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Liquid chromatography/dopant-assisted atmospheric pressure chemical ionization mass spectrometry for the analysis of non-polar compounds

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ABSTRACT

Liquid chromatography dopant-assisted atmospheric pressure chemical ionization (LC/DA-APCI-MS) has been introduced and demonstrated as a promising method for the analysis of non-polar compounds. Polycyclic aromatic hydrocarbons (PAHs) were studied as non-polar representatives. Chlorobenzene, toluene and anisole were explored as dopants with anisole achieving the best results. Using anisole as the dopant, signal to noise ratio (S/N) for PAHs were up to two orders of magnitude better than those achieved with LC/APCI-MS under identical conditions. While predominant $M^{\bullet+}$ ions were observed for fourteen of the sixteen PAHs by LC/DA-APCI-MS, predominant $[M-H]^+$ ions were observed for acenaphthene and fluorene whose structure is different from other PAHs by containing cyclopenta-fused rings formed by methylene ($-CH_2-$) insertion. PAH and solvent adduct ions, e.g., $[M+CH_2CN]^+$, were also observed for a few PAHs. Reactant ion mass spectra of LC/DA-APCI-MS were examined in detail. All three dopants generated chemical background ions at $m/z \leq 250$, which possibly limited the use of high dopant flow rates to improve the S/N of low-molecular-mass PAHs. They also generated predominant $D^{\bullet+}$ ions. In comparison with toluene, almost two and seven times more $D^{\bullet+}$ ions were observed by chlorobenzene and anisole, respectively. In addition to predominant $D^{\bullet+}$ ions, anisole also generated noticeable amount of $[D+H]^+$ ions. Toluene generated not only $[D+H]^+$ but also $[D-H]^+$ ions. Therefore, while a charge exchange reaction could be mainly responsible for the ionization of PAHs, a proton transfer reaction followed by in-source fragmentation could also be responsible.

1. Introduction

Gas chromatography mass spectrometry (GC/MS) [1] is widely used in the analysis of non-polar compounds, but it is not suitable for compounds with low volatility and thermal lability. Liquid chromatography mass spectrometry (LC/MS) is extensively applied in the analysis of polar compounds that are usually non-volatile and thermally labile [2–4], but it has not been widely used in the analysis of non-polar compounds [5]. Polycyclic aromatic hydrocarbons (PAHs), which are potent environmental mutagens and carcinogens, formed from both natural (e.g., biosynthesis and natural combustion) and anthropogenic sources, are one type of non-polar compounds whose analysis can benefit from LC/MS. The United States Environmental Protection Agency (U.S. EPA) has developed a few methods for the analysis of PAHs in different matrixes, e.g., EPA methods 8100 [6], 8270 [7], 8275 [8] and 8310 [9]. These methods include GC with flame ionization detection (FID) [6], GC/MS [7,8], LC/UV and LC with fluorescence detection (FLD) [9], but do not include LC/MS. Analysis of low-molecular-mass (<300 amu) PAHs by GC/MS has been proven successful, but the analysis of high-molecular-mass (>300 amu) PAHs by GC/MS is difficult due to the low volatility of these compounds [10].

Lack of adequate ionization is the main obstacle in the analysis of non-polar compounds by LC/MS. Currently, LC is coupled to MS through three popular atmospheric pressure ionization (API) sources: electrospray ionization (ESI) [11], atmospheric pressure chemical ionization (APCI) [12], and atmospheric pressure photoionization (APPI) [13,14]. ESI is mainly based on acid–base chemistry in solution. Therefore, it is more suitable for ionizing polar compounds, but has difficulty ionizing non-polar compounds. Although APCI was successfully coupled to normal phase liquid chromatography (NPLC) and actually used in the analysis of PAHs in the 1970s [15,16], the use of reversed phase liquid chromatography (RPLC) in LC/MS applications is more important because the use of NPLC is associated with a large number of problems, including limited compatibility with water in the samples, which results

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in limited reproducibility. Because the major ion/molecule reactions in RPLC/APCI-MS involve gas-phase acid–base chemistry [2], proton transfer is recognized as the major ionization mechanism. Therefore, non-polar compounds have been recognized to have difficulty being ionized by APCI due to low proton affinities (PAs) [5].

Of the three API techniques that couple LC with MS, APPI has achieved the most success in ionization of non-polar compounds [17–20]. This technique was developed around the year 2000 [13,14] with a motivation to extend the range of compounds amenable to LC/MS to non-polar compounds. It was believed that single-photon photoionization could be used to directly ionize non-polar compounds not readily accepting a proton as is generally required by ESI and APCI. In practice, however, it was found that direct APPI was inefficient. Instead, dopant-assisted APPI (DA-APPI), where analytes are ionized through gas-phase ion/molecule reactions with photoions formed by a photoionizable dopant supplemental to the LC effluent, has become the de facto standard method of utilizing APPI due to frequent enhancement of sensitivity by 2–3 orders of magnitude in comparison to direct APPI. Due to the nature of generating ions through gas-phase ion/molecule reactions, DA-APPI can be considered as APCI using photoions, e.g., the radical ions of the dopant, as intermediates. The key difference between them, however, is that both proton transfer and charge exchange ionization mechanisms are frequently observed by DA-APPI [17–20]. With DA-APPI, polar compounds can be efficiently ionized by proton transfer ionization mechanism; non-polar compounds can be efficiently ionized by charge exchange ionization mechanism.

This study is an attempt to regenerate the success of LC/DA-APPI-MS in the analysis of non-polar compounds through incorporation of an appropriate dopant into LC/APCI-MS analysis of non-polar compounds. PAHs, which have been much more successfully analyzed by LC/APPI-MS [21–27] in comparison to LC/APCI-MS [28–34], have been chosen as non-polar representatives. We anticipate that our results will demonstrate that LC/DA-APCI-MS can be a good alternative technique for the analysis of non-polar compounds.

2. Experimental

2.1. Reagents

Compressed nitrogen (minimum purity 99.998%) was purchased from Airgas (Bowling Green, KY, USA). HPLC grade water, acetonitrile and toluene were purchased from Fisher Scientific (Suwanee, GA, USA). Certified A.C.S. grade anisole and CHROMASOLV® HPLC grade chlorobenzene were purchased from Sigma–Aldrich (St. Louis, MO, USA). In the LC/DA-APCI-MS analysis, nitrogen was used as both nebulizing and drying gas; water and acetonitrile were used as mobile phase; chlorobenzene, toluene and anisole were used as dopants. Their formula, monoisotopic mass of $[M-H]^+$, $M^{\bullet +}$, $[M+H]^+$, and $[M_2+H]^+$ ions when applicable, ionization energy (IE), proton affinity (PA) in different forms including monomer, dimer, and [M−H] radical when applicable, are listed in Table S-1 in Supplementary Material. The IE and PA values of the monomers were obtained from the NIST Chemistry WebBook [35]. The PA values of their dimers and [M−H] radicals were thermochemically estimated as described previously [36].

16 PAHs designated by U.S. EPA as priority pollutants were purchased as a mixture solution (EPA 610 Polynuclear Aromatic Hydrocarbons Mixture, Catalog No. 4S8743) from Supelco (Bellefonte, PA, USA) and analyzed by LC/DA-APCI-MS after being diluted 20 times with the initial LC mobile phase, i.e., water/acetonitrile (50/50, v/v). They are listed in alphabetical order in Table S-2 in Supplementary Material with their molecular formula, monoisotopic mass, final concentration, IE and PA values. The IE and PA values were obtained from the NIST Chemistry WebBook [35]. Their chemical structure is shown in Fig. S-1 in Supplementary Material.

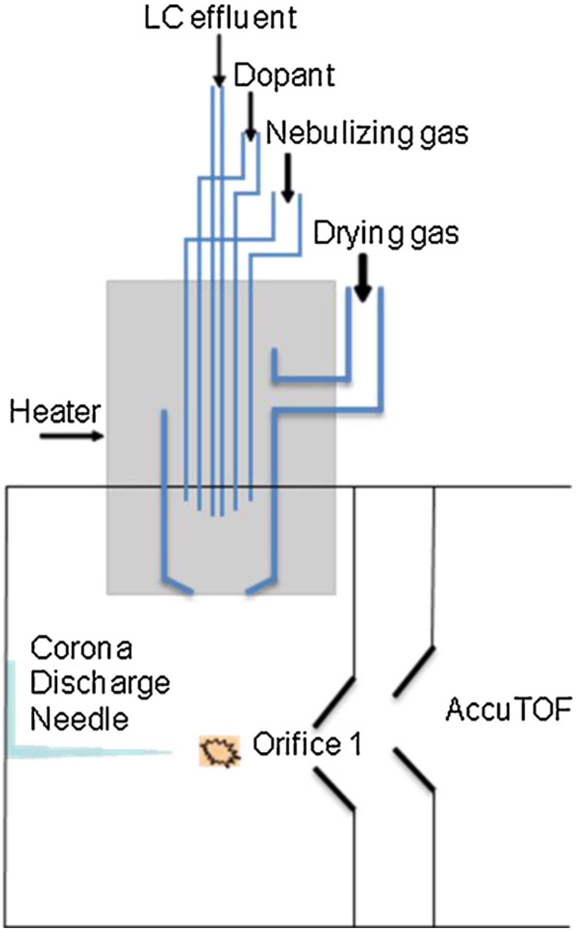

Fig. 1. A schematic of the DA-APCI ion source.

2.2. Apparatus

An Agilent (Santa Clara, CA, USA) 1100 series HPLC system with a quaternary pump and a thermostatted column compartment was used for the separation. It was coupled online to a JEOL (Peabody, MA, USA) JMS-T100LC (AccuTOF^{TM}) orthogonal time-of-flight (TOF) mass spectrometer through a JEOL APCI ion source. The APCI sprayer was modified so that dopant could be delivered coaxially to the LC effluent and mixed at the tip of the APCI sprayer. A schematic of the APCI ion source is shown in Fig. 1. The orifice 1 of the AccuTOF, the corona discharge needle, and the APCI sprayer were in one vertical plane. The distance between orifice 1 of the AccuTOF and the tip of the corona discharge needle, the distance between orifice 1 of the AccuTOF and the center of the LC effluent, and the distance between the center of the LC effluent and the tip of the corona discharge needle was 10, 4.7 and 5.3 mm, respectively. The length of the APCI sprayer was 78.9 mm. The distance between the tip of the APCI sprayer to the horizontal plane of the corona discharge needle and the orifice 1 of the AccuTOF was 36.1 mm. A Harvard Apparatus (Holliston, MA, USA) micro syringe pump was used to deliver the dopant. A Grace Vydac (Deerfield, IL, USA) 201TP5115 C18 column (150 mm × 1 mm × 5 μm) was used for all LC separations.

2.3. LC/DA-APCI-MS

The LC mobile phases were (A) water and (B) acetonitrile. The LC flow rate was 100 μL/min and the LC column was thermostatted at 20 °C. The eluting gradient was as follows: 0 min, 50% B; 4 min, 50% B; 11 min, 100% B; 25 min, 100% B. The injection volume was 20 μL. All the isomeric PAHs were well-resolved, though dibenzo[ah]anthracene and benzo[ghi]perylene were not baseline separated from each other. Dopant flow rates of 0, 5, 10, 20, 50, 100, and 200 μL/min, corresponding to 0, 5, 10, 20, 50, 100, and 200% of the LC flow rate, respectively, were tested. The general controlling parameters for the AccuTOF, which was operated at a resolution power of over 6000 (FWHM, full width at half maximum) for reserpine, were as follows: corona discharge needle voltage, 4600 V; orifice 1 voltage, 85 V; orifice 2 voltage, 10 V; ring lens voltage, 15 V; desolvating chamber temperature, 400 °C; orifice 1 temperature, 100 °C; ion guide RF voltage, 200 V. The flow rates of nebulizing and drying gas were 4.5 and 0.5 L/min, respectively. The spectra recording interval was 1 s. The mass acquisition range was *m*/*z* 30–300 because there were no significant ions observed outside this range. It should be noted that neither the LC separation nor the MS detection were specially optimized for quantification. The ion guide RF voltage of 200 V was specially selected so that small reactant ions down to *m*/*z* 20 could be observed, though it reduced the signal of analyte ions above *m*/*z* 200.

3. Results and discussion

3.1. Analysis of PAHs by LC/APCI-MS

Previously, LC/APCI-MS has been used in PAHs analysis with limited success [28–33]. Both $[M+H]^+$ and $M^{\bullet+}$ ions were observed and the predominance appeared to be dependent on the instrument used. With the use of a SCIEX (Concord, Ontario, Canada) API III triple quadrupole mass spectrometer [28–30], higher-molecular-mass (>200 Da) PAHs generated more abundant $[M+H]^+$ ions relative to $M^{\bullet+}$ ions, while lower-molecular-mass (<200 Da) PAHs produced more abundant $M^{\bullet+}$ ions. With the use of a Fisons Platform (now Micromass, Manchester, UK) benchtop quadrupole instrument [31,32], the mass spectra of benzo[a]pyrene, benzo[ghi]perylene and dibenzo[al]pyrene were dominated by $[M+H]^+$ ions, while the mass spectrum of benzo[c]chrysene was dominated by $M^{\bullet+}$ ion. A Hewlett-Packard (now Agilent, Santa Clara, CA) model 1100 mass-selective detector (MSD) was also used [33], only a minor extent (4–10%) of $[M+H]^+$ ions were observed for all the PAHs studied with the largest being indeno[1,2,3-cd]pyrene and benzo[ghi]perylene.

In our LC/APCI-MS analysis of PAHs, predominant $M^{\bullet+}$ ions were observed for 14 of 16 PAHs studied. The exceptions were acenaphthene and fluorene with predominant $[M-H]^+$ ions. Their structure is different from other PAHs studied by containing cyclopenta-fused rings formed by methylene $(–CH_2–)$ insertion, as shown in Fig. S-1. The $[M-H]^+$ ions should be the fragmentation and/or hydrogen abstraction products of $M^{\bullet+}$ ions based on previous results with chemical ionization (CI) and corresponding interpretation [37]. Representative chromatograms of the 16 PAHs analyzed by LC/APCI-MS are shown in Fig. S-2A in Supplementary Material.

3.2. Analysis of PAHs by LC/DA-APCI-MS

Three popular dopants have been used in LC/DA-APPI-MS analysis of non-polar compounds, i.e., toluene [13,38], anisole [39] and chlorobenzene [27,40]. They were explored in the analysis of PAHs by LC/DA-APCI-MS in this study. It is noted that benzene assisted LC/APCI-MS has also been previously used in the analysis of hydrophobic compounds of significant environmental relevance such as pesticides, benzofurans and dioxin congeners [34]. However, a low-temperature trap had to be used to condense its vapor due to its high toxicity. In addition, another study using benzene as the dopant in DA-APPI-MS has shown that benzene was not an efficient dopant due to its reaction with oxygen [41]. Therefore, the use of benzene as the dopant in this study was excluded.

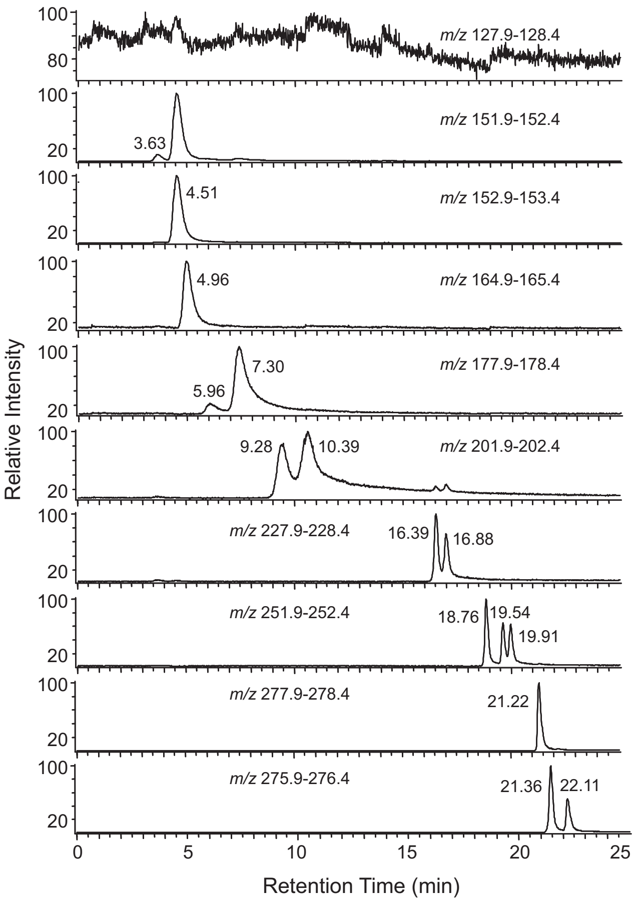

Fig. 2. Representative chromatograms of the 16 PAHs analyzed by LC/DA-APCI-MS with anisole as dopant at a flow rate 50% of the LC mobile phase, i.e., 50 μL/min. Other LC/DA-APCI-MS conditions are described in Section 2. Although the acquisition used a full scan mode from *m*/*z* 30–300, extracted ion chromatograms with mass window of 0.5 *m*/*z* units was used to demonstrate the separation of the PAHs. The elution sequence is naphthalene (*m*/*z* 127.9–128.4), acenaphthylene (*m*/*z* 151.9–152.4), acenaphthene (*m*/*z* 152.9–153.4), fluorene (*m*/*z* 164.9–165.4), phenanthrene (*m*/*z* 177.9–178.4), anthracene (*m*/*z* 177.9–178.4), fluoranthene (*m*/*z* 201.9–202.4), pyrene (*m*/*z* 201.9–202.4), benzo[a]anthracene (*m*/*z* 227.9–228.4), chrysene (*m*/*z* 227.9–228.4), benzo[b]fluoranthene (*m*/*z* 251.9–252.4), benzo[k]fluoranthene (*m*/*z* 251.9–252.4), benzo[a]pyrene (*m*/*z* 251.9–252.4), dibenzo[a,h]anthracene (*m*/*z* 277.9–278.4), benzo[ghi]perylene (*m*/*z* 275.9–276.4) and indeno[1,2,3-cd]pyrene (*m*/*z* 275.9–276.4).

Representative chromatograms of the 16 PAHs analyzed by LC/DA-APCI-MS with chlorobenzene, toluene and anisole as the dopant at a flow rate 50% of the LC mobile phase, i.e., 50 μL/min, are shown in Fig. S-2B and C, both located in Supplementary Material, and Fig. 2, respectively. A comparison of the use of different dopant on the detection of PAHs by LC/DA-APCI-MS, which is represented by the signal-to-noise ratio (S/N) of a PAH at the same loading amount on the LC column, is shown in Fig. 3. The S/N of a PAH was calculated by mass spectrometric software, i.e., MassCenter 1.3.8, using the peak height of the chromatographic peak (*S*) and the standard deviation of the background noise. Error bars of the S/N in Fig. 3 represent the standard deviation (SD) of three

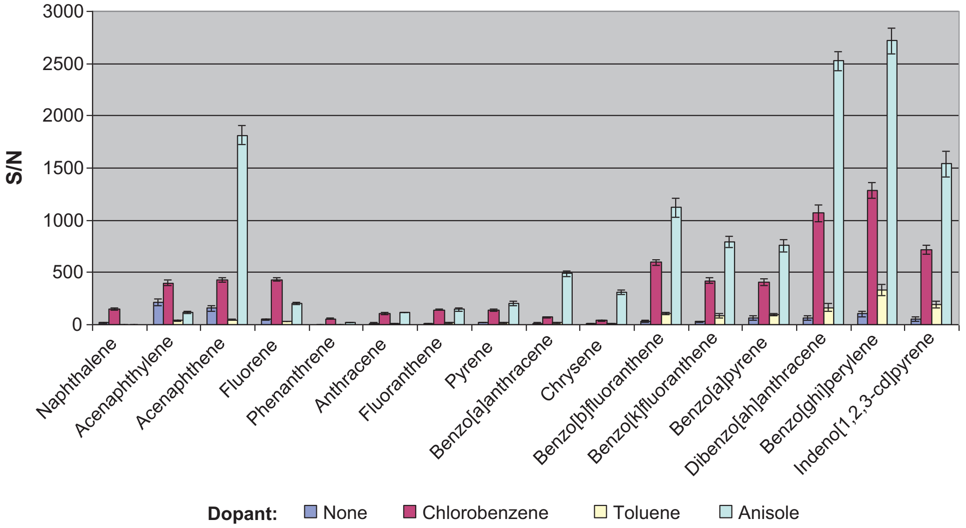

Fig. 3. A comparison of the use of different dopants on the detection of PAHs by LC/DA-APCI-MS. The dopant flow rate was 50% of the LC mobile phase, i.e., 50 μL/min. Other LC/DA-APCI-MS conditions are described in Section 2.

replicates. It should be noted that the relative standard deviation (RSD) of the S/N showed clear dependence on the S/N. Lower RSD were observed with larger S/N and vice versa.

It can be seen from Fig. S-2 and Figs. 2 and 3 that the use of anisole as the dopant achieved the best S/N for most of the PAHs except for a few low-molecular-mass PAHs. These PAHs, including naphthalene, acenaphthylene, fluorene, and phenanthrene, achieved the best S/N when chlorobenzene was used as the dopant. The use of toluene as the dopant provided the worst S/N of all the PAHs. Using LC/DA-APCI-MS with anisole as the dopant, the S/N of PAHs has been improved up to two orders of magnitude than those achieved by LC/APCI-MS.

The effect of dopant flow rate on the detection of PAHs by LC/DA-APCI-MS with chlorobenzene as the dopant is shown in Fig. 4. Error bars of the S/N are not included in Fig. 4 because similar RSDs (shown in Fig. 3), which were dependent on the S/N, can be expected. The tested dopant flow rates were 0, 5, 10, 20, 50, 100 and 200% of the LC flow rate, i.e., 0, 5, 10, 20, 50, 100 and 200 μL/min. It can be seen from Fig. 4 that for PAHs with molecular mass < 170, 170–250 and > 250, the optimum dopant flow rate was 5, 20 and 100 μL/min, respectively.

The effect of dopant flow rate on the detection of PAHs by LC/DA-APCI-MS with toluene and anisole as the dopant is shown in Fig. S-3B and C in Supplementary Material. Toluene's result was similar to that of chlorobenzene. The result with anisole was also close to that of chlorobenzene. However, anisole caused more decrease in S/N of low-molecular-mass PAHs than chlorobenzene, e.g., 63.3 versus 11.5 for acenaphthylene. On the other hand, anisole caused less increases of S/N for high-molecular-mass PAHs than chlorobenzene, e.g., 3.3 versus 4.6 for indeno [1,2,3-cd]pyrene.

The ionization products of the PAHs remained consistent regardless of the type and amount of dopant use. Using the major characteristic ion peak of each PAH, an extracted ion chromatogram, with a mass window of 0.5 *m*/*z* units, was obtained. The retention time of each PAH was then determined. The mass spectrum of each analyte at its maximum chromatographic retention time was subsequently obtained and background subtracted

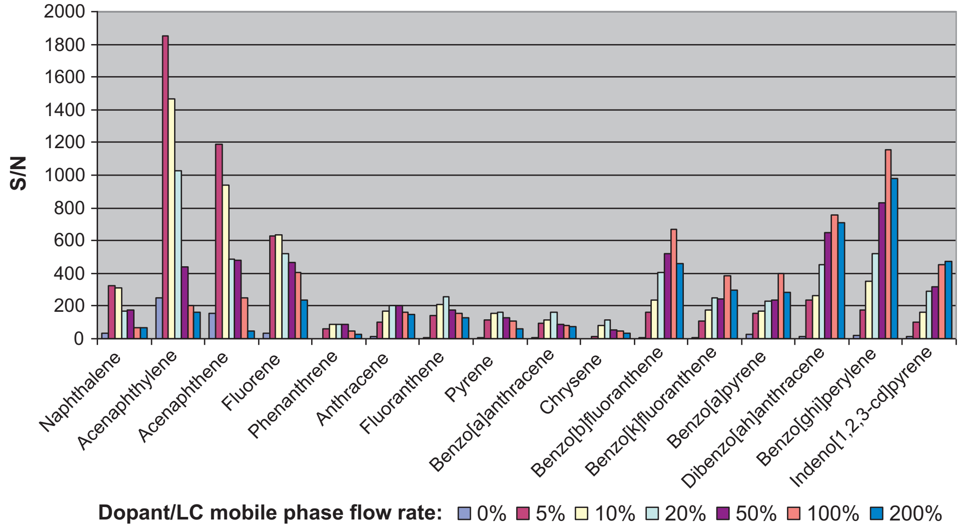

Fig. 4. Effect of dopant flow rate on the detection of PAHs by LC/DA-APCI-MS with chlorobenzene as the dopant. LC flow rate was 100 μL/min. Dopant flow rate was 0, 5, 10, 20, 50, 100 and 200 μL/min. Other LC/DA-APCI-MS conditions are described in Section 2.

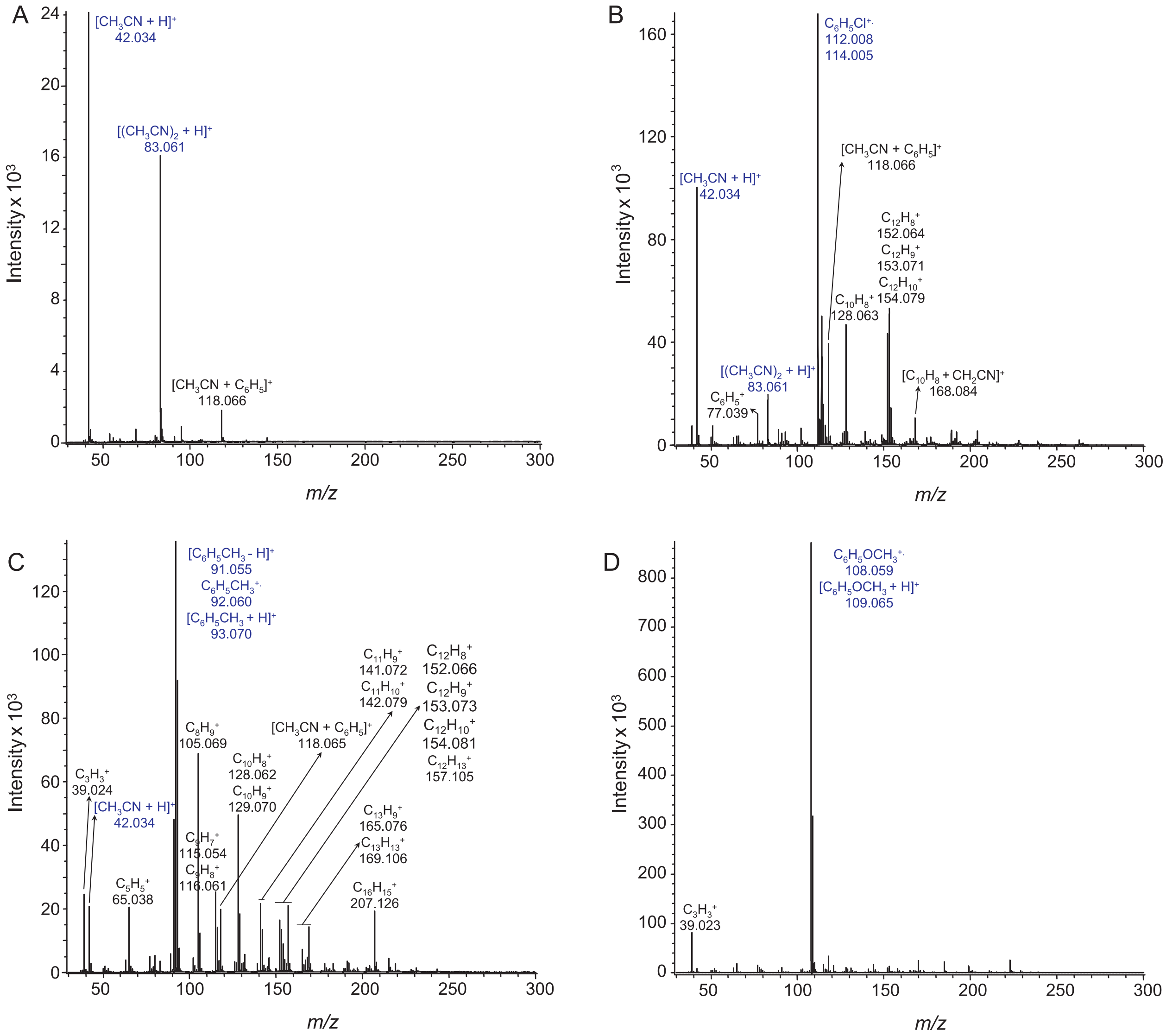

Fig. 5. Representative reactant ion mass spectra of LC/DA-APCI-MS with initial LC mobile phase, i.e., 100 μL/min water/acetonitrile (50/50, v/v), and none (A), chlorobenzene (B), toluene (C) and anisole (D) as dopant at a flow rate 100% of the LC mobile phase, i.e., 100 μL/min. The mass spectrometric controlling parameters are described in Section 2. In Fig. 2A, the spectrum was calibrated with $[CH_3CN{+}H]^+$ and $[(CH_3CN)_2{+}H]^+$ ions. The *m*/*z* 118.066 ion is therefore identified as $[CH_3CN{+}C_6H_5]^+$, not $[(CH_3CN{+}H_2O)_2]^+$. Its presence could not be eliminated and was possibly due to aromatic compounds in the ambient air. In Fig. 2B, the spectrum was calibrated with $[CH_3CN{+}H]^+$, $[(CH_3CN)_2{+}H]^+$ and $[C_6H_5Cl]^{\bullet+}$ ions. In Fig. 2C, the spectrum was calibrated with $[CH_3CN{+}H]^+$, $[C_6H_5CH_3{-}H]^+$, $[C_6H_5CH_3]^{\bullet+}$ and $[C_6H_5CH_3{+}H]^+$ ions. In Fig. 2D, the spectrum was calibrated with $[C_6H_5OCH_3]^{\bullet+}$ and $[C_6H_5OCH_3{+}H]^+$ ions.

by the mass spectrum taken at 2 min. The ionization products of 16 PAHs analyzed by LC/DA-APCI-MS are listed in Table 1 when anisole was used as the dopant at a flow rate 5% of the LC mobile phase, i.e., 5 μL/min. The intensities listed under the $[M{+}H]^+$ ions were mostly due to the corresponding $M^{\bullet+}$ ion isotope, though for the high-molecular-mass PAHs including benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, approximately 5% of $[M{+}H]^+$ ions could be estimated. Predominant $M^{\bullet+}$ ions were observed for 14 of the 16 PAHs studied with acenaphthene and fluorene being the exceptions with predominant $[M{-}H]^+$ ions. A substantial amount of $[M{-}2H]^+$ and $[M{-}H]^+$ ions were observed for 4 of the 14 PAHs, i.e., phenanthrene, anthracene, benzo[a]anthracene and chrysene (the MW 202 and 228 pairs), together with substantial amount of fragment ions, which might indicate the voltage of orifice 1 of the AccuTOF was so high that in-source fragmentation occurred. It is noted that while the *m*/*z* 152.1 ion could be generated from acenaphthylene, it could also be an in-source fragment ion of many PAHs. As a consequence, extracted ion chromatograms of *m*/*z* 151.9–152.4 could show multiple visible peaks (see Fig. S-2B, C and Fig. 2). Substantial amounts of $[M{+}CH_2CN]^+$ ions were observed for naphthalene and acenaphthylene. In fact, whenever *m*/*z* 152.1 ion ($M^{\bullet+}$ ion of acenaphthylene) was observed, its acetonitrile adduct, i.e., *m*/*z* 192.1, was also observed even when the *m*/*z* 152.1 ion was a fragment ion of acenaphthene, fluorene, phenanthrene and anthracene. Noticeable amount of $[M{+}CH_2CN]^+$ ions, i.e., *m*/*z* 242.0, were observed for fluoranthene and pyrene as well.

Other adduct ions were also observed and were most distinct when chlorobenzene was used as the dopant. The $[M{+}C_6H_4]^+$ and $[M{+}C_6H_4Cl]^+$ ions, i.e., *m*/*z* 228.1 and 263.1, were substantial for acenaphthylene with the former clearly visible in Fig. S-2B. In fact, the same $[M{+}C_6H_4]^+$ ion was also observed in small amount with toluene as the dopant. The $[M{+}D{-}H]^+$ ion was also observed in small amount with both toluene and anisole as dopants. The for-

Table 1
Ionization products of 16 PAHs analyzed by LC/DA-APCI-MS using anisole as dopant at a flow rate 5% to the LC mobile phase, i.e., 5 μL/min.

Compound	[a]$[M-2H]^+$	[a]$[M-H]^+$	[a]$M^{\bullet+}$	[a]$[M+H]^+$	Other detected ions: (m/z) intensity%						
[b]Naphthalene	(126.1)21	(127.1)28	(128.1)100	(129.1)11.0	(145.1)7.3	(149.1)15.1	(168.1)46.5				
Acenaphthylene	(150.1)8.5	(151.1)4.5	(152.1)100	(153.1)12.9	(126.1)7.9	(192.1)42.7					
Acenaphthene	(152.1)82.5	(153.1)100	(154.1)14.4	(155.1) 0	(192.1)11.0						
Fluorene	(164.1)1.7	(165.1)100	(166.1)13.8	(167.1)1.2	(152.1)71.8	(153.1)78.1	(154.1)10.1	(168.1)5.2	(192.1)10.9		
Phenanthrene	(176.1)38.1	(177.1)32.9	(178.1)41.2	(179.1)6.9	(152.1)100	(153.1)44.6	165.1 (24.5)	(192.1)17.7			
Anthracene	(176.1)50.2	(177.1)47.0	(178.1)100	(179.1)16.7	(128.1)4.7	(152.1)93.7	(153.1)26.9	(192.1)18.2			
Fluoranthene	(200.1)5.7	(201.1)4.7	(202.1)100	(203.1)15.5	(152.1)15.0	(153.1)8.0	(176.1)8.5	(177.1)5.4	(178.1)7.4	(205.1)6.0	(242.1)11.1
Pyrene	(200.1)2.3	(201.1) 2.5	(202.1)100	(203.1)17.5	(152.1)11.2	(153.1)6.7	(176.1)3.6	(177.1)3.1	(178.1)4.5	(205.1)4.0	(242.1)7.4
Benzo[a]anthracene	(226.1)29.8	(227.1)15.0	(228.1)100	(229.1)15.3	(152.1)3.9	(202.1)22.9					
Chrysene	(226.1)64.9	(227.1)47.9	(228.1)100	(229.1)14.6	(152.1)5.4	(202.1)37.6	(203.1)7.0				
Benzo[b]fluoranthene	(250.1)13.9	(251.1)7.2	(252.1)100	(253.1)23.0	(202.1)6.6	(226.1)4.7					
Benzo[k]fluoranthene	(250.1)8.9	(251.1)2.8	(252.1)100	(253.1)23.8	(202.1)6.8						
Benzo[a]pyrene	(250.1)5.9	(251.1)3.5	(252.1)100	(253.1)22.6	(202.1)5.9	(226.1)3.6					
Dibenzo[a,h]anthracene	***(276.1)18.6***	***(277.1)11.1***	(278.1)100	(279.1)23.1	(252.1)5.5						
Benzo[ghi]perylene	(274.1)0	(275.1)0	(276.1)100	(277.1)31.3	(252.1)17.7						
Indeno[1,2,3-cd]pyrene	(274.1)0	(275.1)0	(276.1)100	(277.1)29.6	(202.1)4.7						

The m/z ***276.1*** and ***277.1*** ions listed for dibenzo[ah]anthracene were observed due to benzo[ghi]perylene which was not baseline separated from dibenzo[ah]anthracene.

[a] (m/z) intensity%. The listed intensities of the $[M+H]^+$ ions included the contribution from the isotope of the $M^{\bullet+}$ ions.

[b] Chlorobenzene was used as the dopant at a flow rate 5% of the LC mobile phase, i.e., 5 μL/min, because the use of anisole as the dopant could not ionize naphthalene.

mation of unnecessary dopant adduct ions makes chlorobenzene a less attractive dopant.

3.3. *Reactant ion mass spectra*

Sunner et al. [42] have described the reactions involved when a corona discharge occurs in air having normal humidity levels. Briefly, the corona discharge generates energetic electrons, which ionize ambient air producing primary ions (mainly $N_2^{\bullet+}$ and $O_2^{\bullet+}$). Water is then ionized by the primary ions. Due to self-protonation and clustering, protonated water clusters dominate the reactant ion mass spectrum [2]. However, during LC/APCI-MS analysis, the difference between the gaseous composition inside the APCI source and ambient air with normal humidity level has to be considered. A modern APCI source is generally an enclosed assembly with gaseous species, which is composed of nebulizer gas, drying gas and LC effluent flowing out of the heated nebulizer at a high flow rate. Therefore, the gas contents of the APCI source are being replaced rapidly. In this study, the combined flow rate of nebulizing and drying gas, i.e., N_2, was 5 L/min. The flow rate of LC mobile phase, i.e., water and acetonitrile, was 100 μL/min. The maximum flow rate of the dopant, i.e., chlorobenzene, toluene or anisole, was 200% of the LC mobile phase. A solvent flow rate of 100 μL/min is approximately equivalent to 0.05 L/min of gas at 25 °C and one atmosphere pressure, assuming an ideal gas, a solvent with molecular weight 50 g/mol and a density of 1.0 g/mL. Therefore, N_2 was the most abundant species inside the APCI source, which should be preferentially ionized by energetic electrons generated from the corona discharge to produce primary ions. The ionization of the rest species inside the APCI source by the primary ions, however, should depend on IEs of these species.

The background mass spectra of LC/APCI-MS and LC/DA-APCI-MS with a dopant flow rate of 100% of the LC mobile phase, i.e., 100 μL/min, were examined. Over the 25 min LC elution, the total ion current (TIC) over the mass acquisition range of m/z 30–300 was not significantly changed (usually less than 10%) regardless of the dopant usage. However, the TIC was significantly lower without a dopant, which could be ascribed to the significantly higher IE of water and acetonitrile than the dopants (see Table S-1). Among the three dopants, the TIC increased as the IE of the dopant decreased. At 2 min retention time, the TIC of the LC mobile phase without a dopant and with chlorobenzene, toluene and anisole as the dopant was 0.25, 6.50, 6.83 and 12.61 × 10^6, respectively.

A representative reactant ion mass spectrum of the LC/APCI-MS analysis with initial LC mobile phase, i.e., 100 μL/min water/acetonitrile (50/50, v/v), is shown in Fig. 5A. Protonated water clusters are absent and protonated acetonitrile monomer and dimer ions are predominant. This result is reasonable because acetonitrile was introduced into the APCI source as part of the LC mobile phase and its PA is much higher than water (see Table S-1). Primary ions are also absent in Fig. 5A because they are highly reactive with short lifetime [43].

Representative reactant ion mass spectra of LC/DA-APCI-MS with initial LC mobile phase, i.e., 100 μL/min water/acetonitrile (50/50, v/v), and dopant, i.e., chlorobenzene, toluene and anisole, at a flow rate of 100% of the LC mobile phase are shown in Fig. 5B–D, respectively. It can be seen that toluene generated many chemical background ions at $m/z \leq 250$ with high intensity. Their relative intensity to the base peak, i.e., its $D^{\bullet+}$ ion, was up to 50%. In comparison to toluene, chlorobenzene generated fewer chemical background ions at $m/z \leq 250$ with slightly lower intensity. Their relative intensity to the $D^{\bullet+}$ base peak was also lower, up to 28%. Anisole appeared to generate many chemical background ions as well. Their intensity was actually only slightly lower than those from chlorobenzene. However, their relative intensity to the $D^{\bullet+}$ base peak was lower than 5%. After careful calibration of the reactant ion mass spectra, accurate masses of the observed chemical background ions with relative intensity $\geq$10% of the base peak were determined and their corresponding elemental composition was estimated and listed in Fig. 5. Most chemical background ions could be considered as either $[M-H]^+$ or $M^{\bullet+}$ ions of hydrocarbons. For example, the ion at m/z 128.06 had the same elemental composition as naphthalene. Other ions, i.e., $[CH_3CN+C_6H_5]^+$ and $[C_{12}H_8+CH_2CN]^+$, could be considered as adduct ions of acetonitrile and hydrocarbons. When chlorobenzene was previously used as reagent gas in chemical ionization (CI), m/z 128, 152, 153 and 154 were also observed in the reactant ion mass spectrum [44]. Previously, acetonitrile was also used as reagent gas in CI and abundant $[M+CH_2CN]^+$ ions were observed [45–47]. It appeared to us that the $D^{\bullet+}$ ions of the dopants could be in-source fragmented. And these fragment ions might attach to other ions and form ions with higher m/z than the $D^{\bullet+}$ ions of the dopants.

The $D^{\bullet+}$ ions of the dopant might be more related to the ionization of the PAHs. As seen in Fig. 5, all the dopants generated predominant $D^{\bullet+}$ ions. In comparison with toluene, about 1.7 times more $D^{\bullet+}$ ions were observed by chlorobenzene when both isotopes of chloride were considered. Anisole generated about 6.9 times more $D^{\bullet+}$ ions than toluene. In addition to predominant $D^{\bullet+}$ ions, anisole also generated 33% $[D+H]^{\bullet+}$ ions. Toluene generated not only 75% $[D+H]^{\bullet+}$ but also 37% $[D-H]^{\bullet+}$ ions. Therefore, anisole generated 3.2 times more $[D+H]^{\bullet+}$ ions than toluene.

Reactant ion mass spectra of LC/DA-APCI-MS with initial LC mobile phase, i.e., 100 μL/min water/acetonitrile (50/50, v/v), and dopant, i.e., chlorobenzene, toluene and anisole, at other flow rates, e.g., 200, 50, 20, 10 and 5% of the LC mobile phase were further examined. The relative abundance of the $[CH_3CN+H]^+$ and $[(CH_3CN)_2+H]^+$ to the dopant ions increased with the decrease of the dopant flow rate, indicating more LC solvent was ionized. The TIC of the dopant ions also decreased with the decrease of the dopant flow rate.

Reactant ion mass spectra of LC/DA-APCI-MS with the same dopant at a constant dopant flow rate were also examined throughout the LC gradient elution. The appearance of the reactant ion mass spectra did not change significantly, but it did reflect the change of the LC mobile phase.

3.4. *Ionization mechanism*

Two typical gas-phase ion/molecule reactions, i.e., proton transfer and charge exchange, have been reported to be responsible for the ionization of PAHs by APCI [48]. Previous LC/APCI-MS studies have attributed PAH-derived $[M+H]^+$ ions to a proton transfer reaction with protonated water clusters and PAH-derived $M^{\bullet+}$ ions to a charge exchange reaction with primary ions [28–33]. In this study, predominant $M^{\bullet+}$ ions of most PAHs were detected by both LC/APCI-MS and LC/DA-APCI-MS. Therefore, it was possible that a charge exchange reaction was mainly responsible, especially in consideration that predominant $D^{\bullet+}$ ions were observed in the reactant mass spectra when a dopant was used (see Fig. 5B–D). However, protonated acetonitrile monomer, toluene and anisole were also observed in the reactant mass spectra. Because acetonitrile and toluene have lower PA than all the PAHs studied (see Tables S-1 and S-2), protonated acetonitrile monomer and toluene could transfer their proton to the PAHs. Protonated anisole may also transfer its proton to the PAHs with higher PA than anisole. The protonated PAHs may then undergo in-source fragmentation, lose hydrogen and became the $M^{\bullet+}$ ions observed. With a high orifice 1 voltage, i.e., 85 V, used in this study, both reactions would eventually contribute to the same ionization product, i.e., $M^{\bullet+}$ ions, which was beneficial to improve S/N in the detection of PAHs. In order to fully understand the ionization mechanism, further experiments are needed.

4. Conclusion

LC/APCI-MS is not well suited for the analysis of non-polar compounds, which has been demonstrated in the analysis of PAHs by others and again in this study. However, this study has further demonstrated that PAHs can be well analyzed when LC/APCI-MS is performed with an appropriate dopant. Chlorobenzene, toluene and anisole were explored as the dopant and anisole achieved the best results. Using LC/DA-APCI-MS with anisole as the dopant, the S/N of PAHs has been improved up to two orders of magnitude than those achieved by LC/APCI-MS.

Reactant ion mass spectra showed that both the LC solvents and dopants could be ionized by LC/DA-APCI-MS. However, the ionization of the LC solvents did not pose a problem for LC/DA-APCI-MS in the analysis of PAHs except that it promoted the formation of solvent adduct ions, i.e., $[M+CH_2CN]^+$ ions, for a few PAHs. In addition, chemical background ions at $m/z \leq 250$ were observed for all three dopants, which possibly limited the use of high dopant flow rates to improve the S/N of low-molecular-mass PAHs by LC/DA-APCI-MS. A charge exchange reaction could be mainly responsible for the ionization of PAHs due to the observation of predominant $M^{\bullet+}$ ions, but a proton transfer reaction followed by in-source fragmentation could not be ruled out.

Nevertheless, LC/DA-APCI-MS seemed to be a good alternative technique for the analysis of non-polar compounds, especially for the analysis of PAHs with high-molecular-mass (>300 amu), because GC/MS has difficulties due to their low volatility. In order to better understand the ionization mechanism, strength and limitations of LC/DA-APCI-MS in comparison to LC/DA-APPI-MS, a direct comparison between them using an identical LC/MS system should be performed. A full optimization and validation of a quantification method of PAHs by LC/DA-APCI-MS, which could not be carried out in this study due to its scope, can then be completed. Subsequently, the achieved limit of detections (LODs) and limit of quantifications (LOQs) can be compared with GC/MS methods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2011.01.028.

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