - H]^-$) is initiated by thermal electrons formed in the photoionization of toluene. In addition, the formation of oxidation products shows that oxygen plays an important role in negative ion APPI.

The molecular anion of 1,4-naphthoquinone can be formed directly by electron capture or by charge exchange. Oxygen, present in the ionization region, has a positive EA of 0.451 eV³³ and is ionized by the thermal electrons to superoxide ion (O_2^{*-}). Because the EA of oxygen is relatively low, it may well react further by charge exchange. Charge exchange between O_2^{*-} and 1,4-naphthoquinone is thermodynamically favored, since the EA of 1,4-naphthoquinone (1.813 eV)³³ is significantly higher than that of O_2 . The oxidation product $[M - H + O]^-$ of m/z 173 is formed when O_2^{*-} reacts with neutral 1,4-naphthoquinone (reaction 9) or when neutral O_2 reacts with M^- of 1,4-naphthoquinone (reaction

10). These reactions are analogous to those presented earlier by Horning et al.³⁹ for *p*-nitrochlorobenzene. The ions $[M - 2H + O]^-$ of m/z 172 and $[M - 2H + 2O]^-$ of m/z 188 are formed by further oxidation of m/z 173.

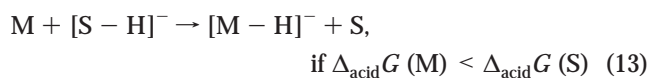
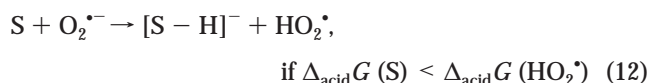
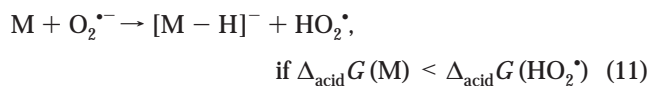
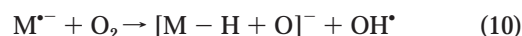
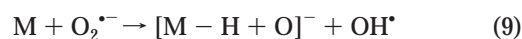
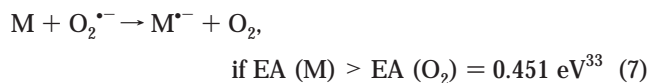
Figure 3 shows that $M^{\bullet-}$ of 1,4-naphthoquinone was formed approximately with the same efficiency in solvents 1 and 3–7. However, the formation of oxidation products was more abundant and the overall ionization efficiency higher in methanol and methanol/water (solvents 4 and 6) than in acetonitrile and acetonitrile/water (solvents 5 and 7). Also, the formation of oxidation products was clearly reduced in hexane and acetonitrile in comparison to all the other solvents. The addition of acetic acid, ammonium acetate, or ammonium hydroxide inhibited the formation of $M^{\bullet-}$, resulting in a high relative abundance of the oxidation products. This may be due to the fact that the addition of protons or ammonium ions consumes the thermal electrons by the following reduction reactions: $2H^+ + 2e^- \rightarrow H_2$ and $2NH_4^+ + 2e^- \rightarrow 2NH_3 + H_2$. As there is a decreased number of thermal electrons, the electron capture reaction becomes less favored. The decreased number of thermal electrons was probably also the reason for the decreased overall ionization efficiency with solvents that contain acetic acid or ammonium acetate. However, the addition of ammonium hydroxide did not change significantly the overall ionization efficiency (Figure 3).

Although the spectra of 1,4-naphthoquinone measured in less polar and aprotic solvents (hexane and acetonitrile) showed abundant $M^{\bullet-}$ ion and only weak ions formed by the oxidation reactions, ionization in the nonpolar and aprotic chloroform was an exception: instead of $M^{\bullet-}$, only the oxidation product $[M - H + O]^-$ was detected (Figure 3), at a very low intensity. This was probably due to the insufficient number of thermal electrons for the ionization of 1,4-naphthoquinone by electron capture, since chloroform itself has positive EA (0.622 eV)³³ and therefore efficiently captures thermal electrons. The lack of thermal electrons inhibits the formation of $O_2^{\bullet-}$, and therefore, the formation of $[M - H + O]^-$ also decreases. Hence, the overall ionization efficiency of 1,4-naphthoquinone in chloroform is poor.

In the gas phase, $O_2^{\bullet-}$ ion is a relatively strong base⁴⁰ and it may therefore take a proton from many organic compounds. The $O_2^{\bullet-}$ ion, its hydrated forms, or a solvent cluster ion can ionize the analyte directly by proton transfer or it can initiate the formation of deprotonated reactant ions (Table 6). Proton transfer from the analyte to the deprotonated reactant ion is possible, if the gas-phase acidity of the analyte exceeds the acidity of the reactant gas molecule, i.e., if the $\Delta_{acid}G$ (analyte) is lower than $\Delta_{acid}G$ (reactant gas). This criterion is met for 2-naphthol and 2-naphthaleneacetic acid; hence, they formed $[M - H]^-$ ions.

In the negative ion mode, the use of chloroform as a solvent totally inhibited the ionization of both 2-naphthol and 2-naphthaleneacetic acid. Again, the high EA of chloroform (0.622 eV)³³ inhibits the formation of $O_2^{\bullet-}$, and thus, the deprotonation of 2-naphthol and 2-naphthaleneacetic acid failed to occur. Also, the addition of acetic acid and ammonium acetate suppressed significantly the deprotonation of 2-naphthol (Figure 3) and 2-naphthaleneacetic acid. Even small additions of acetate-containing

buffers (0.1%) resulted in the formation of abundant CH_3COO^- ion or its cluster ions as indicated by the APPI-MS spectra of solvents 9, 11, and 12 (Table 6). This is because CH_3COOH is a stronger gas-phase acid ($\Delta_{acid}G = 1429$ kJ/mol)³³ than HO_2^{\bullet} .⁴⁰ As a consequence, proton transfer to CH_3COO^- from the analyte is less exothermic or even endothermic compared to the proton transfer between $O_2^{\bullet-}$ and the analyte. This may partly explain the decreased sensitivity for 2-naphthol and 2-naphthaleneacetic acid in acetate-containing buffers. The reactions leading to the analyte ionization in negative ion mode can be summarized as follows:



Because the role of oxygen is very important in negative ion mode, the sources of oxygen should be known. Oxygen may originate from atmosphere or it can exist as traces in the nebulizing gas (nitrogen) or in the solvents. The negative ion spectrum of naphthoquinone was recorded by using only high-purity nitrogen (99.999%) as all gases in APPI; then another spectrum was recorded by changing the nebulizing gas to air. The measurements were done with hexane as the solvent. The results showed that when air was used as the nebulizing gas, the relative abundances for $M^{\bullet-}$ and $[M - H + O]^-$ were 48 and 100%, respectively. With high-purity nitrogen, the relative abundance of $M^{\bullet-}$ was increased to 100% and that of $[M - H + O]^-$ decreased to 81%. The results show clearly that the gases used in APPI may have a significant effect on the oxidation reactions and even small residues of oxygen may be enough for the formation of oxidation products.

CONCLUSIONS

In our APPI experiments, the use of dopant (here, toluene) was necessary to achieve high ionization efficiency in both positive and negative ion modes. Reactions leading to ionization of the analyte in APPI are therefore likely to be initiated by the photoionization of the dopant. In positive ion APPI, the ionization of the analyte can occur either by proton transfer or by charge exchange. If the PA of a solvent molecule (or its cluster) is higher

(39) Dzidic, I.; Carroll, D. I.; Stillwell, R. N.; Horning, E. C. *Anal. Chem.* **1975**, *47*, 1308–1312.

(40) Dzidic, I.; Carroll, D. I.; Stillwell, R. N.; Horning, E. C. *J. Am. Chem. Soc.* **1974**, *96*, 5258–5259.

than that of benzene radical $C_7H_7^{\cdot}$ (deprotonated toluene radical cation, $C_7H_8^{+\cdot}$), the main reactant ion in the system is a protonated solvent molecule that can transfer a proton to the analyte if the PA of the analyte is higher than that of the solvent molecule. If the PA of the solvent molecule is lower than that of $C_7H_7^{\cdot}$, then the $C_7H_8^{+\cdot}$ ion remains in the system and ionization of the analyte by charge exchange is favored, if the IE of the analyte is lower than that of C_7H_8 . Because the charge exchange depends only on IEs and not on PAs, nonpolar compounds that mainly have low PAs can be ionized in APPI.

In negative ion mode the ionization of the analyte can occur by electron capture, by charge exchange, by oxidation, or by proton transfer—all initiated by thermal electrons formed in the photoionization of toluene. The compounds with high EAs can form molecular anions either by electron capture or by charge exchange. The formation of the oxidation product $[M - H + O]^-$ of 1,4-naphthoquinone showed that molecular oxygen plays an important role in negative ion APPI. Compounds of high gas-phase acidity may undergo proton transfer. The electron capture provides high selectivity for compounds of high EA in the analysis. However, proton transfer may deteriorate the selectivity, for example, in bioanalysis. Therefore, the selectivity in negative ion

mode might be enhanced by eliminating the possibility of proton transfer by minimizing the number of oxygen atoms or other solvent components capable of undergoing proton transfer.

Impurities and possible reaction products of $C_7H_8^{+\cdot}$ and solvent molecules complicate the ionization process in APPI. Also, the excitation of aromatic compounds by photons may make them more reactive than expected by their thermochemical data. Despite these uncertainties, it can be concluded that selectivity and sensitivity can be adjusted by careful optimization of solvent compositions in LC/APPI-MS.

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